Virologic failure and HIV-1 drug resistance among antiretroviral therapy recipients in an urban resource-limited setting - the South African Virologic Evaluation (SAVE) study

Ziad El-Khatib
“What is best in me I owe to my mom”

Barack Obama

ما هو من أفضل صفاته يعود فضله لأمي

باراك أوباما
In sub-Saharan Africa, more than four million HIV-infected people have been initiated on antiretroviral therapy (ART); most only have access to first line-treatment, and few ART clinics can provide second-line regimens to everyone in need due to the cost of equipment to detect virologic failure and the cost of alternative drugs. Hence, sustaining the usage of first-line regimens is crucial. More than 25% of ART recipients are residents of South Africa. The overall aim of this thesis was to study determinants of virologic failure and the development of drug resistance among ART recipients in South Africa.

**Article I:** We assessed, longitudinally, risk factors for incomplete adherence in a cohort of HIV-infected women (n=154) initiating ART and examined the association between adherence to ART and virologic response. Seven per cent had a viral load (VL) >400 copies/ml at month 6 on ART. Incomplete adherence was associated with lower education (p=0.01) and lack of financial support from a partner (p=0.02) after adjustment for confounders. Only when adherence levels dropped below 80% was there a significant association with viremia in the group overall, although adherence <95% was associated with viremia among those exposed to single-dose nevirapine (sdNVP).

**Article II:** Risk factors for virologic failure were assessed among long-term ART recipients in a cross-sectional study in Soweto, among 998 patients receiving ART for >12 months. Fourteen per cent (n=139) of line-one ART recipients (n=883) had VL >400 copies/ml; 12% (102/882) on first-line vs 33% (37/115) on the second-line regimen were viremic. Two-thirds vs one-third on line-one vs line-two ART had drug-resistance mutations (DRM). A history of poor adherence, concurrent HIV/TB treatment, being at a public clinic and not having a refrigerator at home were risk factors for virologic failure on treatment.

**Article III:** In a retrospective cohort study among 456 non-nucleoside reverse transcriptase inhibitor (NNRTI) recipients in Soweto, we assessed the association between coming late for drug refill visits, as one of the World Health Organization Early Warning Indicators (WHO-EWIs), and treatment failure. After a median of 15 months on ART, 19% (n=88) and 19% (n=87) had failed virologically (here defined as two repeated VL>50 copies/ml) and immunologically (as defined by WHO) respectively, and both types of failure were associated with coming late to drug refill. In the final multivariable model risk factors for virologic failure were incomplete adherence and previous exposure to sdNVP or any other antiretrovirals (ARVs). In Kaplan-Meier analysis the virologic failure rate by month 48 was 19% (adherent) vs 37% (non-adherent).

**Article IV:** Risk factors for HIV-1 DRM development and persistence of viremia were assessed among 43 NNRTI-recipients with a VL >400 copies/ml after a minimum of 12 months on ART. Sequences were obtained from 38/43. Of those, 82% had 1-7 DRM. In bivariate analysis remote exposure to sdNVP or prior ARVs; higher CD4 cell counts; lower VL; and >6 months of virologic failure were significantly associated with number of DRM. Among 25 viremic patients that were continued on an NNRTI-containing regimen despite viremia, 12 (48%) re-suppressed after a median of 8 months, 6 with K103N and 3 with M184V. Thirteen (52%) had continued virologic failure, which was significantly associated with detectable VL >6 months prior to study enrollment and number of DRM.

**Conclusion:** Overall, about 1 in 10 failed virologically in these urban township settings in Johannesburg, South Africa. Most patients failing virologically after long-term ART had at least one DRM. Intensive adherence support appears particularly important among women with pre-exposure to ARVs and patients showing virologic failure. Adherence to drug refills works as an early warning indicator for both virologic and immunologic failure, and can be used in settings where measurement of viremia is unavailable.

**Keywords:** HIV-1, South Africa, Soweto, antiretroviral therapy, subtype C, adherence, virologic failure, drug resistance, World Health Organization, early warning indicators.
LIST OF ARTICLES

I  Ziad El-Khatib, Anna Mia Ekstrom, Ashraf Coovadia, Elaine J. Abrams, Max Petzold, David Katzenstein, Lynn Morris, Louise Kuhn.
Adherence and virologic suppression during the first 24 weeks on antiretroviral therapy among women in Johannesburg, South Africa – a prospective cohort study.
BMC Public Health, 2011; 11 (1): 88

II  Ziad El-Khatib, Anna Mia Ekstrom, Johanna Ledwaba, Lerato Mohapi, Fatima Laher, Alan Karstaedt, Salome Charalambous, Max Petzold, David Katzenstein and Lynn Morris.
Viremia and drug resistance among HIV-1 patients on antiretroviral treatment: a cross-sectional study in Soweto, South Africa.
AIDS 2010; 24 (11): 1679-1687

III  Ziad El-Khatib, David Katzenstein, Gaetano Marrone, Fatima Laher, Lerato Mohapi, Max Petzold, Lynn Morris, Anna Mia Ekstrom.
Adherence to drug-refill is a useful early warning indicator of virologic and immunologic failure among HIV patients on first-line ART in South Africa.

IV  Ziad El-Khatib, Allison K. DeLong, David Katzenstein, Anna Mia Ekstrom, Johanna Ledwaba, Lerato Mohapi, Fatima Laher, Max Petzold, Lynn Morris and Rami Kantor
Drug resistance patterns and virus re-suppression among HIV-1 subtype C infected patients receiving non-nucleoside reverse transcriptase inhibitors in South Africa.
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# List of Abbreviations

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<th>Acronym</th>
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<tr>
<td>AIDS</td>
<td>Acquired Immunodeficiency Syndrome</td>
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<td>ANC</td>
<td>Antenatal care</td>
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<td>ART</td>
<td>Antiretroviral treatment</td>
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<td>ARVs</td>
<td>Antiretroviral drugs</td>
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<td>CD4</td>
<td>A protein marker on the surface of a special type of T lymphocytes</td>
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<td>CD4 cell count</td>
<td>Indicating the level of T-helper cells</td>
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<td>CI</td>
<td>Confidence interval</td>
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<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<td>DOT</td>
<td>Directly observed therapy</td>
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<td>DRM</td>
<td>Drug-resistance mutations</td>
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<td>EWIs</td>
<td>Early warning indicators</td>
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<td>HIV</td>
<td>Human immunodeficiency syndrome</td>
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<tr>
<td>LPV/r</td>
<td>Lopinavir (PI), ritonavir-boosted</td>
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<tr>
<td>mDOT</td>
<td>Modified directly observed therapy</td>
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<td>MR.SAVE©</td>
<td>Medical records review – South African Virologic Evaluation</td>
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<td>MSM</td>
<td>Men having sex with men</td>
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<td>NICD</td>
<td>National Institute for Communicable Diseases</td>
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<td>NNRTI</td>
<td>Non-nucleoside reverse transcriptase inhibitor</td>
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<tr>
<td>NRTI</td>
<td>Nucleoside reverse transcriptase inhibitor</td>
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<td>OR</td>
<td>Odds ratio</td>
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<td>PHRU</td>
<td>Perinatal HIV Research Unit</td>
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<tr>
<td>PI</td>
<td>Protease inhibitor</td>
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<td>PLWH</td>
<td>People living with HIV</td>
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<td>PMTCT</td>
<td>Prevention of mother-to-child transmission</td>
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<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
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<td>SAVE</td>
<td>South African Virologic Evaluation</td>
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<td>sdNVP</td>
<td>Single-dose nevirapine</td>
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<td>TAM</td>
<td>Thymidine analogue mutation</td>
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<td>TB</td>
<td>Tuberculosis</td>
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<tr>
<td>UNAIDS</td>
<td>The Joint United Nations Programs on HIV and AIDS</td>
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<td>VL</td>
<td>Viral load</td>
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<td>WHO</td>
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My first encounter with an AIDS patient occurred in 1995 while I was working as a nursing assistant in Karak Noah, Lebanon. It was the first time I became aware of the medical society’s stigma against HIV and AIDS patients. At the time, we were uninformed about the disease and how to manage this case. The patient was discharged and had to travel outside of Lebanon to seek HIV care. After the patient was discharged, we were required to burn all the bed sheets. The experience resonated with me.

Another experience which made an impression upon me after that was while working an internship within medical laboratory sciences. We had to screen nightclub dancers in Beirut for HIV every third month. Again I noticed a stigma about them at the hospital.

People used to reach out about the HIV topic in a different way. My impression was that they had a stigma about it, because its “reputation” is that it is transmitted sexually. Because of this stigma and ignorance on the issue, there were no affordable treatments for affected patients.

In 2001 I attended a conference on “safe sex on campus” in Las Vegas, USA. The discussion was on how to protect teenagers from becoming infected by HIV in a city where buying sex is not a crime.

In 2002 I came to Umeå University, Sweden, to pursue my master’s studies in Epidemiology and International Health. In 2005 I worked with Medecins Sans Frontieres (MSF) as a flying laboratory technician in the Sobbat Corridor, south east of Sudan. This became an eye-opener for me with regard to HIV and lack of access to medicine. Every time someone discussed access to antiretroviral therapy (ART), three arguments always popped up against the ART roll-out: “limited access to drugs, adherence and virologic failure”.

Following these eye-opening experiences, when I returned to Sweden I started to conduct internet research on those in the forefront of HIV research. It was there that I located Professor David Katzenstein’s work and contact information, and I emailed him about my interests and found that he was eager to collaborate on a research project. When discussing this topic with a colleague from MSF, Dr Christian Unge, I came to know about my PhD supervisor, Dr Anna Mia Ekström, and her new HIV research group at the Division of Global Health/IHCAR. In October-November 2005 I visited 13 ART programs in South Africa and Zimbabwe. We discussed the feasibility of starting an HIV drug resistance network. It was there that I met my co-supervisor Professor Lynn Morris. This was the first step towards starting my PhD project at two treatment clinics at Chris Hani Baragwanath Hospital in Soweto, South Africa.

All in all I spent three years in Johannesburg to start up the project, which included making infectious diseases/HIV-related trips to Ghana and Zimbabwe. My passion for MSF remained. I was invited by the MSF-South Africa office to be a board member. This gave me the chance to remain active in the organization and in the debate around access to ART. I realised it was a helpful combination between research and humanitarian assistance. When I returned to Sweden, I wanted to sustain this combination of work, so I went into the board elections of MSF-Sweden and joined them.

Almost every week or every second week I used to see an announcement for the funeral of an undergraduate student. When I asked the staff about it, they used to say that they have to attend funerals, almost every weekend. The reasons for the deaths were suspected to be HIV-related. It is therefore my hope that this thesis work will be able to contribute new knowledge to assist individuals who have tested HIV positive and who are receiving ART.
BACKGROUND

By 2010, the United Nations AIDS agency (UNAIDS) estimated that 33.4 million people were living with HIV (PLWH) [1], >50% of whom are resident in sub-Saharan Africa (Figure 1; Appendix 1). The acquired immune deficiency syndrome (AIDS) as an end-stage of HIV infection in the absence of antiretroviral therapy (ART) is a major contributor to adult mortality in sub-Saharan Africa [1]. The HIV epidemic has reduced life expectancy, impaired economic development and reversed population growth in certain countries in southern Africa [2].

Figure 1. Estimated number of HIV-infected adults on ART and their virologic outcomes

The estimates (upper value) made after the UNAIDS [3], WHO [1], Boulle et al. [4], Hoffmann et al. [5], Hosseinipour et al. [6], Marconi et al. [7], Rosen et al. [8] and Coetzee et al. [9].

In southern Africa transmission among adults mainly occurs through concurrent sexual relationships [10-13]. At the time of sexual contact, the virus crosses the mucosal barrier of the vagina, vulva, penis or rectum by first coming into contact with tissue antigen presenting cells (also known as dendritic cells or Langerhans cells). These cells adsorb virus that has successfully penetrated the mucosa and serve as a Trojan horse [14], delivering the virus to susceptible lymphocytes by releasing it into lymphatic tissues. Then the virus binds to a permissive CD4 cell which may migrate to the lymphatic tissue, and initiate infection. As HIV replicates, more and more CD4 cells are destroyed.

The average risk of transmission without any type of prevention is estimated to be up to 9 per 1000 coital vaginal sexual relationships [12]. However, transmission risk depends on the amount of virus, i.e. the viral load (VL), which in turn is subject to large individual variation but also dependant on the phase of HIV infection (Figure 2). During acute and early infection adults have high VL levels in the plasma and genital secretions, which may contribute to a significant fraction of transmission. This is illustrated in the acute and early phases, where in the first 6 months of infection there is an increased plasma VL associated with increased risk for transmission [15]. Following early infection, which may be associated with flu-like
symptoms, there is an asymptomatic phase estimated to last on average from 7 [16] to 10 [17] years. Individuals with HIV infection gradually develop increasingly severe immunodeficiency and risk for opportunistic infections and malignancies as the CD4 cell count falls below 350 cells/mm³. The VL level and risk of transmission are higher during the acute and AIDS phase (Figure 2).

Women are at a higher risk than men of acquiring HIV during heterosexual intercourse due to both biologic reasons [18], such as the larger mucosal surface area exposed to seminal fluid, and social reasons such as lower negotiation power when it comes to sex in general and safe sex in particular.

Apart from behavioral and social interventions aimed at reducing vulnerability and risk behavior, prevention of HIV infection may also be achieved through different biomedical interventions [19]. For adults these include using condoms, male circumcision [19-21], ART [22], ART-based microbicides [23] and, most recently, pre-exposure prophylaxis with ART.

ART also effectively reduces the risk of mother-to-child transmission (MTCT) among HIV-infected pregnant women. Combination ART given to the woman during pregnancy combined with treatment postpartum to mother and/or the infant until the end of the breastfeeding period has been shown to reduce MTCT by up to 90% [24-26]. A few countries, such as Uganda [27] and Thailand [28], have shown that it is possible to successfully control the HIV epidemic by using different social and biomedical interventions, including promoting one sexual partner and using condoms [29].

Yet there is no cure or vaccine for HIV. However, with ART, HIV has become a chronic disease.

The development of ART may be viewed as evolving through three different eras, depending on the type of antiretrovirals (ARVs) and their potency [31]. Era one utilised the by then only known effective drug, zidovudine, as a nucleoside reverse transcriptase (NRTI) monotherapy, between 1987 and 1991. However, zidovudine drug-resistance mutations (DRM) were quickly selected after initiating patients on treatment [32-34]. In era two, between 1991 to 1995 [31], zidovudine, zalcitabine, lamivudine and didanosine were used as dual NRTI therapy with modest

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Figure 2. Illustration of the relationship between VL, risk of transmission and viral evolution post-HIV infection

Source: Simon et al. [30]
improvements in virologic and immunologic outcome compared to NRTI monotherapy [32, 35-37]. Era three, from 1995 to the present [31], includes a combination of at least three different drugs from at least two different classes. ART can reduce the viral replication cycle below a detectable level. This reduces the risk of viral replication and the risk of development of DRM [38].

Although the pharmaceutical industry was active in the development of ART, these drugs were not affordable in low- and middle-income countries. However, based on time for medicine patents ending, generic drug manufacture and activist pressure, the estimated cost of drugs in developing countries decreased from US $10,000 to US $350 per person per year [39]. In 2003 the WHO put forward a target of 3 million HIV-infected patients to be initiated on ART by the end of year 2005, known as the “3-by-5” initiative [40]. The WHO clinical guidelines [41] at the time of the data collection of this study recommended initiating patients on ART based on CD4 cell counts of less than 250 cells/mm$^3$ or presence of any AIDS symptoms. However, in 2010 around 5 million HIV-infected individuals are estimated to be receiving ART, the majority of them (4 million; 80%) being residents of sub-Saharan Africa (Figure 1). One million (25%) of those 4 million live in South Africa.

At the time of this study, triple therapy-based ART regimens are orally administered and recommended as two different line regimens: (i) first-line regimens include the combination of one non-nucleoside reverse transcriptase inhibitor (NNRTI) (efavirenz or nevirapine) and two NRTIs (zidovudine or stavudine, in combination with lamivudine) [30, 40] (Figure 3); or (ii) in the case of virologic failure, patients are recommended to be switched into a second-line regimen, including one protease inhibitor (PI) (lopinavir/ritonavir or saquinavir/ritonavir) and two NRTIs (abacavir or tenofovir, and didanosine) [30, 40] if available.

![Figure 3. Main first-line regimen used among ART recipients in 36 low- and middle-income countries by December 2008](source: WHO progress report, 2009)

**HIV-1 life cycle**

HIV infection is initiated at a cellular level when the envelope glycoprotein 120 (gp120) binds to CD4 T-cells through the primary receptor (CD4) on the cell surface (Figure 4). Then, using a
second receptor, which is usually either CCR5 or CXCR4, there is fusion and release of the viral nucleocapsid core into the cell cytoplasm. Upon entering the cytoplasm of a permissive cell, reverse transcriptase (RT) enzyme contained within the viral nucleocapside starts the reverse transcription, to convert the viral RNA into a double-stranded DNA. When a complete, a double-stranded cDNA copy has been formed, viral integrase (a part of the RT) carries the viral DNA to the nucleus and through a complex process facilitates the integration of viral DNA genomic sequences to random sites in the host cell DNA. Once there is an integrated copy of HIV proviral DNA in the cell, this is termed latent infection at the cellular level. This means that the cell harbors proviral DNA without the expression of viral proteins or viral antigens on the cell surface. After a variable period of latency, which may be many years, the activation of the CD4 cell in which there is proviral DNA triggers reactivated viral transcription.

When the cell is activated, the transcription of the integrated DNA may be initiated. The viral mRNA is transcribed to make the proteins that are assembled within the host cell, and genomic viral RNA molecules are packaged into a nucleocapsid core. The virus particles are released through the host cell membrane, resulting in an enveloped virus particle expressing gp120 on the surface, which is a protein-lipid membrane from the host, completing the virus maturation cycle.

Figure 4. HIV-1 life cycle and points of interventions for the NNRTI and PI-based regimens
Source: Simon and Ho [42]

HIV-1 drug resistance

HIV-1 drug resistance is one of the major factors associated with virologic failure [43]. HIV replicates at a very high rate, with billions of copies created on a daily basis. At every replication cycle there is the possibility of single mutations, potentially including drug-resistant variants,
due to the high levels of errors associated with reverse transcriptase [44, 45]. The genetic barrier to drug resistance is defined as the number of mutations required to overcome drug pressure and eventually develop drug resistance. Patients receiving NNRTIs can achieve high levels of resistance through single base-pair mutations, leading to a single amino acid change, conferring cross-resistance within the class. Thus, NNRTIs are known to have a low genetic barrier. Certain mutations can be resistant to more than one drug, known as cross-resistance (Appendix 2).

Four recent southern African studies among NNRTI recipients identified treatment failure by virologic or immunologic criteria [5, 7, 46, 47]. The prevalence of HIV drug resistance ranged from 62% to 95% [5-7, 46, 47]. In the first year of ART, Marconi et al. [7] in KwaZulu-Natal (n=115) and Orrell et al. [46] in Cape Town (n=110) identified DRM among 83% and >87% of their patients respectively. In longer-term studies, Hoffmann et al. [5, 48] in South Africa (n=68) and Hosseinipour et al. [6] in Malawi (n=94) reported DRM after a median of >36 months, among 62% and 95% respectively. Wallis et al. [47] in Johannesburg reported DRM among 84% of 226 viremic patients; however, duration of treatment was not reported.

**HIV-1 subtypes**

HIV may be divided into two different subtypes: HIV-1 and HIV-2. HIV-1, in turn, is divided into three major groups: group M (main), group O (outlier) and group N (non-M, non-O) [49]. The global epidemic is fuelled mainly by group M. Group M has 10 subtypes (A to K). Sub-Saharan Africa is predominated by HIV-1 subtype C, which is causing >50% of the global HIV-1 epidemic (Figure 5).

![Figure 5. Global distribution of HIV subtypes](Source: Taylor et al. [50])

The ability of the virus to replicate, known as ‘fitness’, [51] is related to different factors depending on its environment, either related to the immune system or drug pressure [52, 53]. In vitro data from India show that subtype C is more fit than subtype A [54].
Virologic outcomes among subtypes: This is not a totally understood area [50]. When it comes to HIV drug resistance development, K103N, M46L, I84V, Y181C and Y188C are reported to be more prevalent in subtype C than in other subtypes [55-58]. The D30N is reported to be common in subtype B [57]. The most common mutation in subtype B was thymidine analogue mutation (TAM). [57]. Subtype B is predominant in high-income countries and subtype C is predominant in low- and middle-income countries; therefore, patients might be exposed to different drugs. In terms of virologic outcomes, studies from Canada [59], France [60] and the United Kingdom [61] found no significant difference between subtype B and other subtypes.
“When I run out of drug options, I kiss my viremic patients on both cheeks, send them home and pray for the best for them” (Medical doctor’s statement on managing viremic patients with a lack of drug options, Johannesburg, 2009)

Previous guidelines recommended ART to be initiated at <200 CD4 cells/mm³ with the median CD4 cell count of ~100 cells/mm³, pre-ART initiation [4]. Overall, more than 70% of HIV patients initiated on ART in sub-Saharan Africa are women. However, the majority of HIV patients in sub-Saharan Africa only have access to one line regimen. Therefore, sustaining them on the same line regimen is crucial for the success of this only ART regimen.

Access to VL and drug resistance testing

The aim of ART is to suppress viral replication as much as possible [62]. There is no final consensus about the definition of treatment failure [63, 64]. In high-income countries clinical guidelines recommend keeping VL <50 copies/ml [65]. In resource-limited settings, virologic failure has been defined as either VL >400 copies/ml, two repeated VL >1000 copies/ml or >5000 copies/ml [5, 7, 24, 47, 66, 67]. However, virologic monitoring is not accessible among most of the ART programs in sub-Saharan Africa. Where VL laboratory diagnostics are available, mainly in South Africa, virologic failure is defined as two repeated VL >1000 or >5000 copies/ml [68].

In the absence of VL testing, the recommendations are to use clinical symptoms or CD4 cell count as a proxy for virologic failure [41]. The WHO has formulated three criteria used to define immunologic failure: (i) a CD4 cell count of 100 cells/mm³ post-6 months on ART, (ii) a reduction to or CD4 cell count level below the pre-ART CD4 cell count level, post-6 months on ART, or (iii) a 150% decrease from the on-treatment peak CD4 cell count [24]. Individual patients may be offered second-line therapy based on these criteria. There is growing evidence that relying only on CD4 cell count assessment is neither sensitive nor specific for virologic failure [6, 69, 70]. Switching patients who are failing immunologically although they are virologically suppressed, may be an expensive manoeuvre.

In high-income countries, viremic patients are assessed for the presence of DRM, using routine laboratory assays. In low- and middle-income countries this is not affordable. Data on HIV drug resistance coming from the latter settings were from research-based studies. In 2010 a large systematic review on HIV drug resistance was done among 8376 line-one recipients in limited-resource settings [71]. There was a significant difference in NNRTI drug resistance prevalence rates at week 48 among infrequently and frequently monitored patients (88% (95% CI 82.2–92.9) and 61% (95% CI 48.9–72.2) (p<0.01) respectively). Yet there is no access to HIV drug resistance testing in limited-resource settings. There is a need for low-cost diagnostics to sustain use of the first-line regimen.
Drug resistance mutations are assessed by using the International AIDS Society-USA list [72] for all drug classes (Appendix 2), that is updated on yearly basis. There are also different online drug resistance algorithms that can be used, for free, to interpret the genotypic mutation results [73].

**Adherence to ART**

The WHO defines adherence as “the extent to which a person’s behavior – taking medication, following a diet, and/or executing lifestyle changes, corresponds with agreed recommendations from a health care provider” [74].

**Adherence assessment**

It is not feasible to assess drug concentrations in plasma, therefore adherence is used as a proxy to monitor drug exposure, although there is no “gold standard” to measure adherence. HIV ART-related research work has reported the use of different methods (Figure 6), including:

(i) **Pharmacy drug-refill appointment:** As part of its strategy to monitor and evaluate the emergence of HIV drug resistance, the WHO [75, 76] recommends using seven early warning indicators (EWIs). One of them is to assess coming late for pharmacy refill visits as proxy for adherence to ART. Patients are usually dispensed their pills on a monthly basis, i.e. with around 30 days of drug supply [75]. This assessment is done by using the formula \([\frac{(Actual\ date\ coming\ for\ drug\ refill\ visit - Appointment\ date\ to\ be\ back\ to\ the\ clinic) \times 100}{total\ number\ of\ days\ since\ ART\ initiation}\].

(ii) **Self-reported adherence:** This is based on asking patients for approximate adherence to ART, using one of the following methods:

   (a) **Self-reported:** Patients are asked about the number of missed pills during the last four days or last weekend [77].

   (b) **Visual analogue scale:** Patients’ adherence to ART is assessed using an ordinal scaling system for adherence level [78]. They are shown a line ranging between 0% and 100%, and are asked to self-assess their adherence using this line.

(iii) **Pharmacy pill count:** This measure of adherence is recommended by the WHO [Adherence = \((\frac{Number\ of\ pills\ dispensed - Number\ of\ pills\ returned}{Number\ of\ pills\ prescribed\ daily\ \times\ Number\ of\ days\ between\ pharmacy\ visits})\ \times\ 100\)]\. A pill count can be done either at the time of the pharmacy visit, i.e. when patients come back for their drug refill visit, or by visiting/contacting patients to count their pills (known as ‘unannounced pill count’).

    **Observed therapy:** (a) **Directly observed therapy (DOT)** - based on observing patients while taking their ART pills, by health staff, a relative or a treatment buddy, on a regular basis [79];

    (b) **Modified DOT (mDOT)** - similar to DOT, but patients are observed on certain occasions only [79].

(iv) **Medical electronic monitoring system:** Pill boxes have an electronic chip that registers the date and time of when the pill box is opened.

(v) **Therapeutic drug monitoring:** By assessing the drug concentration in plasma or hair, the concentration of some ARVs may be measured [80]. However, this method is rarely used to assess adherence due to large individual variation.
In sub-Saharan Africa adherence to ART is reported to be associated with different health outcomes [81], including virologic failure [82-89], change in CD4 cell count after ART initiation [83, 90-92], lower health cost [93], death [94], and loss-to-follow-up [91, 92, 94].

To achieve viral suppression, continuous combined-therapy pressure is required [24] (Figure 7). Adherence to ART is important, due to the risk of HIV drug resistance development, which leads to treatment and ultimately clinical and immunologic failure in limited-resource settings. Therefore, to sustain virologic suppression, ART recipients are recommended to maintain a very high level of adherence [88, 95]. An extensive review by Mills et al. [96] showed that adherence levels are generally higher among patients on ART in low- and middle-income countries compared to high-income countries. However, patients are usually prescribed ART on a monthly basis, which means they have to come every month for drug refills. Patients who do not have any more pills at home and come >24 hours late for their drug refill visits can be at risk of low-drug pressure [94]. The NNRTIs have a longer half-life concentration in the blood than the NRTIs. After >24 hours have passed from the last dose of NRTIs and NNRTIs, there may be differential drug exposure in the blood, i.e. a monotherapy for NNRTIs alone, which gives a window of opportunity for the virus to replicate in the presence of suboptimal drug pressure. This will be reflected, eventually, in an increased number of DRM and a higher VL. Repeated treatment interruptions increase the risk of virologic failure and DRM (Figure 7).
Barriers to adherence

There are different explanations of barriers to adherence to ART. An extensive review by Mills et al. [98] reports the following three main reasons related to patients: simply forgetting; being busy/distracted; and being away from home. As for health services, barriers to adherence included: financial constraints; pharmacy drug stock-out [99, 100] and not understanding the treatment.

Bartlett et al.’s [101] systematic review of clinical trials found that 60-80% of NNRTI recipients (no exposure to any ARVs, pre-ART initiation) had VL ≤50 copies/ml at week 48 when having 5-6 pills per day. The proportion of NNRTI recipients reaching suppression has decreased to 20% in studies administering from 7 up to 12 pills per day. Having high number of pills per day is considered a pill burden, and is associated with incomplete adherence [102-106].

Claxton et al.’s [106, 107] extensive review of 76 studies found an inverse relationship between number of doses per day (i.e. number of times patients are required to take their pills per day) and adherence to treatment (Figure 8). The NNRTI has a longer-half life than NRTIs - therefore patients have to take two doses of NRTIs and one dose of NNRTIs per day, when administered ART in the form of three different drugs.

![Graph](image)

*Figure 8. Adherence level (%) in relation to number of doses per day*

*Source: Claxton et al. [107]*

Previous exposure to single-dose nevirapine or any ARVs

Women may have been exposed to sdNVP during their pregnancy, which may jeopardise their virologic suppression on ART [108] if they were initiated on ART within 12 months of their exposure to sdNVP [109].

More than 600 000 children (mostly in sub-Saharan Africa) are estimated to be infected with HIV at the time of birth [3, 110, 111]. In 2008, the WHO and South African guidelines recommended administering a single 200 mg dose of nevirapine to HIV-infected women in labour and a single 2 mg/kg dose of nevirapine to their infants within 72 hours of birth, to reduce the risk of MTCT [110, 112, 113]. However, this may select HIV-1 DRM for the nevirapine and can jeopardise the ART outcome when women are initiated on therapy [109, 110, 113]. The recent WHO guidelines recommend initiating pregnant women on ART [24], which will reduce both risks of MTCT and virologic failure for these women.
Tuberculosis/HIV coinfection

Tuberculosis (TB) is a global public health problem, especially among HIV-infected patients. In sub-Saharan Africa more than 50% of reported TB cases have also been diagnosed with HIV (Figure 9). And TB has been reported as the main cause of death among HIV patients [1, 114]. At the time of the study, guidelines for treating TB were the same for HIV-infected and uninfected patients, recommending 6-12 months of a rifampicin-based multiple drug combination regimen, depending on the location of the TB infection and the presence of TB drug resistance [115]. In a recent review by Lawn et al. [116], TB incidence rates on ART were shown to decrease after ART initiation. However, baseline CD4 cell count, sex and socio-economic conditions still influence TB acquisition on ART. The success of TB/HIV therapy can be jeopardised due to either drug-drug interaction and/or the increase in pill burden for patients [102, 114, 117].

![Figure 9. Proportion of persons infected with TB (per 100 000) vs proportion of population infected with HIV-1](source: www.gapminder.org)

Most ART care is carried out in parallel to TB treatment, i.e. TB/HIV patients will be seeking care at two different clinics [118]. Seeking treatment at two different locations might add an additional cost onto patients for transport. This can be a major barrier for patients dually infected and receiving treatment for these two diseases [119]. Additional burdens can be the pill burden, adherence, overlapping side-effects, drug toxicities and drug-drug interactions [118].
Primary DRM

Primary DRM can be due either to patients being infected with HIV-1 DRM, known by transmitted drug resistance, or post-exposure to sdNVP as part of the prevention of mother-to-child transmission (PMTCT) programs.

The WHO considers >5% of primary DRM as a public health concern [75]. Blower et al. [120] developed a model predicting virologic failure due to transmitted DRM. A recent study by Wittkop et al. [121] showed that patients with any type of HIV drug resistance, pre-ART initiation, are at higher risk for virologic failure during ART.

The National Institute for Communicable Diseases (NICD) in Johannesburg started HIV drug resistance surveillance from 2002 in Gauteng province, followed by two other provinces, KwaZulu-Natal and Western Cape. The surveillance program includes women attending antenatal care clinics who have no history of parity. So far the prevalence of DRM has been <5% in South Africa [122, 123].
SOUTH AFRICA

Country context

The Republic of South Africa consists of nine provinces, with a total area size of 1,219,080 km², located in the southern tip of the continent of Africa. The country has an estimated population size of ~50 million. The most densely populated province is Gauteng, which includes the cities of Johannesburg and Pretoria, with an estimate of 614 persons per km² (Table 1).

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total population [125]</td>
<td>49,99 million</td>
</tr>
<tr>
<td>Proportion of females [125]</td>
<td>51%</td>
</tr>
<tr>
<td>Proportion of persons earning less than US $2 per day [126]</td>
<td>43%</td>
</tr>
<tr>
<td>Unemployment rate [127]</td>
<td>25%</td>
</tr>
<tr>
<td>Gini coefficient [128]</td>
<td>0.68</td>
</tr>
<tr>
<td>HIV prevalence rate among adult* population [1]</td>
<td>28%</td>
</tr>
<tr>
<td>Total adult literacy** rate [129]</td>
<td>88%</td>
</tr>
<tr>
<td>TB incidence rate [130]</td>
<td>948 per 100,000</td>
</tr>
<tr>
<td>Number of patients per nurse [131]</td>
<td>714 : 1</td>
</tr>
<tr>
<td>Number of persons per doctor</td>
<td>1300 : 1</td>
</tr>
</tbody>
</table>

*Age 15-49 years; ** Can read and write.

The approximate gross national income per capita is US $8,900 [2], and there is a very large inequality in income (gini coefficient equal to 0.68) [127]. The country achieved a democratic government in 1994, after 46 years of apartheid when the majority was not afforded civil, political or economic rights. Today, the country is described to be going into a “bipolar” epidemiologic transition [132, 133]; this is the phenomenon of a middle-income country facing, on the one hand, the burden of non-communicable diseases such as cancer and cardiovascular diseases, as well as a large burden of injuries, communicable diseases and health conditions related to poverty: HIV, gastro-enteritis, TB, pneumonia and child malnutrition, [132, 134, 135].

HIV in South Africa

The first case in South Africa was reported in 1983, believed to be associated with transmission between men having sex with men (MSM). This was followed by hundreds of cases among the MSM community in the mid-1980s, associated with subtype B infection [136]. In this population HIV infection was part of a cosmopolitan spread of infection among the international MSM community. However, in the early 1990s HIV infection shifted to the heterosexual community [137] carrying subtype C [136]. The HIV epidemic in South Africa is considered one of the fastest
growing globally [136]. However, recent UNAIDS estimates indicate that the HIV prevalence is stabilizing in South Africa [138].

The HIV prevalence among sexually active adults aged 15-45 years has increased 30 times, from 1% in 1990 to around 30% in 2005 [139] where, at younger ages, >70% of infected individuals are women. Twenty-three per cent of men aged 15 to 24 years are estimated to have had more than one sexual partner in a 12-month period of time, where the probability of using a condom with intercourse varies from 20% to 60% [140]. Today the HIV prevalence is approximately 18% [128], and 5.6 million residents of South Africa are estimated to be infected with HIV [138].

**Figure 10. HIV prevalence in South African provinces**

*Figure source: WHO*

**Figure 11. Number of HIV infected individuals per km² in South Africa**

**ART roll-out in South Africa**

The political history of South Africa has had an influence on ART scale-up. In 1994, when South Africa became a democratic country, the HIV prevalence was ~4% [3]. At that time, President Nelson Mandela was faced with the political priorities of the country, including reconciliation, and he did not tackle the HIV issue [137, 142, 143].

The next president, Thabo Mbeki, was challenging the scientific community about the lack of evidence showing that HIV causes AIDS. Therefore the South African Government was labelled as “being in denial” [137, 144], supported by an international network of “AIDS denialists”. Subsequent recommendations came from the former Minister of Health, Dr. Manto Tshabalala-Msiman, to use olive oil and lemon juice against HIV. Estimates by Chigwedere et al. show that earlier action from policy makers could have saved >300,000 HIV-infected patients [145].

In 2004 South Africa started its ART roll-out. The South African clinical guidelines recommend using a first-line regimen including two NRTIs plus one NNRTI, mainly stavudine + lamivudine + [efavirenz or nevirapine] [146]. In case of first-line treatment failure, patients are to be switched to a second-line regimen of two new NRTI drugs [didanosine or tenofovir] + [abacavir or lamivudine] and a boosted protease inhibitor, usually lopinavir/retonavir (kaletra) [147].

In the case of virologic failure, guidelines recommend counseling patients on adherence first. If virologic failure persists over two repeated laboratory check-up visits, it is recommended that patients are switched to a second-line regimen [146]. Treatment is anchored on maintaining first-line therapy to avoid the last and more expensive option. Protease inhibitors cost up to around US $3 per day per patient, with an additional requirement for expensive nucleoside combinations, in comparison to US $1 per day per patient for the first-line regimen [148].
The overall aim of this study is to increase knowledge about virologic failure and the development of drug resistance among ART recipients in South Africa (Figure 12).

**Specific objectives**

1. To identify risk factors for poor adherence and virologic failure among ART recipients (articles I and II);

2. To examine the association between the WHO-EWIs and virologic outcome (article III); and

3. To assess predictors to persistent mutations among ART recipients failing their therapy (article IV).

*Figure 12. Conceptual framework for factors influencing virologic failure and HIV-1 drug resistance development*

*After Nachega et al. [147]; Friedland et al. [148] and Rathgebe and Vlassoff [149]*
<table>
<thead>
<tr>
<th>Article aim (article number)</th>
<th>Outcome (article number)</th>
<th>Study population</th>
<th>Drug class-containing regimen (sample size)</th>
<th>Setting</th>
<th>Design and method</th>
<th>Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Define risk factors for poor adherence during the first 6 months on NNRTIs (I)</td>
<td>Adherence (I)</td>
<td>Women on ART for 6 months</td>
<td>NNRTI (n=154)</td>
<td>One outpatient HIV clinic</td>
<td>Location: Coronation Hospital, urban Johannesburg</td>
<td>Secondary data analysis - non-randomised cohort study</td>
</tr>
<tr>
<td></td>
<td>Virologic failure (II, III)</td>
<td>ART recipients; for ≥12 months</td>
<td>NNRTI (n=883)</td>
<td>Two outpatient HIV clinics, Location: Chris Hani Baragwanath Hospital, Soweto</td>
<td>Cross-sectional study Administered questionnaire</td>
<td>Descriptive; multivariable logistic regression analysis</td>
</tr>
<tr>
<td>Define risk factors for virologic failure and pattern of DRM (II)</td>
<td>Immunologic failure (III)</td>
<td>Subgroup of article II</td>
<td>NNRTI (n=456)</td>
<td>One of the outpatient HIV clinics, enrolled in article II Location: Chris Hani Baragwanath Hospital, Soweto</td>
<td>Retrospective longitudinal study Medical records review</td>
<td>Kaplan-Meier survival analysis Cox regression analysis</td>
</tr>
<tr>
<td>Define the association between coming late for drug refill visits and virologic vs immunologic failure (III)</td>
<td>HIV-1 drug resistance (II, IV)</td>
<td>Subgroup of article II</td>
<td>NNRTI (n=431)</td>
<td></td>
<td>Retrospective longitudinal and follow-up study Medical records review</td>
<td>Descriptive; Ordinal logistic regression; Fisher’s exact and Wilcoxon rank sum tests</td>
</tr>
</tbody>
</table>
METHODS

My role in field work and data collection

Overall the duration of field work for this thesis was 3 years. This included study design, piloting of instruments, site recruitment, training and supervision of four field workers, coordinating with three laboratory scientists and two laboratory managers, and shadowing medical doctors, nurses and pharmacists.

Article I is based on a secondary analysis of data derived from a non-randomised trial conducted at Coronation Hospital (Figure 13) between July 2004 and May 2006. I visited the clinic and pharmacy several times, where I shadowed medical doctors and pharmacists to get a better understanding of their day-to-day work. Also, I attended over a 10-month period a weekly epidemiology session with the staff of Coronation Hospital clinic (Article I) and Nthabiseng clinic (one of the clinics in Article II). They presented and discussed their operational research projects, which gave me a deeper understanding of their work.

![HIV prevalence estimates by district among antenatal clinic attendees, Gauteng, 2007.](image)

**Figure 13.** HIV prevalence by districts in Gauteng province and the location of the study sites
*Adapted from the Department of Health, South Africa [150]*

For Articles II, III and IV (Figure 13), as will be described below, data collection involved different data sources. First I went into the ethics certificate requirements for Gauteng province and became certified to conduct research studies there. For medical records review, I spent around 3 months trying to extract and use the clinics’ datasets. Each clinic has a different system for archiving medical records. Also different database software was used over time, and it was not possible to
merge the datasets. Eventually it became easier to develop special software for medical records review and data entry. First a draft was developed and piloted, then the data-entry software was developed using EpiData [153, 154]. The software’s name was Medical Records Review – South African Adherence and Evaluation study (MR.SAVE)© [155]. Field workers received intensive training, which involved piloting an administered questionnaire (Articles II, III and IV). I was doing data entry every evening in order to give them feedback the following day on any errors in filling out the questionnaire.

For the medical records review (Articles III and IV), field-workers were trained on how to review medical records. For the laboratory data two field workers were also trained, mainly by the nurse laboratory, on how to extract the laboratory data (VL and CD4 cell count).

**Challenges in the field**

For Article I, it took me time to understand the dataset of a study that I was not involved in. For Articles II, III and IV, both clinics are located at the premises of the same hospital (walking distance between them around 5 minutes). Challenges were related to the study logistics and the context of each clinic.

At the non-governmental organization clinic, called the Perinatal HIV Research Unit (PHRU), I first spent around three months reviewing their electronic medical register. They used the Fuchia database system [156] and then switched to a new system. They had technical problems in migrating their dataset from the old to the new database software. I tried to help with the migration of the dataset, but realised it would be more time-efficient to review the medical records instead. The public clinic (called Nthabiseng) used a different database system. This was a relational database system where they used the patient’s name as the key ID for accessing medical records, combined with the patient’s personal number as a control. Due to patient confidentiality we did not collect patients’ private information on our questionnaires. We tried to use patients’ file numbers to link these datasets, but it was not possible to find the proper data. Also, sometimes we found that patients were registered as dead or there were duplicated names or personal numbers. Therefore, Nthabiseng clinic could not be included in Articles III and IV.

Due to the security and safety measures at these clinics, the research team was asked to work during day time and only during week days. I had to adhere to these regulations, which delayed the field work such as the medical records review.

**Study areas and populations**

All of the studies were conducted in the city of Johannesburg, located in Gauteng province, with an estimated HIV prevalence of around 30%, and HIV prevalence in Johannesburg itself of 20-30% (Figure 13). However, the clinics provide health care up to radius of 25 km, which covers the neighbouring districts, with HIV prevalence is up to 34%. It is estimated that 100-150 HIV patients live per km² in the city of Johannesburg (Figure 13).

**Study sites**

**Article I**

During July 2004 through to May 2006 [109], HIV-infected women were identified at one adult HIV clinic in Johannesburg (Figure 13). Inclusion criteria included being: (1) ARV-naïve women
with a CD4 cell count <200 cells/mm³, (2) with a CD4 cell count <350 cells/mm³ plus a WHO stage III or stage IV condition. Such women were eligible to receive ART. Exclusion criteria were acute hepatitis, elevated liver function test values of grade II or more and a history of nevirapine toxicity. Additional participants were referred from other HIV treatment programs near this clinic. Women were defined as “exposed” to sdNVP from their after medical record history, or “unexposed” to it if confirmed by medical records and the study participant [109].

**Articles II, III and IV**
The studies were conducted at two outpatient clinics at Chris Hani Baragwanath Hospital (the largest in Africa) located in Soweto outside Johannesburg, and serving a population of 4 million people [157]. Both clinics are affiliated to the Faculty of Health Sciences at the University of the Witwatersrand in Johannesburg, and had access to ART through clinical research trials before the National ART scale-up in 2004. The first is a donor-funded treatment access clinic called the Perinatal HIV Research Unit (PHRU), located in Chris Hani Baragwanath Hospital [158]. At the time of the study the clinic staff consisted of five medical doctors, two nursing assistants and two counselors, managing around 1500 ART recipients with 50 daily visits [66]. The second is a public clinic [159] with eight medical doctors, four nurses, one nursing assistant and seven counselors managing an estimated 200 HIV patient visits per day from approximately 3500 patients on ART (i.e. around 440 patients for each medical doctor) [66].

The clinics generally provided care according to the South African National Antiretroviral Guidelines [160], which at that time provided for CD4 and VL monitoring 6-monthly, and confirmations of VL >5000 copies/ml in 3-6 months.

**Study designs and data collection**

**Article I**
A questionnaire was administered at study enrollment prior to treatment initiation. This included questions on education level, socio-economic indicators, household members, previous pregnancies, previous live births and number of living children, marital status, quality of life and exposure to sdNVP. Viral load and CD4 cell counts were measured pre-treatment and WHO clinical stage was determined. Women attended the clinic monthly to 24 weeks, and at each visit VL was measured and an adherence assessment was done by the study pharmacist. At week 24 another questionnaire was administered, monitoring life events and quality of life since ART initiation. CD4 cell counts and VL ≤400 or >400 copies/ml were the primary treatment outcomes.

Pill counts were performed by the study pharmacist, who counted the number of remaining pills for each drug separately at each drug refill visit. Refills were scheduled at weeks 2, 4, 8, 12, 16, 20 and 24 from the start of treatment. Pill count-based adherence was assessed by calculating the average combined pill count for the three drugs at each visit, using the formula \[
\text{Adherence} = \frac{(\text{Number of pills dispensed} - \text{Number of pills returned}) \times 100}{(\text{Number of pills prescribed daily} \times \text{Number of days between pharmacy visits})}.
\]

The adherence level of the women was assessed at the seven time-points. Patients were defined as incompletely adherent if they ever had an average pill count of returning >5% of their prescribed pills to the pharmacy, indicating an adherence level of <95% adherence at any visit. We also performed additional analyses of levels of adherence indicated by the percentage of returned pills from 0% to >5%, >10%, >20% and >30% on the outcome of VL >400 copies/ml at week 24. This analysis was done first among all women and then among women exposed vs not exposed to sdNVP.
Patients were asked a battery of questions to assess quality of life, where the sum of questions varied from 12 (very good quality of life) to 55 (the poorest level of quality of life). These responses were categorised as 1) 12-23 (good), 2) 24-35 (intermediate) or 3) 36-55 (poor), to analyse the relationship between quality of life, adherence and virologic response.

Article II
During March-September 2008 we recruited patients through posters in the clinic and pharmacy waiting areas written in the three most widely spoken languages in Soweto: English, isiZulu (Appendix 3) and Sesotho (Appendix 4). Patients interested in the study were provided with an information sheet as well as South African Rands 50 (US$ 5) as transport reimbursement.

The following inclusion criteria were applied: at least 18 years old; being on ART for at least 12 months; and consenting to participate in the study. We initially enrolled 1000 patients (500 at each site), but reduced the number to 998 individuals after discovering that two patients were interviewed twice at the public clinic.

The interview questionnaire was developed in English, translated into isiZulu (Appendix 3) and Sesotho (Appendix 4) and then back-translated into English. After piloting, the final questionnaire included 59 questions with a total of 210 items covering socio-economic background, disclosure, TB treatment and adherence during the previous weekend [161], which served as a proxy for recent adherence. Two research assistants trained in nursing and public health and fluent in all three languages interviewed the patients, each interview took on average 15 minutes. The questionnaire data were entered into a database using EpiData [153, 154]

Article III
All patients receiving an NNRTI-containing regimen at PHRU were assessed retrospectively (Figure 14), which included year of HIV diagnosis, pre-ART initiation characteristics (VL, CD4 cell count, pre-exposure to sdNVP or other ARVs), current and previous TB therapy, dates for drug refill visits and treatment interruptions. VL and CD4 cell count had been measured every sixth month on average. Medical records were not available for two of the patients and they were excluded from the longitudinal analysis, leaving 456 patients for data analyses. A survey form, called Medical Records-SAVE (MR.SAVE©), was developed, piloted and modified [155] for data collection. EpiData [153, 154] was used for data entry.

Article IV
Consenting patients that were enrolled in the study for Article II, at the PHRU clinic only, were also used for Article IV. Persistent virologic failure and resuppression were defined as VL>400 copies/ml and return to ≤400 copies/ml, respectively, at follow-up.

Medical records were reviewed retrospectively to extract information on potential risk factors that may be associated with DRM or resuppression, including age, gender, year of HIV diagnosis, ART regimens and dates, history of sdNVP or other ART exposure, TB treatment, pre-ART initiation VL and CD4 cell counts, WHO stage prior to ART initiation and any treatment interruptions in the last 6 months prior to study enrollment. Virologic failure prior to study enrollment was defined as VL>400 copies/ml at either of the prior two visits in the last 12 months. Poor adherence was considered to be returning more than 7 days late for the drug refill appointment pre-study enrollment. An instrument was designed and tested to extract information from medical records using EpiData [154, 155].
Figure 14. Study design for articles II, III and IV

Pre-data collection

October 2006 – March 2007
Inviting sites and presenting the study design for the staff

April 2007
Completed the Witwatersrand ethics certificate requirements

May – June 2007
Applying to the PHRU internal ethics research board

July 2007
Applying to the Witwatersrand University ethics committee

August – October 2007
Recruiting and training field worker

Data collection

September – November 2008
Epi tool design, piloted and developed for medical records review (clinic one only)
Medical Records SAVE (MR SAVE) ©

November 2008 to December 2009
Medical records review (article III)

November 2008 to December 2009
Follow-up study on failures (article IV)

November 2007 – February 2008
Questionnaire design and study piloting

Cross-sectional study

March – July 2008
Data collection, clinic one (n=500 patients)

July – September 2008
Data collection, clinic two (n=498 patients)

Risk factors for virologic failure (article II)

Feedback to clinics

February 2011
Presenting articles and discussing results with the staff
Laboratory assessments

**Articles I, II and IV:** A 10 ml blood sample was drawn by the clinic nurse at the time of interview. Then plasma viral levels were assessed using the Amplicor HIV-1 Monitor Test, v1.5 (Roche Molecular Diagnostics, Basel, Switzerland) lower limit of detection of 400 copies/ml. CD4 cell counts were done using a BD FACSCount (Becton Dickinson BioSciences, Immunocytochemistry Systems, San Jose, California, USA).

For HIV genotyping (Articles II and IV), viral RNA was isolated from plasma samples with VL >400 copies/ml using the MagNa Pure LC Total Nucleic Acid Isolation kit (Roche Diagnostics, Indianapolis, Indiana, USA) on the MagNa Pure Automated System (Roche Molecular Diagnostics) and sequenced using an in-house assay at the NICD [123].

**DRM and susceptibility scoring**

Assessment of HIV DRM was done using the Stanford HIV database genotypic resistance algorithm. Major DRM were then coded using the International AIDS Society list from December 2009 (Appendix 2) [72] (Articles II and IV). HIV-1 subtype classifications were done using Rega version 2.0 [162] (Articles II and IV). Sequence quality was confirmed prior to analysis by inspecting sequences for possible frame shifts, high numbers of ambiguous nucleic and/or amino acids, extreme levels of pair-wise genetic distances, and atypical amino acids or stop codons (Article IV). To predict phenotypic drug resistance the Stanford HIV database scoring system was applied [163] and a resistance score calculated as: (i) susceptible (0-9) to potentially low-level (10-14); (ii) low (15-29); (iii) intermediate (30-59); and (iv) a high level of resistance (score ≥60) (Article IV).

**Definitions**

**Virologic failure**

In Article I we defined virologic failure as a VL >400 copies/ml at month 6, post-ART initiation. In Article II we enrolled patients at any time beyond 12 months after ART initiation and defined virologic failure as a VL >400 copies/ml at study enrollment. Article III, based on a retrospective longitudinal analysis, defined virologic failure as two repeated VL >50 copies/ml, post-3 months of ART initiation. Finally, in Article IV virologic failure was defined as VL >400 copies/ml at study enrollment (the same as in Article II). Persistence of viremia was defined as VL >400 copies/ml at the follow-up study.

**Immunologic failure**

This was only used as an outcome in Article III and defined by using the WHO criteria of having either: (i) a CD4 cell count <100 cells/mm³ after 6 months on ART; (ii) a CD4 cell count ≤ CD4 pre-ART after 6 months on ART; or (iii) >50% reduction from the on-ART peak CD4 cell count [40]

**Incomplete adherence**

In Article I first pill count-based adherence was assessed by calculating the average combined pill count for the three drugs at each visit, using the formula [Adherence = [(Number of pills dispensed - Number of pills returned) x 100]/(Number of pills prescribed daily x Number of days between pharmacy visits)]. Then patients were defined as incompletely adherent if they ever had
an average pill count of returning >5% of their prescribed pills to the pharmacy, indicating an adherence level of <95% adherence at any visit.

In Article II patients were defined as incompletely adherent if they reported missing taking any of their pills the weekend prior to study enrollment.

In Article III patients were dispensed ART monthly, and at scheduled doctors’ visits patients were given projected monthly pharmacy refill dates and the date of the next doctor’s appointment. At each refill visit pharmacy staff dispensed pills and recorded the date. To estimate adherence we calculated the total number of days where the patient came late for the drug refill visits divided by the total duration on ART. The formula was: [The number of days late for drug refill visit = (Date when the patient came for drug refill - Date of the pre-scheduled appointment indicated on the patient’s medical record)]. The results were then summarised for repeated refill visits to get the cumulative number of days coming late per client. To estimate adherence the following formula was used [The cumulative number of days coming late x 100) / Total number of days the patient was assumed to be exposed to ART given the dispensed number of pills].

In Article IV incomplete adherence was defined as coming >7 days late for the drug refill visit prior to study enrollment.

Data analysis

Article I
Characteristics of the participants (including demographics, socio-economic and pre-treatment clinical factors) were compiled. Associations between incomplete adherence, virologic failure and these other characteristics were assessed using unadjusted odds ratios (ORs) with 95% confidence interval (CI) and p values. Backward-selection multivariable logistical regression analysis was performed including factors with p values ≤0.10 and then repeated for those variables yielding p values ≤0.05. Associations between incomplete adherence at different thresholds and virologic failure were assessed using unadjusted ORs with 95% CI.

Article II
We grouped patients by the type of line regimen, first-line vs. second-line, and then a number of variables were examined as potential predictors of virologic failure. For each variable we first calculated the adjusted OR, 95% CI and p value controlling for sex, education, type of clinic, age and self-reported adherence during the last weekend, which is denoted as the basic adjusted model. The presented adjusted ORs for the five variables above are derived from a regression model including only these covariates. All variables with p values of 0.15 or less in the basic adjusted model were added into a backward selection multiple logistical regression analysis (inclusion criteria was p≤0.05). This model is denoted the final model. The adherence variable was included in the model regardless of p value.

Article III
Descriptive analyses including median (inter-quartile median) for numerical variables, frequencies and proportions for categorical variables were performed. Bivariate analyses to assess risk factors for virologic and immunologic failure were performed using Pearson Chi square and Fisher’s exact tests. Thereafter variables with a p value ≤0.10 were added into a multivariate logistical regression model, and those with a p value <0.05 were considered significant in the final multivariate model, calculating OR and 95% CI. However, the variables sex and age were always maintained in the final multivariate models to account for possible remaining confounders.
Kaplan Meier survival analysis was done using months as the time unit to assess time to virologic and immunologic failure on ART among all patients. Known pre-ART risk factors, exposure to sdNVP [108, 109, 164] or any type of ARVs, CD4 cell count and age [4, 164, 165] were adjusted for using Cox regression analysis for virologic failure. For immunologic failure, Cox regression analysis was done by adjusting for the same above-mentioned variables plus any virologic failure. Due to co-linearity between sex and pre-ART CD4 count, sex was not included in the final survival model.

Finally, we assessed the median gain in CD4 cell count during ART on a 6-monthly basis (range +/- 3 months) among 1) patients with incomplete vs complete cumulative drug refill adherence; and 2) patients with virologic failure vs suppression up to 36 months on ART only, due to data availability.

**Article IV**

Risk factors were examined for associations with two drug resistance outcomes: (i) number of all-class DRM at enrollment; and (ii) continued viremia versus re-suppression at follow-up. Associations between viral resuppression and presence of any DRM, number of NRTI and NNRTI DRM and the total number of DRM at study enrollment were examined. Due to the small sample size, bivariate analysis was performed without adjusting for confounding variables, and the results must be interpreted with this in mind.

Ordinal logistic regression was used to examine the association between risk factors and the number of DRM at enrollment expressed as OR and 95% CI. Unlike Poisson regression, ordinal logistic regression can be fitted to zero-inflated data and does not assume that the events (i.e. accumulation of DRM) are independent and occur at a constant rate. Each model was checked to ensure that the assumption of proportional odds between successive DRM categories was met.

To examine risk factors associated with persistent virologic failure at follow-up, Fisher’s exact tests were used for categorical risk factors (OR, 95% CI) and Wilcoxon rank sum tests for continuous risk factors (difference in median, 95% CI). P values less than 0.05 were considered statistically significant.

**Statistical software**

STATA/SE College Station, Texas (version 10.1) [164] was used in Articles I, II, III and IV; Graphpad Prism (version 4.0c) [165] was used in Article III and R (version 2.11.1) [166] was used in Article IV.
Article I
The original study was approved by the institutional review boards of Columbia University, New York, USA and the Research Ethics Committees at the University of the Witswatersrand, Johannesburg, South Africa (IRB00001233) [109].

Articles II, III and IV
In April 2007 the training course requirements for the Introduction of Clinical Trials and Good Clinical Practice (Accreditations number MDB08/250/01/2007 and MDB08/237/01/2007) were completed (Figure 14).

At the time of study enrollment (Article II) patients that were interested in the study met with one of the research team individually. They were provided with information about the study, including assurance of the confidentiality of their personal information. Patients’ names and signatures to the consent forms (Appendix 5) were kept in a locked drawer at the NICD. Only the chief of the unit and I had access to them. No personal information was captured on the questionnaire form. Finally study participants were provided with 50 South African Rand (US $5) as transport reimbursement.

The studies were approved by the Research Ethics Committees at the University of the Witswatersrand, Johannesburg, South Africa (M070721) and the regional Medical Ethics Board in Stockholm, Sweden (Protocol 2008/3:7).
Figure 15. Key results: Factors associated with virologic failure, DRM and persistence of virologic failure among NNRTI recipients (article number)

Factors typed in **Bold**: Higher risk; Factors typed in *Italic and underlined*: Lower risk
The findings from Articles I-IV are presented following each specific aim for this PhD thesis (please see summary in Figure 15).

**Risk factors for poor adherence and virologic failure**

**Article I**

This study was a secondary data analysis for a study originally designed to look at the impact of sdNVP on ART outcome at month 6 at Coronation Hospital, but patients were invited from the neighbouring clinics to be enrolled in this study.

The study included 147 women, where the majority (88%) were born in South Africa. More than half of them (53%) were below 30 years old and 63% of them had less than 11 years of schooling. Although 83% were single at the time of study enrollment, 75% of all women had been pregnant at least twice and less than half of them (40%) had at least two living children at home. In terms of their economic situation, 38% of single women and 84% of married women self-reported having any type of financial support from a partner or husband. The majority had more than four of the pre-specified household assets: indoor tap water (50%), toilet (42%), electricity (84%), a refrigerator (66%), radio (82%), television (82%) or a landline telephone (11%), defining higher socio-economic status.

Thirty-seven per cent (n=55) of women showed incomplete adherence. In multivariable analysis, two factors remained significantly associated with incomplete adherence (Figure 16): education level ≤ grade 11 (OR 2.6; 95% CI 1.2-5.6; p=0.01), and not receiving financial support from a partner or a husband (OR 2.3; 95% CI 1.1-4.8; p=0.02).

![Figure 16. Summary of factors associated with incomplete adherence among women receiving line one regimen (Article I)](image-url)
Seven per cent (n=11) of the women had virologic failure after 6 months on ART, and more than half (n=6) of these women had a history of incomplete adherence. We found significant associations between virologic failure and incomplete adherence during the first 24 weeks on therapy (Figure 17) (OR=2.1; 95% CI 0.5-9.3; p=0.33), an education level below or equal to grade 11 (OR 2.9; 95% CI 0.6-28.0; p=0.21), living in an informal settlement (OR 2.2; 95% CI 0.5-9.6; p=0.21) and a death in the family during the first 24 weeks on ART (OR 3.3; 95% CI 0.7-14.0; p=0.06). As has been reported previously, there was no significant difference in viral suppression between women exposed to sdNVP 18-36 months earlier relative to exposed women with a pregnancy within the same interval [109].

Overall, there was a significant association between VL >400 copies/ml and ever having an adherence level <80% (p=0.01) or <70% (p=0.01). Next, we assessed the correlation between VL >400 copies/ml and adherence defined using varying thresholds stratifying women based on their exposure to sdNVP. For women who had received sdNVP, there was a significant association between VL >400 copies/ml at week 24 and adherence levels <95%, <90%, <80%, and <70% (p ≤0.03). For women who had not been exposed to sdNVP, there was no significant association at any threshold.

The most common self-reported reasons for missing taking pills were being away, being busy with other things and forgetting.

<table>
<thead>
<tr>
<th>Article I</th>
<th>OR (CI95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pill count &lt;80%*</td>
<td>4.8 (1.0 - 20.8)</td>
</tr>
<tr>
<td>Article II</td>
<td>Missed pills last weekend*</td>
</tr>
<tr>
<td>Concurrent TB/HIV therapy*</td>
<td>6.4 (2.2 - 18.8)</td>
</tr>
<tr>
<td>Lack of refrigerator at home**</td>
<td>6.7 (1.2 - 37.5)</td>
</tr>
<tr>
<td>Attending public clinic**</td>
<td>4.6 (1.8 - 11.3)</td>
</tr>
<tr>
<td>Article III</td>
<td>ART experienced*</td>
</tr>
<tr>
<td>Cumulative adherence &lt;95%*</td>
<td>2.8 (1.2 - 6.7)</td>
</tr>
</tbody>
</table>

* Line 1 regimen; ** Line 2 regimen

Figure 17. Summary of factors associated with virologic failure (Articles I, II and III)

Article II

In 2008 we assessed risk factors associated with virologic failure for first- and second-line regimens among 998 patients in Soweto, South Africa. Eighty-eight percent of these patients were on first-line regimen and the rest (12%) were receiving the second-line regimen at two HIV clinics at Chris Hani Baragwanath Hospital in Soweto, outside Johannesburg.

Three-quarters of the study participants were women (75%), with a median age 41 years. Overall, 64% of patients had been on ART for more than 3 years. The proportion of patients with VL >400 copies/ml was 14% (139/998). Of the 139 viremic samples, 129 (93%) were successfully amplified. Seventy-eight per cent of viremic patients had at least one DRM and 52% (67/129) harbored both NNRTI and NRTI resistance mutations.
For NNRTI recipients: The majority (88%; 883/998) were on an NNRTI-based regimen, and 12% (102/883) of these patients had virologic failure (Figure 17). The related risk factors included concurrent TB treatment (OR 6.4; 95% CI 2.2-18.8; p<0.01) and a recent history of incomplete adherence (OR 2.7; 95% CI 1.3-5.6; p=0.01). Analyzing the existence of DRM or wild-type viremia, we found that 96% of those with DRM reported complete adherence. In contrast, only 63% of patients exhibiting wild-type virus reported incomplete adherence (p<0.01).

PI recipients: The rest (12%; 115/998) were receiving a PI-based regimen and 33% (37/115) had virologic failure (Figure 17). The related risk factors included attending a public clinic (OR 4.6; 95% CI 1.8-11.3; p<0.01) and not having a refrigerator at home (OR 6.7; 95% CI 1.2-37.5; p=0.03).

Coming late to pharmacy visits as an early warning indicator for virologic and immunologic failure

Article III
This study included a total of the 456 NNRTI recipients also enrolled in the study for Article II. Most of the patients (79%) were diagnosed with HIV between 2001 and 2004 (median 2003), 14% before 2000 and the remaining 7% between 2005 and 2008. Pre-ART initiation, 51% (222/434) had a CD4 cell count of ≤100 cell/mm3 and 41% (172/421) had a VL of ≥100 000 copies/ml. Overall, the median time on ART was 44 months (inter-quartile median 38-48; 1,510 person-years) and 77% (349/456) were women. Eighteen per cent (80/445) had been exposed to ARVs previously; 15% of the women (52/349) had received sdNVP for PMTCT with a median time before ART initiation of 15 months, and 6% of all patients (28/446) had received ART before starting the current ART regimen. Approximately half (48%, 199/414) had been treated for TB before ART initiation.

Virologic failure
Overall, 19% (88/456) met the criteria for virologic failure. There was a significant difference in time to virologic failure between patients with complete vs incomplete adherence. By month 12 on ART, the failure rate was similar (7% vs 8% among patients with incomplete and complete adherence respectively), but by month 48 the difference in failure rate had reached statistical significance between the groups, 19% vs 37% respectively, i.e. more than one-third of the patients with incomplete adherence failed virologically (log rank p value=0.02). After adjustment for CD4 cell count, age and exposure to any ARVs pre-ART initiation in the Cox regression analysis (Figure 18), the virologic failure rate among patients with incomplete adherence was 11% at month 12 on ART, while only 2% of patients with complete adherence failed virologically after 1 year (log rank p value <0.01). At month 48 the virologic failure rate was 43% among patients with incomplete adherence and 18% among those with complete adherence (log rank p value <0.01).

In bivariate analysis, being exposed to any type of ARV including sdNVP prior to ART initiation or incomplete adherence was significantly associated with virologic failure (p=0.04). These two factors remained significant after adjustment for confounding by age and sex in the multivariable analysis model. The OR of virologic failure (Figure 17) among patients with incomplete adherence almost tripled (OR 2.8, 95% CI 1.2-6.7) and doubled among those exposed to any type of ARV prior to ART initiation (OR 2.1, 95% CI 1.2-3.9). However, exposure to sdNVP alone did not reach statistical significance. The level of CD4 cell count or VL pre-ART initiation, were not significantly associated with subsequent virologic failure on
ART in bivariate analysis, and therefore not included in the multivariable model. In a separate analysis there was no significant association between patient demographics, socio-economic or clinical data, and, adherence to drug refill visits.

Figure 18. Cox regression analysis for time to virologic failure by level of cumulative adherence to drug refill visits

**Immunologic failure**
Overall, 87/456 (19%) of the patients met one or more of the definitions of immunologic failure based on CD4 cell count [24]. Kaplan Meier survival analysis demonstrated an overall immunologic failure rate of 27% by month 48. The risk of immunologic failure was 41% vs 19% among those with incomplete and complete adherence respectively after 48 months on ART (log rank p value=0.02). After adjustment for CD4 cell count, age and exposure to any ARVs pre-ART initiation and for virologic failure in the Cox regression analysis, patients with incomplete adherence had an immunologic failure rate of 90% at month 48, while the corresponding figure among patients with complete adherence was only 11% (Figure 19) (log rank p value<0.01).

Figure 19. Cox regression analysis for time to immunologic failure, by level of cumulative adherence to drug refill visits
In bivariate analysis immunologic failure was associated with incomplete adherence (p=0.04), gender (p=0.03), and low education level (p=0.03). However, none of these variables remained significant in the final multivariate logistic regression model. More than one-third (37%; 32/87) of patients failing immunologically were also found to be viremic. Among those with immunologic failure there was no significant difference in CD4 cell count or VL pre-ART initiation between the 32 viremic and 55 non-viremic patients.

**DRM among patients who were viremic on ART**

**Article II**

Of 94 viremic patients on a first-line regimen, 64%, 81% and 2% had evidence of NRTI, NNRTI and PI resistance respectively; M184V/I and K103N were the most prevalent mutations (62% and 48% respectively) and 16% had TAMs (Figure 20).

![Figure 20. List of HIV DRM (%) among viremic patients and receiving NNRTI-containing regimen](image)

Among the 35 viremic patients receiving the second-line regimen 29%, 54% and 6% had mutations associated with NNRTI resistance (mainly K103N), NRTI resistance (mainly M184V/I), and major PI mutations respectively. The 2 patients with PI mutations harbored either Q58E, L90M or N88S (Figure 21). All PI recipients were on lopinavir/ritonavir, except for one patient receiving ritonavir and atazanavir.
Article IV

This study included a total of 431 patients who had been on an NNRTI-containing regimen for at least 12 months, where 75% were females, 96% were born in South Africa, 90% had above primary school education and the median age at study enrollment was 38 years. Ninety-one percent were receiving efavirenz-based therapy and 9% a nevirapine-based therapy.

Before ART initiation the median VL and CD4 count were 71 995 (range 1078 to >500 000) copies/ml and 93 (range 1 to 444) cells/mm³ respectively. At study enrollment patients had received ART for a median of 45 months (range 13 to 152) and the CD4 cell count increased to 419 (range 16 to 1270) cells/mm³.

Thirty-eight of 43 samples were successfully genotyped; 31/38 (82%) had at least one DRM and 24 (63%) had ≥3 DRM (Figure 22). Five (13%) had K103N alone; 2/38 (5%) had M184V and K103N; and 10/38 (26%) had three mutations with M184V/I, K103N and an additional NNRTI mutation. Finally 14/38 (37%) had ≥4 mutations: 13/14 (93%) M184V/I and 8/14 (57%) K103N, all with ≥2 NNRTIs and most with >1 TAM or other NRTI mutations. Overall, 8/38 (21%) had one or more TAMs, 3 had A62V or V75I and only 1 patient had K65R.

Several risk factors were associated with increased numbers of DRM. Patients with prior exposure to either sdNVP or other ART had more mutations than those not previously exposed. The ordinal regression OR was 3.8 (95% CI 1.1 to 15.2; p=0.03), i.e. it was 3.8 times more likely for patients with prior ART exposure to have ≥1 vs 0, ≥2 vs ≤1, ≥3 vs ≤2 DRM and so on. The number of DRM was positively associated with being female (OR 5.6; 95% CI 1.3 to 24.5; p=0.02), having a higher CD4 cell count (OR 1.7 per 100 CD4 cells; 95% CI 1.1 to 2.7; p=0.02) and having detectable VL at one of two earlier scheduled visits (OR 8.4; 95% CI 1.9 to
The association with gender was mainly explained by prior exposure to PMTCT, mainly sdNVP, among the women. Only one male had any prior exposure to ART. The number of DRM was negatively associated with coming late for the drug refill visit in the last month (OR 0.1; 95% CI 0 to 0.5; p=0.01) and with VL such that for participants with 1 log unit higher VL the odds of having a higher number of DRM was 0.5 (95% CI 0.2 to 1.0; p=0.04). Finally, the median VL of the 7 patients with no DRM was 83 000 copies/ml compared with a median VL of 6510 copies/ml among those with at least one DRM, providing evidence of existing but incomplete drug pressure among those with DRM.

**Risk factors for persistent virologic failure and DRM at follow-up**

Follow-up data and samples were available for 25/43 (58%) of the viremic patients, after a median of 8 (range 4 to 10) months. Persistent virologic failure at follow-up in 13/25 (52%) patients was associated with a detectable VL at the two visits prior to study enrollment (p<0.01) and the number of DRM at study enrollment (OR 2.36; 95% CI 1.11 to 5.02; p=0.04), particularly NRTI mutations (OR 3.68; 95% CI 1.11 to 12.17; p=0.05). All 13 patients had genotypic resistance, with six additional DRM acquired at follow-up, leading to high-level predicted resistance to efavirenz and/or nevirapine (100%) and lamivudine (100%) and intermediate to low predicted resistance to etravirine in 7/13 (54%). Although viremic, one patient did not have any DRM at study enrollment, but with continued treatment and presumably better adherence, three DRM were selected at follow-up with a persistent, albeit lower, VL for 9 months. At study enrollment 9 of these patients had failed first-line regimens with full predicted susceptibility to NRTIs.

Twelve of the 25 (48%) patients resuppressed at follow-up, after a median of 8 additional months on treatment with the same NNRTI (mostly efavirenz)-based regimen. Comparison of clinical and laboratory characteristics of these 12 patients with the 13 who had persistent virologic failure showed no significant differences in sex, median CD4 and VL prior to ART initiation. At study enrollment 3/12 (25%) resuppressed patients could not be amplified and 3 (25%) had no DRM. However, the remaining 6 patients who were re-suppressed had NNRTI DRM, 3 had K103N, 1 had K103N and M184V and 1 had K103N, V106M and M184V. The sixth patient had three NNRTI and three NRTI mutations. Thus, 6 patients with high-level NNRTI resistance and 3 patients with high-level NNRTI and lamivudine resistance achieved resuppression while continuing the same first-line regimen.

![Figure 22. Summary of factors associated with a) drug resistance at enrollment and b) persistence of virologic failure at follow-up (first-line regimen, Article IV)](image_url)
DISCUSSION

The discussion is outlined according to the key findings.

TB/HIV therapy

Virologic failure may relate to multiple factors such as reduced ART adherence during severe illness or increased pill burden. In addition to exacerbation of side-effects and drug interactions with rifampicin, which may reduce NNRTI and PI levels in plasma concentrations (Article II) [169]. In 2008, Pienaar [170] found that patients receiving TB/HIV therapy were 2.1 times (95% CI 1.17 to 3.91) more likely to show poor adherence to ART. Also, it is conceivable that adherence to concomitant ART and TB treatment is made difficult for patients when care is not integrated, which increases waiting times and transport costs. In 2009 a review by Lawn et al. [116] showed that TB incidence rates on ART decrease over time, but baseline CD4 cell count, sex and socio-economic conditions still influenced TB acquisition on ART. Given that UNAIDS reports that more than half of TB patients are HIV-infected and that in this study most patients had previously received TB treatment, better integration of HIV and TB care should be encouraged.

Self-reported adherence

We used self-reported adherence for the weekend prior to study enrollment (Article II). All NNRTI recipients who reported recent poor adherence were viremic at study enrollment. Rosenblum et al. [171] showed that the longer ART recipients virologically suppressed, the less likely they are to become viremic after missing taking any of their pills.

Potential barriers to incomplete adherence endorsed by study participants were similar in Articles I and III. They reported three main reasons for missing their medication: being away from home, being busy with other things and simply forgetting. This confirms findings from an extensive systematic review of barriers to adherence by Mills et al. [98]. However, the most common reason for missing pills, i.e. being away from home, varied in importance over the first 24 weeks (Article I). Other reasons for missing daily medication remained similar over the first 2-20 weeks on ART, but diminished in importance by week 24.

Patients used a combination of methods to remember to take their pills on time, including mobile phone alarms, relying on their own memory, relying on a close friend/relative or a partner to remind them, or pill-boxes (Article III).

Coming late for drug refill-visits

ART is a life-long undertaking, and finding feasible and affordable means for early detection of treatment failure is crucial to sustain first-line therapy effectiveness. In Article III we reported that the estimated proportion of patients failing virologically was 2-3 times higher among patients coming late for their drug refill visits compared to those with adherence to drug refills of above 95% (Figure 17).
Prior exposure to sdNVP or any ARVs pre-ART initiation

Previous exposure to other ARVs was associated with increased risk of virologic failure (Article III), and our multivariable analysis provided ample evidence that sdNVP [172-175] or other ARVs [176] may predispose to virologic failure and emergence of HIV DRM among women treated with NNRTI-based ARVs.

In Article IV patients with prior exposure to either sdNVP or other ART were found to have more mutations than those not previously exposed. With further analysis of exposure to sdNVP among women, we found a borderline association between exposure to sdNVP and virologic failure, albeit years afterwards and with a period of suppression, consistent with the results of a recent study from the Western Cape, South Africa [108], and another report of a significant association between detection of minority NNRTI mutations and treatment failure, even after 18 months had elapsed since sdNVP [109]. These findings are not entirely in line with other reports from sub-Saharan Africa and Asia that suggest that in the short-term, administration of ART >12 months after sdNVP may not jeopardise the efficacy of NNRTI-based ART [177, 178].

Current guidelines recommend that pregnant women with CD4 cell counts <350 cells/mm³ receive ART rather than sdNVP, so there is less basis to be concerned about the impact of sdNVP on later treatment outcomes [24].

Public clinic

The increased frequency of virologic failure among patients at the public clinic compared to the NGO, may reflect the higher workload, a higher patient-to-provider ratio and more limited resources in the public sector. Fielding et al. [179] made a similar finding when comparing 39 HIV clinics in South Africa that used the same clinical guidelines. They speculate that virologic failure is related to long waiting times at the clinic and/or pharmacy and lack of resources to follow-up patients for their clinic/drug refill appointments. In addition, the patients attending the public clinic were more financially and socially vulnerable than their peers at the NGO clinic; however, they reported higher adherence levels compared with the NGO clinic.

Lack of refrigerator at home

We found that patients who did not own a refrigerator were more likely to fail their second-line regimen. At the time of the study lopinavir/ritonavir was used in South Africa as a prescription of three pills to be taken twice daily formed as soft gel capsules requiring refrigeration [180]. This highlights the need for heat-stable formulations, particularly in areas with poor socio-economic conditions.

Virologic resuppression, persistence of virologic failure and drug resistance evolution

Although K103N and other NNRTI resistance mutations confer high-level NNRTI resistance [65, 163], 6 patients reported in Article IV who harbored such mutations, resuppressed VL after 4 to 10 months with no change in their first-line regimen (Figure 15; Figure 22). Patients coming >7 days late for drug refill visits were at higher risk for DRM accumulation (Figure 15; Figure
This implies that failure to collect ART does work as a proxy for drug intake over time. Monitoring the extent to which ART sites function through EWIs such as adherence to on-time drug refills may be the highest priority in order to minimise preventable HIV drug resistance, according to the WHO [75, 76]. Three patients had high-level resistance to two drug classes, with the addition of the M184V mutation conferring resistance to lamivudine. These findings extend observations by Hoffmann and colleagues, who reported 11 males with either NNRTI and NRTI mutations who resuppressed with continued first-line regimens, raising questions about potential reuse or continuation of those medications in certain circumstances [5]. The observation that successful resuppression was strongly associated with recent virologic failure and a low number of DRM seems logical, but should be confirmed prospectively in larger studies.

Thirteen patients found to be viremic both at enrollment and at follow-up had a longer duration of virologic failure on treatment and a higher number of DRM. These findings substantiate the observation that resistance evolves as a function of continued, albeit suboptimal, drug pressure due to reduced adherence, treatment interruptions or both. Mutations accumulated, with a rise in high-level predicted 2-drug class resistance, despite the relatively short time between sequences (8 months) and a median of 3 DRM per patient. This is consistent with observations in HIV-1 subtype B [181, 182]. Some of the accumulated DRM were associated with etravirine resistance, conferring intermediate resistance to this second-line drug after first-line regimen failure, suggesting the need for further studies of the use of this NNRTI in subsequent regimens [183]. We also compared the patterns of DRM found here to 418 published sequences from adult patients failing first-line regimens in subtype C studies, mostly from southern Africa and India [6, 7, 47, 184-187], accessed in July 2010 from the Stanford HIV Sequence Database [163]. Patterns of DRM, including the overall frequency of any DRM (82% in the current study vs 83% among published sequences), ≥1 NRTI resistance mutations (11% vs 9%), the prevalence of K103N (55% vs 42%), M184V/I (66% vs 74%), and K65R (3% vs 6%) were not significantly different. However, the data presented here demonstrated significant differences in the frequency of ≥1 NNRTI mutation (58% vs 40%, p=0.03) and a lower rate of TAM (21% vs 37%, p=0.05) compared to the published sequences. These modest differences may be ascribed to differences in clinical management strategies, specific drug combinations, duration of virologic failure or chance.
In real-life research it is not possible to do a “perfect study” [188-190]. In epidemiologic studies internal validity indicates how accurately the study outcomes have been estimated [189]. Errors of validity can be either random or systematic. Studies with little random error are described as precise, while those with few systematic errors are described as valid (or non-biased). Study accuracy includes both validity and precision. Internal validity precedes external validity. External validity indicates the generalisability of the study, i.e. the extrapolation of findings outside the study population. Internal validity is dependent on the presence of selection bias, information bias or confounding.

### Selection bias

Selection bias is a systematic error that cannot be adjusted for in the analysis. Overall, this is related to how study participants were selected/participated in the study. The process of selection bias may include any or all of the following steps of selecting study participants: whether our study population represents the general population (coverage bias), the representation of the study population of the sampling frame (sampling bias), and the effect of the self-selection of study participants who did not participate (non-participation bias).

In Article I the study population for the original study was enrolled based on convenience sampling. Women were referred to the study from different clinics surrounding Coronation Hospital [109]. This was a clinical research study in which attention and resources were devoted to reinforcing adherence [191], so it is likely that incomplete adherence is more prevalent in a routine HIV care setting, meaning that the women may not be representative of other women in the area, i.e. affecting the external validity (coverage bias).

In Article II patients receiving ART for more than 12 months were approached, on a volunteer basis, and enrolled. We announced about the study in the waiting area and the pharmacy. We also had informative posters about the study displayed in visible parts of these rooms. Three languages were consistently used: English, isiZulu and Sesotho. Patients approached the research project team if they have decided to know more about the study. Almost all individuals that approached us and were eligible for the study did participate in the study. We could not assess which patients were eligible if they did not approach us.

In addition, since one of our inclusion criteria was to be an ART recipient for more than 12 months, we missed those who failed virologically and then dropped out of the program before reaching 12 months on treatment. Given that a substantial proportion of patients enrolled on ART in similar urban settings are expected to drop out early [192], this would lead to an underestimation of the true virologic failure rate among NNRTI recipients in the current assessment.

In Articles III and IV, all study participants were from the NGO clinic since data were either missing or unavailable at the public clinic. Both clinics are located on the same premises and provide services to patients from the same geographical area and similar low socio-economic backgrounds (Article II). At the time of study enrollment (Article II), ART recipients at the public clinic tended to have a lower CD4 cell count and to have less social support. This might be related to patients being initiated later on ART. In addition, the public clinic uses the South
African National Health Laboratory Services that provides VL measures using a higher limit for detection of viremia (400 copies/ml). The NGO clinic uses a private diagnostic laboratory that provides VL measures with a lower limit of detection of 50 copies/ml. This might have influenced the adherence counseling for the patients. Therefore we would not be able to test the association of coming late for drug refill visits with virologic failure.

In article IV, we planned to recruit patients when they came back for their clinic follow-up visit, but only managed to meet and enroll a smaller group of patients (25/43). Given a lack of consent to phone patients who did not come back for follow-up as planned, we were unable to find out the reasons why patients did not come back for their clinic visit and could not rule out the possibility of non-participation bias. However, the results are similar to those in other subtype C-related studies, as described in detail in Article IV.

**Information bias**

This type of bias is related to how the information is collected or how the disease is measured in a study population, and whether this differs between comparison groups - for example, validity of an instrument, questions asked or laboratory assays used to screen a disease.

In Article II we used an interviewer-administered questionnaire. Field workers spoke at least three of the most common languages in Soweto (English, isiZulu and Sesotho). The interview questionnaire was also piloted, translated and back-translated into these three languages. Although information bias might have occurred among patients with other mother tongues, it is unlikely to have had any major influence on data quality.

Social desirability bias could potentially have occurred during the interviews (Articles I and II), i.e. study participants might have reported information that they thought the interviewers wanted to hear, rather than the truth. However, for Article I patients were informed that interviews were done without linking the data to medical records. In Article II the field workers were not part of the clinic staff, and no personal information was collected on the survey forms. We assume that these measures reduced the risk of social desirability bias, and moreover, it is unlikely that patients with virologic failure/DRM responded differently. Also, since we did not use self-reported adherence in Articles III and IV, the risk of differential information bias in terms of exposure assessment is low.

We used the International AIDS Society mutations list [72], December 2009, to define major resistance mutations in Articles II and IV. This list is updated annually, based on the opinion of a panel of HIV drug resistance experts (Appendix 2). Thus certain mutations of clinical importance may have been missed unless identified on this list. The Stanford HIV database [163] algorithm was used to predict the phenotypic HIV drug resistance. Since this algorithm is updated regularly, the level of sensitivity may vary. Using phenotypic assays would give a more accurate result; however, this was not possible at the time of the study due to technical and cost reasons.

**Confounding factors**

Confounding is a factor or bias that is associated with both the dependent and independent variables [193]. Different factors can influence virologic failure [149] and HIV-1 drug resistance development [194], apart from poor adherence to ART, such as age, lifestyle, genetic characteristics, hepatitis C coinfection, menstrual cycle disorders/menopause, gender, etc. [149]. Many different factors also influence incomplete adherence, apart from those studied in this thesis, such as clinical symptoms, appearance-associated signs, depression, regimen
complexity, disability, change in daily lifestyle, physical disability, etc. [149].

In Article I we initially aimed to adjust for the presence of the K103N mutation, pre-ART initiation. However, because of a too small sample size, we were not able to adjust for it and used exposure to sdNVP instead as proxy. At the time of writing Article II, no retrospective data was available to adjust for patients’ characteristics, pre-ART initiation (e.g. CD4 cell count, VL, sdNVP or ART exposed) in the multivariable model.

In Article IV we wished to adjust for potential confounding, but due to the small sample size the analysis remained unadjusted, explaining why female gender stood out as a risk factor while in fact it was previous exposure to any type of ARV during pregnancy that was the main reason for DRM.

Figure 23 is a snapshot from a 2007 heading in The Sowetan newspaper, which created serious discussion in the media during my stay in South Africa.

Similarly, the recommendations of the former Minister of health, Dr. Manto Tshabalala-Msimang, to use herbal medicine (Box 1), and the fact that many patients also seek care at traditional healers and combine herbs with ART, could also have influenced adherence and treatment outcomes. However, we lacked data on these potential confounders and could not adjust for any of these factors, that are mainly associated with a lack of adequate knowledge on side-effects and the capacity of an individual to interpret deterring information in the media. Such knowledge and capacity could have affected men and women as well as lower vs higher educated people differently. However, it is more likely that this sort of random ‘noise’ causes a non-systematic bias if anything, ie biasing the results towards the null hypothesis.

Figure 23. Picture of a male patient receiving ART in Soweto who had gynaecomastia
Source: The Sowetan newspaper, 2007

Box 1. Citation from a medical record for a patient reported to be taking vegetables after the recommendation of former Minister of Health of South Africa:

“... patient says she is eating large amounts of garlic, carrots after listening to Minister of Health. I have advised her to eat moderate amounts of each food group.”
RECOMMENDATIONS

As ART scale-up continuous, there is a need for long-term planning to ensure that patients can be maintained on first-line ART for as long as possible. However, a proportion of patients will eventually always experience virologic failure. This requires the availability of low-cost diagnostic tools to screen VL and HIV drug resistance.

So far most of the ART programs in sub-Saharan Africa have limited access to alternative regimens due to high cost. This hinders switching patients who are failing their first-line regimen into second-line options in time. Reducing the cost of second-line regimens is therefore a necessity.

The history of using triple HIV treatment therapy on a larger scale in African is still young and policy makers and providers need to learn more about adapting resource-poor health systems to the problems faced by ART recipients on life-long treatment e.g. the higher risk of non-communicable diseases that affects this group, adding to the double burden of South Africa. This requires strengthening health systems overall, especially public clinics, including improving the medical records archiving system, investing in human resources and affordable diagnostics.
CONCLUSIONS

- Overall, the virologic response among long-term NNRTI recipients in Soweto was good.

- Women exposed to sdNVP prior to ART may require higher adherence levels to reach virologic suppression when initiated on ART.

- Routinely collected adherence to drug refill appointments could be good and low-cost indicator for predicting virologic and immulogic failure.

- Viremic patients may require a second VL testing within 3-6 months, including intensive adherence counseling.

- There was a discrepancy between immunologic and virologic failure. Thus health care providers without access to VL testing may need to consider patients with immunologic failure more carefully before switching them to a second-line regimen.

- Additional adherence counseling to ensure long-term success on ART may be required for the following groups of patients:
  - Patients on concurrent TB/HIV treatment;
  - Patients exposed to either sdNVP or any type of ARVs prior to the initiation on ART; and
  - Patients with lower education and less social support.
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APPENDICES

Appendix 1. Gapminder HIV chart 2009
Appendix 2. International AIDS Society-USA list of DRM by drug type
### South African Virologic Evaluation

**Appendix 3 Questionnaire form, isiZulu language (Articles II, III and IV)**

#### Personal Information & Patient Background

1. **Usuku lokuzalwa (usuku/inyangana/unyaka)** / Date of birth (day/month/year) __ / __ / __
2. **Ubudala beminyaka** / Age __
3. **Ubulili** / Sex ( ) 1. Owesimane / Woman ; ( ) 2. Owesilisa / Man

#### Uzalelwwe kuliphi izwephi / What is your country of birth?

- ( ) 1. ENingizimu Afrika / South Africa
- ( ) 2. Ezimbabwe / Zimbabwe
- ( ) 3. Elesotho / Lesotho
- ( ) 4. Esawazi / Swaziland
- ( ) 5. Botswana / Botswana
- ( ) 6. Enamibia / Namibia
- ( ) 7. Emozambique / Mozambique
- ( ) 8. Kwenye indawo / Other

#### Ufundwе wafika kuliphi ibanga lenfundo? / What is your first language?

- ( ) 1. Angikaze ngiya esikhathi / Never been to school ; ( ) 2. Esikoleni sephephesweni / Primary school
- ( ) 3. Isikoleni seSekondary / Secondary school ; ( ) 4. Emfuleni em photographed amakholishe / Tertiary ; ( ) 5. Okunye / Other

#### Uhlala endaweni enjani / Do you live in

- ( ) 1. Endlini / House ; ( ) 2. Ifulethi / Flat ; ( ) 3. Endaweni engalelele (emkhukhweni) / Informal dwelling (shack)
- ( ) 4. Ekameleni ohlala kuyo nabanye / Shared room ; ( ) 5. Kwende indawo / Other

#### Ngabe yini ulwimi iwakho? / What is your age?

- ( ) 1. isiZulu ; ( ) 2. isiXhosa ; ( ) 3. Isivenda ; ( ) 4. isiSiswati ; ( ) 5. isiTsonga ; ( ) 6. isiShona ; ( ) 7. isiNdebele ; ( ) 8. isiZulu ; ( ) 9. isiXhosa ; ( ) 10. IsiThonga ; ( ) 11. isiPedi ; ( ) 12. isiShona ; ( ) 13. Olunye

#### Isimo somendo / Marital status

- ( ) 1. Angiganile / Sing ; ( ) 2. Ngesiweziwelele / Divorced/Separated ; ( ) 3. Umphakathi_ORD / Widow/widower
- ( ) 4. Ngishadile / Married ; ( ) 5. Ubudlelwane ngezocansi / A sexual relationship ; ( ) 6. Ukhulalisana / Cohabitation
- ( ) 7. Okunye / Other

#### Ngabe ube nophathina abangaki bezocansi ezinyangeni ezingu-3 ezedlule? / How many sexual partners did you have during the last 3 months? __ / __

- ( ) 1. Owesimame / Woman ; ( ) 2. Owesilisa / Man

#### Uhlala endaweni enjani / Do you live in

- ( ) 1. Inside tap
- ( ) 2. Inside toilet
- ( ) 3. Ugesi (i-elektrisithi) / Electricity
- ( ) 4. Ifriji
- ( ) 5. Umsakazo (irediyo) / Radio
- ( ) 6. i-TV
- ( ) 7. Ulayini wocingo (wefoni) yaseendlini / Landline phone

#### Uhlala endaweni enjani / Do you live in

- ( ) 1. Yebo ( ) 2. Cha

#### Uhlala endaweni enjani / Do you live in

- ( ) 1. Impompi yasendlini ngaphakathi / Inside tap
- ( ) 1. Yebo ( ) 2. Cha
- ( ) 2. Ithoyilethi yangaphakathi / Inside toilet
- ( ) 1. Yebo ( ) 2. Cha
- ( ) 3. Ugesi (i-elektrisithi) / Electricity
- ( ) 1. Yebo ( ) 2. Cha
- ( ) 4. Ifriji / Fridge
- ( ) 1. Yebo ( ) 2. Cha
- ( ) 5. Umsakazo (irediyo) / Radio
- ( ) 1. Yebo ( ) 2. Cha
- ( ) 6. i-TV
- ( ) 1. Yebo ( ) 2. Cha
- ( ) 7. Ulayini wocingo (wefoni) yasendlini / Landline phone
- ( ) 1. Yebo ( ) 2. Cha

#### Uhlala endaweni enjani / Do you live in

- ( ) 1. Yebo ( ) 2. Cha

#### Uhlala endaweni enjani / Do you live in

- ( ) 1. Yebo ( ) 2. Cha

#### Uhlala endaweni enjani / Do you live in

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- ( ) 1. Yebo ( ) 2. Cha

#### Uhlala endaweni enjani / Do you live in

- ( ) 1. Yebo ( ) 2. Cha

#### Uhlala endaweni enjani / Do you live in

- ( ) 1. Yebo ( ) 2. Cha
Appendix 3.

17. UMA KUNGU YEBO, ngabe watshela (wadalulela) bani ngesimo sakho? (bheka konke okusebenzayo) / IF YES, who did you disclose your status to? (check all that apply)
   ( ) 1. Umama/Ubaba /Mother /Father; ( ) 2. Umamazala/Ubabazala /Mother-in-law /Father-in-law
   ( ) 3. Ingane yakini / Sibling(s); ( ) 4. Uphathina / Partner; ( ) 5. Umngani / Friend
   ( ) 6. Izigan / Children; ( ) 7. Amyane amalunga omndeni/Ilunga lomndeni /Other family/household Member
   ( ) 8. Uqama / Employer; ( ) 10. Omunye umuntu / Other

18. Ngabe yini isimo sakho ngezomsebenzi? / What is your employment status?
   ( ) 1. Angisebenzi / Not employed -> gyiya kunombolo 20 / go to number 20
   ( ) 2. Ngitathethe umhlalaphansi / Retired -> yiya kunombolo 20 / go to number 20
   ( ) 3. Ngisebenza / Employed; ( ) 4. Ngisebenza nsuku zonke amatoho / Work on daily labour basis

19. Usebenza kancani? / How do you work?
   ( ) 1. Ngokuphelele / Full-time; ( ) 2. Ngezinye izikhathi / Part-time; ( ) 3. Ukuziqaasha / Self-employed; ( ) 4. Okunye / Other

20. Uyitholaphi imali imali yekuphila lisanga? (kwmukeli ukuthi uhe nezinto othola kuzo imali ezingupehezuka kokukodwa) / What are your sources of income? (check all that apply)
   ( ) 1. Uthola imali ngokwakho -> yiya kunombolo 22 / Self; go to number 22
   ( ) 2. Uphathina / Parent -> yiya kunombolo 22 / go to number 22
   ( ) 3. Ingane yakini / Sibling -> yiya kunombolo 22 / go to number 22
   ( ) 4. Imali yempesheni -> yiya kunombolo 22 / Child support grant -> yiya kunombolo 22 / go to number 22
   ( ) 5. Umxhaso (igranti) yokukhubazeka / Disability grant -> yiya kunombolo 21 / go to number 21
   ( ) 6. Ingane yakini / Sibling -> yiya kunombolo 22 / go to number 22
   ( ) 7. Unzali / Parent -> yiya kunombolo 22 / go to number 22
   ( ) 8. Kokunye / Other
   ( ) 9. Akukho / None -> yiya kunombolo 22 / go to number 22

21. Uma kungu yebo, ngabe sekuyisikhathi esingakani umphila ngomxhaso (ngengeri) yokukhubazeka? / If yes, how long you have been on the disability grant?

<table>
<thead>
<tr>
<th>Ungafaka usuku eyaqala ngalo (usuku-inyanga- unyaka)</th>
<th>Either date started (dd-mm-yyyy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noma inani lezinyanga / Or number of months</td>
<td></td>
</tr>
</tbody>
</table>

22. Ngabe watshela ingeniiso (income) ekingakani ngenyanga edlule? / What was your own total income for last month?
Amarandi / Rand

Imibuzo egendene nama-ARV / ARVs related

23. Ngemva kokuba waze ngesimo sakho se-HIV kwathatha isikhathi esingakani ukuba uqale ukusebenzisa ama-ARV? / How long, before starting your ARVs, did you know about your HIV status?
   ( ) 1. 4 wamawo /4 weeks; ( ) 2. Itoyana eyodwa ukuwa kwesizingu-5 /1-5 months
   ( ) 3. Itoyana eyodwa ukuwa kwesizingu-6 / 6-12 months; ( ) 4. Itoyana eyodwa ukuwa kweminyaka engu-2 /1-2 years
   ( ) 5. Itoyana eyodwa ukuwa kweminyaka engu-5 / More than 5 years

24. Ngabe waya kuhlelo lokuzilungiselela (readiness programme) ngaphambili kokuba uqale ukusebenzisa ama-ARV? / Did you go into a readiness program before starting to take your ARVs?
   ( ) 1. Yebo / Yes; ( ) 2. Cha / No

25. (Kwabesimame), ngabe ukhulelwokwamanele / (For women), are you currently pregnant?
   ( ) 1. Yebo / Yes; ( ) 2. Cha / No

26. Ngabe welashelwa i-TB owamanele / Are you currently receiving TB treatment?
   ( ) 1. Yebo / Yes -> yiya kunombolo 29 / go to number 29; ( ) 2. Cha / No

27. Ngabe wake welashelwa i-TB owesikhathi esedlule / Have you been treated for TB before?
   ( ) 1. Yebo / Yes -> yiya kunombolo 29 / go to number 29; ( ) 2. Cha / No

28. Ngabe uke waholelwa (wathestelwa) i-TB maduzane nje / Have you been recently tested for TB?
   ( ) 1. Yebo / Yes; ( ) 2. Cha / No

29. Ngabe yebo kudokotela wangsase, ifameni/ikhenisi/okunye, isibhedelela sikahulumeni/ikliniki noma inyanganyana yeisintu, selokhu waqala ukusebenzisa ama-ARV's kule kliniki? (bheka konke okusebenzayo) / Have you visited a private doctor, a pharmacist/chemist/other public hospital/clinic or a traditional healer, since you started ARVs at this clinic? (check all that apply)
   ( ) 1. Udobokotela wangsase / Private doctors; ( ) 2. Ifameni/ikhenisi / Pharmacist/chemist; ( ) 3. Abalaphi besintu / Traditional healer
   ( ) 4. Isibhedlela sikahulumeni/ikliniki / Public hospital/clinic; ( ) 5. Cha, angikaze nakanye / No, not at all
**Appendix 3.**

**Instructions for interviewer: read this slowly and clearly to the patient, to build trust with him/her**

'Ngithanda ukukubuza imibuzo negendlela ophuza ngayo ama-ARV akho. Ngidinga ukucondisisa ngokuthi empeni abantu benzani ngemithi yabo. Ungakahathazeki ngokungitshele ukuthi awuwaphuzi onke amaphilisi akho. Abanye abantu baphuza yonke imithi yabo njengoba beyalwelwe (prescribed) kanti abanye kungenenzeka bengayiphuzi yonke imithi yabo. Ngidinga ukwazi ngokuthi empeni kwenzeka ini, hhayi lokhu okucabangayo, 'Ngifuna ukuzwa/.. I would like to ask you some questions about the way you have been taking your ARVs. I need to understand what people are really doing with their medicines. Do not worry about telling me that you don't take all your pills. Some people take all of their medications as they are prescribed while others may not take all of their medications. I need to know what is really happening, not what you think I "want to hear."

30. Ngicela ungikhombise kulebhodi ye-cartoon, ukuthi yiwaphi ama-ARV owasebenzisayo okwamanje /Please point to me on this cartoon board, which ARVs you are currently taking

<table>
<thead>
<tr>
<th>Igama lomuthi (drug)</th>
<th>Name of drug</th>
<th>Inani LAMAPHILISI oyalelwed ukuwaphuza isikhathi nesikhathi (amaphilisi amangami ngomthamo) /i-dose eyodwa</th>
<th>Inani LEZIKHATHI oyalelwed ukuthi uyiphuze ngalo amaphilisi akho ngosuku (ama-dose ngosuku)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Number of PILLS prescribed to be taken each time (pills per dose)</td>
<td>Number of TIMES prescribed to be taken per day (doses per day)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>*</td>
<td>*</td>
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<tr>
<td></td>
<td></td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

31. Amaphilisi ongawaphuzanga/ Missed pills

<table>
<thead>
<tr>
<th>Igama lomuthi (drug) we-HIV</th>
<th>Name of HIV drugs</th>
<th>Kumaphilisi oyalelebe ukuwaphuza, ngabe mangaki amaphilisi owaweza awangawaphuzu /how many pills did you miss</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>*</td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td>*</td>
</tr>
</tbody>
</table>

32. Amaphilisi owathathe ngesikhathi /Pills taken on time

<table>
<thead>
<tr>
<th>Igama lomuthi (drug) we-HIV</th>
<th>Name of HIV drugs</th>
<th>Ngabe mangaki amaphilisi owaphuza ngesikhathi (okusho isikhathi esciche sibe yihola elilodwa +/- 1 wesikhathi oyalelwed sona) /how many drug pills did you take on time (i.e. within +/-1 hour of the prescribed time)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>*</td>
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<td></td>
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<td>*</td>
</tr>
</tbody>
</table>

33. Ngabe amanye ama-ARV akho anemiyalalele yesipesheli, efana nokuthi 'aphuze nokudla' noma 'aphuze ungakadli' noma 'aphuze neziphuzo' fluids (eziningi)? / Do any of your ARVs have special instructions, such as “take with food” or “take on an empty stomach” or “take with plenty of fluids”?

- Yebo /Yes;
- Cha /No

34. Uma kungu yebo kumbuzo 33, ngabe wayilandela kangi imiyanalele yesipesheli esikhathi esedlule sezinsukuezine /If Yes to question 33, how often did you follow those special instructions over the last four days?

- Angikazi /Never;
- Kwezinye izikhathi /Sometimes;
- Uhafu wesikhathi /Half of the time;
- Izikhathi eziningi /Most of the time;
- Nqazo zonke izikhathi /All of the time.

35. Abanye abantu bathola ukuthi bayakhohlwa ukuphuza amaphilisi abo ngama-weekend (ngempelasonto) Ngabe weqa ukuphuza ama-ARV akho nge-weekend (ngempelasonto) edlule? /Some people find that they forget to take their pills over the weekend. Did you miss any of your ARVs last weekend?

- Yebo /Yes;
- Cha /No

36. Ngabe kunini ngesikhathi esedlule lapho oweqa khona awangaphuza ama-ARV akho? /When was the last time you missed taking any of your ARVs at all?

- Evikini eledlule /Within the past week
- Izikosi elilodwa /1-2 weeks ago
- Amaviki angu -2 ukuya kumaviki angu -4 edlule /2-4 weeks ago
- Inyanga eyolwathi ukuya kwezinhle ezedlule /More than 3 months ago
- Nqazo zonke izikhathi /Never skip medication

---

<table>
<thead>
<tr>
<th>Igama lomuthi (drug) we-HIV</th>
<th>Name of HIV drugs</th>
<th>Izolo /Yesterday</th>
<th>Usuku olungaphambili kwayizolo (izinsuku ezingu-2 edlule) /Day before yesterday (2 days ago)</th>
<th>(izinsuku ezingu-3 ezedlule) /3 days ago</th>
<th>(izinsuku ezingu-4 ezedlule) /4 days ago</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>*</td>
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</tr>
</tbody>
</table>

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NGICLA UNGIKHOMBISE KULEBHODI YE-CARTOON, UKUTHI YIWAYAHI AMA-ARV OWASEBENZISA YOKWAMANJE

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<table>
<thead>
<tr>
<th>Igama lomuthi (drug)</th>
<th>Name of drug</th>
<th>Izolo /Yesterday</th>
<th>Usuku olungaphambili kwayizolo (izinsuku ezingu-2 edzule) /Day before yesterday (2 days ago)</th>
<th>(izinsuku ezingu-3 ezedlule) /3 days ago</th>
<th>(izinsuku ezingu-4 ezedlule) /4 days ago</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>*</td>
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<td>*</td>
</tr>
</tbody>
</table>

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Appendix 3.

37. Abantu kuyenzenka beqe bangaphuzi imithi yabo ngenxa yezizathu ezhelukahlukene. Nanti uhla lwenzinye izizathu zokuthi kungani weqa ungaphuzi imithi yakho. Ngabe kukangaki lafho owake weqa awangaphuzu imithi yakho ngoba (circle one response for each question/ reason given) / People miss taking their medications for various reasons. Here is a list of possible reasons why you may miss taking your medications. How often have you missed taking your medications because you (circle one response for each question/reason given)

1. Wawunegqho ekhaya? / Were away from home?
2. Wawubhizi nezinye izintor? / Were busy with other things?
3. Wakhohlwa? / Simply forgot?
4. Ayemaningi amaphilisi okumele uwaphuze? / Had too many pills to take?
5. Wawugwema (avoid) imiphumela engemihle yemithi (side effects)? / Wanted to avoid side effects?
6. Wawungafuni ukuthi abanye abantu baqaphele (banothise) ukutbeka okuphoyi zomithi yende? / Did you want others to know you were taking medication?
7. Wawushintshe izinto ozenza njalo imithi? / Reason for not taking medication?
8. Wawuzwa sengathi umuthi unguphoyizeni/uyinikufana? / Felt like the drug was toxic/harmful?
9. Wawuphethwwe ubuthongo/wawulele nesikuphaza umuthi? / Felt depressed/overwhelmed?
10. Wawuza uye (yebo)? / Do you remember to come to your appointment today (cross all that apply)
11. Wawunandi emoyeni (depressed)/waqedeka amandla? / Felt good/better?
12. Wawungafuni ukuthi abanye abantu baqaphele (banothise) ukutbeka okuphoyi zomithi yende? / Did you want others to know you were taking medication?
13. Wawubhizi nezinye izinto uzinto izikhathi? / Other? (state one main reason)
14. Wawungafuni ukuthi abanye abantu baqaphele (banothise) ukutbeka okuphoyi zomithi yende? / Did you want others to know you were taking medication?
15. Wawucindezeleke emoyeni (depressed)/waqedeka amandla? / Felt good/better?
16. Wawungafuni ukuthi abanye abantu baqaphele (banothise) ukutbeka okuphoyi zomithi yende? / Did you want others to know you were taking medication?
17. Wawufuna ukonga amaphilisi akho? / Wanted to save extra pills?
18. Wawucabanga ukuthi imithi yama bhaxa? / Did you want to keep CD4 level low to stay on disability work?
19. Wawushintshe izinto ozenza njalo imithi? / Reason for not taking medication?
20. Wawufuna ukonga amaphilisi akho? / Wanted to save extra pills?

Nongama (Engangcwane) / (Option 4)
Kwanele / (Option 5)
Kwazolile (Gumulungunzi) / (Option 6)

| Ama-appointment asekliniki nama izikhatho zokuya eklini / Clinic appointments |

38. Ngabe ukhumbule kanjani ukuthi uze eklini nambahle nge-appointment yakho nomi isikhathi okumele uze ngaso (cross all that apply) / How did you remember to come to your appointment today (cross all that apply)

( ) 1. Usuku lokobuhle eklini lalihalwe ekhadini lasekliniki / Appointment date written on clinic register card
( ) 2. Ubhala phansi kwidayamakhulu/ibhuku lamaplace / You write it down in a diary/appointment book
( ) 3. Uzama ukukhumbula, okusho ukuthi wethembemse ukuthi uyokhumbula / You try to remember, i.e. you rely on your memory
( ) 4. Usebenzisa icellphone yakho ukukhumbubuzisa / You use your mobile phone
( ) 5. Umngani osondelene naye kakulu/ishishobho osondelene nako kakulu siyakukhumbuza / A close friend/close relative reminds you;
( ) 6. Uppinathina wakho uyakukhumbuza / Your partner reminds you
( ) 7. Umngani wakho casebemini uyakukhumbuza / Your friend at work reminds you
( ) 8. Okanye / Other

39. Esikhathini sezinyanga ezingu-6, ngabe uke weqa angawangakwi-appointment vaselinkeni (okusho ukuthi ukuyobonana nodokotlela noma unesi) / During the last 6 months, have you ever missed a clinic appointment (i.e. to see your doctor or nurse)?

( ) 1. Yebo / Yes ; ( ) 2. Cha /No / go to number 43 ; ( ) 3. Angikaze / Don't remember
( ) 9. Akusebenzi lafha / Not applicable

40. Cishe kungaba zikhathika ezininga lafho weqa khona angawangakwi-appointment vaselinkeni ezinyangeni ezingu-6 ezedlule? / Approximately, how many times have you missed your clinic appointment in the last 6 months? Inani lezikathi / Number 77
41. Uma uke weqa awangaya kwi-appointment yabo, ngabe kakhona umuntu wasekliniki noma wefamasi owaxhumana nawe ukuulkhushela ukuthi weqa awangaya kwi-appointment yabo yokuya kwhona? /If you have ever missed an appointment, did someone from the clinic or the pharmacy contact you to tell you that you missed your appointment? 
( ) 1. Yebo /Yes; ( ) 2. Cha /No; ( ) 9. Akusebenzi lapha /Not applicable

42. Ngabe kube yini isizathu sakho sokuthi ungezi kwi-appointment obekelwe yona? /What has been your reason for not coming to the scheduled appointment?

<table>
<thead>
<tr>
<th>Reason</th>
<th>Yebo (1)</th>
<th>No (2)</th>
<th>Sometimes (3)</th>
<th>Often (4)</th>
<th>Not applicable (9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Ubungenayo imali yezindleko zezinto zokugibela /You could not afford the cost of transportation</td>
<td>( ) 1</td>
<td>( ) 2</td>
<td>( ) 3</td>
<td>( ) 4</td>
<td>( ) 9</td>
</tr>
<tr>
<td>2. Yibanga elise ukuya khona, kanti futhi anginaso isikhathi sokuya /It is long distance to get here and you did not have time for it</td>
<td>( ) 1</td>
<td>( ) 2</td>
<td>( ) 3</td>
<td>( ) 4</td>
<td>( ) 9</td>
</tr>
<tr>
<td>3. Uphathina wakho wakwalela ukuthi uze lapha /Your partner refused you to come here</td>
<td>( ) 1</td>
<td>( ) 2</td>
<td>( ) 3</td>
<td>( ) 4</td>
<td>( ) 9</td>
</tr>
<tr>
<td>4. Wavela wakhohlwa /You simply forgot</td>
<td>( ) 1</td>
<td>( ) 2</td>
<td>( ) 3</td>
<td>( ) 4</td>
<td>( ) 9</td>
</tr>
<tr>
<td>5. Wawungaphilile kahele nakhona wawudinga isikhathi sokuphumula ekhaya /You were not feeling well and needed to rest at home</td>
<td>( ) 1</td>
<td>( ) 2</td>
<td>( ) 3</td>
<td>( ) 4</td>
<td>( ) 9</td>
</tr>
<tr>
<td>6. Wawuzizwa uhlile kahele/unempilo nakhona u zijwa ukuthi awuyidigile imithi /You felt fine/healthy and felt you did not need the medications</td>
<td>( ) 1</td>
<td>( ) 2</td>
<td>( ) 3</td>
<td>( ) 4</td>
<td>( ) 9</td>
</tr>
<tr>
<td>7. Wawuhambile ungekho ngazikhathi zamaholi /You were away for holidays</td>
<td>( ) 1</td>
<td>( ) 2</td>
<td>( ) 3</td>
<td>( ) 4</td>
<td>( ) 9</td>
</tr>
<tr>
<td>8. Wawuhambile ungekho uhambwe ngomsebenzi /You were away for work</td>
<td>( ) 1</td>
<td>( ) 2</td>
<td>( ) 3</td>
<td>( ) 4</td>
<td>( ) 9</td>
</tr>
<tr>
<td>9. Ifamasi yayingenayo imithi kanti kwakufanele ubuye kamuva /Pharmacy ran out of drugs and you had to come back later</td>
<td>( ) 1</td>
<td>( ) 2</td>
<td>( ) 3</td>
<td>( ) 4</td>
<td>( ) 9</td>
</tr>
<tr>
<td>10. Wawungakholewa ukuthi imithi ingakusiza ube ngocono /You did not believe the medicine can help you to get better</td>
<td>( ) 1</td>
<td>( ) 2</td>
<td>( ) 3</td>
<td>( ) 4</td>
<td>( ) 9</td>
</tr>
<tr>
<td>11. Esinye isizathu? /Other</td>
<td>( ) 1</td>
<td>( ) 2</td>
<td>( ) 3</td>
<td>( ) 4</td>
<td>( ) 9</td>
</tr>
</tbody>
</table>

43. Ngabe izikhathi zamahora okuvulwa kwekliniki zilula nokuhambelana nawe? /Are the opening hours of this clinic convenient for you? 
( ) 1. Yebo /Yes; ( ) 2. Cha /No

44. Esiyathini sezinyanga ezingu-6 ezedule, ngabe uke eqa awangaya ukuyothola ama-ARV akho efamasi eklini
/During the last 6 months, have you ever missed your ARV refill at the pharmacy/clinic? 
( ) 1. Yebo /Yes; ( ) 2. Cha /No → yiya kunombolo 49 /→ go to number 49
( ) 3. Awukhumbulile /You don't remember
( ) 9. Akusebenzi lapha /Not applicable

45. Cische kungaba zikhathi ezingakho laho weqa khona awangaya ukuyothola amanye ama-ARV akho efamasi/eukhathi ezinyangeni ezingu-6 ezedule? /Approximately, how many times have you missed your ARV refill at the pharmacy/clinic during the last 6 months
Inani lezikhathi /Number ________

46. Esiyathini sezinyanga ezingu-6 ezedule, ngabe kuke kwenzeka kanku laho ugebuzo ongabo ukucafanisiso akho ebhokisi lamaphilisi? /During the last 6 months, how often you spent more than one day without any drugs at all in your pillbox? 
( ) 1. Cische esuku olulodwa njalo ngenyanga eyodwa /Almost one day every month
( ) 2. Cische njalo kanye ezinyangeni ezingu-2 /Almost once every 2nd month
( ) 3. Cische njalo kanye ezinyangeni ezingu-2 /Almost once every 2nd month
( ) 4. Akukaze kwenzeka nhlobo /Never at all
Appendix 3.

47. Sicela uchaze izizathu ezingu-3 zokuthi kungani weqa awangeza ngesikhathi ukuzothola eminye imithi yakho yama-ARV /Please state up to 3 reasons why you missed coming on time to refill your ARVs.

<table>
<thead>
<tr>
<th>Isizathu sokuqala (1)</th>
<th>Reason 1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Isizathu sesibili (2)</th>
<th>Reason 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Isizathu sesithathu (3)</th>
<th>Reason 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

48. Uma uke weqa awangaya ukuzothola eminye imithi yakho futhi, ngabe kukhona umuntu wasefamasi owaxhumana naye ukuzuthela ukuthi weqe isikhathi awangayi kw-appointment yakho yokuya khona? /If you have ever missed medication refill, did they come from the pharmacy to tell you that you missed your appointment?

( ) 1. Yebo /Yes ; ( ) 2. Cha /No ; ( ) 9. Akusebenzi lapha /Not applicable

49. Manje sesizokubuza ukuthi ngabe ukuhumbela canjani ukuva eekliniki/eefamasi ukuvyethatha amaphilisi akho esikhathini sezinyanga ezingu-6 ezedlule (cross all that apply) /Now we will ask you how did you remember to come to the clinic/pharmacy to pick-up your pills during the past 6 months (cross all that apply)

( ) 1. Usebenzisa iibhokisi lamaphilisi /You use a pill box
( ) 2. Ubhala phansi kwidayari/kwibhuku lam-appointment /You write it down in a diary/appointment book
( ) 3. Uzama ukuhumbula, okusho ukuthi wethembe ukuthi uzokhumbula /You try to remember, i.e. you rely on your memory
( ) 4. Usebenzisa icellphone yakho ukukhumbuza /You use your mobile phone
( ) 5. Umngani osondelene naye kakhulu wakukhumbuza/ A close friend/close relative reminds you
( ) 6. Uphathina wahko ukuakhumbuza /Your partner reminds you
( ) 7. Umngani wahko emsebenzini ukuakhubuzanti /Your friend at your work reminds you
( ) 8. Okanye /Other

50. Okanye (Sicela usichazele ukuthi, ngabe ukuzothola eminye imithi yakho?) /Other (Please share with us, how you normally recall your appointment?)

51. Ngabe amahora okusebenza kwakwakwamfani alula kuwe ukuthi uzothola eminye imithi yakho? /Are the opening hours of this pharmacy convenient for your drug refill?

( ) 1. Yebo /Yes ; ( ) 2. Cha /No

52. Manje sesizokubuza ukuthi ngabe ukuhumbela canjani ukuhumbula akho esikhathini esikhathini samaviki angu-4 edlule ngokuthi ufacele indlela okhumbule ngayo ukusukela ku-0 (okuqindela engasizi ukhumbuza ukuwa ku-10 okuqindela esexenza kakhulu ekukhumbuzeni) /Now we will ask you how you used to remember to take your pills on time during the past 4 weeks (check all that apply)

( ) 1. Usebenzisa iibhokisi lamaphilisi /You use a pill box
( ) 2. Ubhala phansi kwidayari/kwibhuku lam-appointment /You write it down in a diary/appointment book
( ) 3. Uzama ukuhumbula, okusho ukuthi wethembe ukuthi uzokhumbula /You try to remember, i.e. you rely on your memory
( ) 4. Usebenzisa icellphone yakho ukuakhumbuza /You use your mobile phone
( ) 5. Umngani osondelene naye kakhulu wakukhumbuza/ A close friend/close relative reminds you
( ) 6. Uphathina wahko ukuakhumbuza /Your partner reminds you
( ) 7. Umngani wahko emsebenzini ukuakhubuzanti /Your friend at your work reminds you
( ) 8. Okanye /Other

53. Okanye (Sicela usichazele ukuthi, ngabe ukuhumbela canjani ukuhumbula canjani ukuhumbula?) /Other (Please share with us, how you normally recall that you have an appointment?)
The following questions ask about symptoms you might have had during the past four weeks. We will ask you to rate the severity of the symptoms.

### Appendix 3.

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Coding Severity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Do not have this symptom</td>
<td>1</td>
</tr>
<tr>
<td>Have this symptom but not bothers me</td>
<td>2</td>
</tr>
<tr>
<td>It bothers me a little</td>
<td>3</td>
</tr>
<tr>
<td>It bothers me a lot</td>
<td>4</td>
</tr>
<tr>
<td>It bothers me terribly</td>
<td>5</td>
</tr>
<tr>
<td>Not applicable</td>
<td>9</td>
</tr>
</tbody>
</table>

#### 1. Ukudinwa kakhulu nasemqondweni (fatigue) kanye nokungabi namandla (i-energi)? /Fatigue or loss of energy?

#### 2. Ukuba nefisa (umalaleveu), ukugodola kakhulu nama ukujuluka? / FEVERS, CHILLS OR SWEATS?

#### 3. Ukuzizwa unesiyezi noma inhloko yakho ilula (lightheaded)? / Feeling dizzy or lightheaded?

#### 4. Ubuhlungu, ukungobi namizwa (numbness) noma ubuzaze (tingling) ezandleni noma ezinyaweni? / Pain, numbness or tingling in the hands or feet?

#### 5. Ukukhathazwa ukukhohlwa (ukungakhumbuli)? / Trouble remembering?

#### 6. Ukuwa kwezinwele noma ukushintsha kwendlela ukonambitheka nokuzwakala ngayo? (rash), ukoma kwesikhumba noma ukuluma (itching)?

#### 7. Ubuhlungu, ukungabi

#### 8. Ukuwa kwezinwele noma ukushintsha kwendlela ukobukeka ngayo? (fat deposits), noma ukukhuluphala? / Changes in the way your body looks, such as fat deposits or weight gain?

#### 9. Ukuwa kwezinwele noma ukushintsha kwendlela ukobukeka ngayo? (fat deposits), noma ukukhuluphala? / Changes in the way your body looks, such as fat deposits or weight gain?

#### 10. Ukuwa kwezinwele noma ukushintsha kwendlela ukobukeka ngayo? (fat deposits), noma ukukhuluphala? / Changes in the way your body looks, such as fat deposits or weight gain?

#### 11. Ukuwa kwezinwele noma ukushintsha kwendlela ukobukeka ngayo? (fat deposits), noma ukukhuluphala? / Changes in the way your body looks, such as fat deposits or weight gain?

#### 12. Ukuwa kwezinwele noma ukushintsha kwendlela ukobukeka ngayo? (fat deposits), noma ukukhuluphala? / Changes in the way your body looks, such as fat deposits or weight gain?

#### 13. Ukuwa kwezinwele noma ukushintsha kwendlela ukobukeka ngayo? (fat deposits), noma ukukhuluphala? / Changes in the way your body looks, such as fat deposits or weight gain?

#### 14. Ukuwa kwezinwele noma ukushintsha kwendlela ukobukeka ngayo? (fat deposits), noma ukukhuluphala? / Changes in the way your body looks, such as fat deposits or weight gain?

#### 15. Ukuwa kwezinwele noma ukushintsha kwendlela ukobukeka ngayo? (fat deposits), noma ukukhuluphala? / Changes in the way your body looks, such as fat deposits or weight gain?

#### 16. Ukuwa kwezinwele noma ukushintsha kwendlela ukobukeka ngayo? (fat deposits), noma ukukhuluphala? / Changes in the way your body looks, such as fat deposits or weight gain?

#### 17. Ukuwa kwezinwele noma ukushintsha kwendlela ukobukeka ngayo? (fat deposits), noma ukukhuluphala? / Changes in the way your body looks, such as fat deposits or weight gain?

#### 18. Ukuwa kwezinwele noma ukushintsha kwendlela ukobukeka ngayo? (fat deposits), noma ukukhuluphala? / Changes in the way your body looks, such as fat deposits or weight gain?

#### 19. Ukuwa kwezinwele noma ukushintsha kwendlela ukobukeka ngayo? (fat deposits), noma ukukhuluphala? / Changes in the way your body looks, such as fat deposits or weight gain?

#### 20. Ukuwa kwezinwele noma ukushintsha kwendlela ukobukeka ngayo? (fat deposits), noma ukukhuluphala? / Changes in the way your body looks, such as fat deposits or weight gain?

#### 21. Ukuwa kwezinwele noma ukushintsha kwendlela ukobukeka ngayo? (fat deposits), noma ukukhuluphala? / Changes in the way your body looks, such as fat deposits or weight gain?

#### 22. Ukuwa kwezinwele noma ukushintsha kwendlela ukobukeka ngayo? (fat deposits), noma ukukhuluphala? / Changes in the way your body looks, such as fat deposits or weight gain?

#### 23. Ukuwa kwezinwele noma ukushintsha kwendlela ukobukeka ngayo? (fat deposits), noma ukukhuluphala? / Changes in the way your body looks, such as fat deposits or weight gain?
Manje sesizokuhuza imibuzo maqondana nokuyathatha imithi kanye nokuyothatha imithi kanye nokuyathatha nge-appointment aseklinik i

Now we will ask you questions in relation to picking up the medicine and coming to clinic appointment.

55. Namhlanje, ngabe uze ekliniki ngosuku olindelwe ngalo ukuthi uze ekliniki? /Today, did you come on the expected date for your visit?
   ( ) 1. Yebo /Yes
   ( ) 2. Cha, uze ngaphambi kosuku obekelwe lona ukuza ngalo /No, you came earlier than the appointed date
   ( ) 3. Cha, uze ngemuva kosuku obekelwe lona /No, you came late
   ( ) 4. awukhumbuli /You do not remember

56. Ngabe kukuthathe isikhathi esingakanani namhlanje ukuza ekliniki /How much time did it take you today to come to the clinic?
   Ihora: Amaminithi /Hour: Minutes ( : )

57. Ngabe uze kanjani ekliniki namhlanje? /How did you come today to the clinic?
   ( ) 1. Nge-Minitaxi (itekisi elincane) /Minitaxi ; ( ) 2. Ngebhasi /Bus ; ( ) 3. Ngetekisi /Taxi
   ( ) 4. Ngemoto yami / My own ca ; ( ) 5. Ngokuhamba /Walking ; ( ) 6. Okunye / Other

58. Ngabe bekumele ucele emsebenzini ukuze ukwazi ukuza ekliniki namhlanje? /Did you need to take time off from work to be able to come today to clinic?
   ( ) 1. Yebo /Yes ; ( ) 2. Cha /No ; ( ) 3. Awusebenzi /You are unemployed

59. Kwesinye isikhathi abantu bayeka noma baphazamisa ukuphuza kwabo ama-ARV ngenxa yeziizathu ezehlukene.
   Ngabe uke wena nomaxaphazamisa ukuphuza kwakho ama-ARV ngoba udokotela ugebhineni wenzu kanjalo, noma ngoba wena uzithethele isinqumo? /Sometimes people stop or interrupt taking their ARVs for different reasons. Did you ever stop or interrupt taking your ARVs because your doctor wanted you to, or because you decided yourself?
   ( ) 1. Angikaze ngikwenze lokho nhlobo /Never stopped at all ; ( ) 2. Udokotela /Doctor ; ( ) 3 Mina ngokwami /Self

Ngiyabonga!
Thank you!

Time interview ended (hh:min) __ : __       Total duration of interview (min) _____

Interviewer: ___________________ Signed: ___________________
<table>
<thead>
<tr>
<th>Number</th>
<th>Question</th>
<th>Options</th>
<th>Language Options</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Date of birth (day/month/year)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Bong / Sex</td>
<td>( ) 1. Mosadi / Woman ; ( ) 2. Monna / Man</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>What is your country of birth?</td>
<td></td>
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<tr>
<td>5</td>
<td>Na o fihletse maemo afe a thuto? / What is your highest education level?</td>
<td>( ) 1. Afrika Borwa / South Africa ; ( ) 2. Zimbabwe ; ( ) 3. Lesotho ; ( ) 4. Swaziland ; ( ) 5. Botswana ; ( ) 6. Namibia ; ( ) 7. Mozambique ; ( ) 8. E nngwe</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Na o dula / Do you live in</td>
<td></td>
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<tr>
<td>7</td>
<td>Puo ya hao ya lapeng ke efe? / What is your first language?</td>
<td>( ) 1. isiZulu ; ( ) 2. isiXhosa ; ( ) 3. Tshivenda ; ( ) 4. Siswati ; ( ) 5. Setswana ; ( ) 6. Sesotho ; ( ) 7. English</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Boemo ba lenyalo / Marital status</td>
<td>( ) 1. O mong / Single ; ( ) 2. O hladile/le arohane / Divorced/Separated ; ( ) 3. O mohlolohadi (wa monna/mosadi) / Widow/widower ; ( ) 4. O nyetse / Married ; ( ) 5. O na le eo o nang le dikamano tsa thobalano le yena / A sexual relationship ; ( ) 6. O na le eo o helang mmoho le yena / Cohabitation ; ( ) 7. Ho hong / Other</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Ke balekane ba bakae bao o bileng le thobalano le bona dikgweding tse 3 tse fetileng? / How many sexual partners did you have during the last 3 months?</td>
<td>( ) 1. Ee, ka dinako tse ding / Yes, all the time ; ( ) 2. Ee, ka dinako tse ding / Yes, sometimes ; ( ) 3. Tjie, ho hang / No, not at all ; ( ) 4. Ha o na bonnete / Not sure</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Haebe o bile le molekane wa thobalano ya mong kapa ba bangata, o ile wa sebedisa dikhondomo? / If you had one or more than one sexual partner, did you use condoms?</td>
<td>( ) 1. Ee, ka dinako tsohle / Yes, all the time ; ( ) 2. Ee, ka dinako tse ding / Yes, sometimes ; ( ) 3. Ha o na bonnete / Not sure</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Na le na le efe kapa efe ya tse latelang lapeng leno / Do you have any of the following in your home</td>
<td>( ) 1. Pompo ya ka tlung / Inside tap ; ( ) 2. Nhlanwa ya ka tlung / Inside toilet ; ( ) 3. Motlakase / Electricity ; ( ) 4. Forijzi / Fridge ; ( ) 5. Radio / Radio ; ( ) 6. TV ; ( ) 7. Tholefouyi ya ka tlung / Landline phone</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Lelapa leno le na le dipha posisi tse kae? / How many rooms are there in your home?</td>
<td>( ) 1. Pompo ya ka tlung / Inside tap ; ( ) 2. Nhlanwa ya ka tlung / Inside toilet ; ( ) 3. Motlakase / Electricity ; ( ) 4. Forijzi / Fridge ; ( ) 5. Radio / Radio ; ( ) 6. TV ; ( ) 7. Tholefouyi ya ka tlung / Landline phone</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Ntle le wena, ke paloyohle ya bana ba bakae le batho ba baholo ba bakae ba phelang le wean / Besides you, how many children and adults in total live with you</td>
<td>( ) 1. Pompo ya ka tlung / Inside tap ; ( ) 2. Nhlanwa ya ka tlung / Inside toilet ; ( ) 3. Motlakase / Electricity ; ( ) 4. Forijzi / Fridge ; ( ) 5. Radio / Radio ; ( ) 6. TV ; ( ) 7. Tholefouyi ya ka tlung / Landline phone</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Na ho na le e mong ya dulang lapeng leno ya nang le HIV? / Is there anyone else living in your household who has HIV?</td>
<td>( ) 1. Ee / Yes ; ( ) 2. Tjie / No / flela nomorong ya 16 / go to number 16 ; ( ) 3. Ha o tsebe / Do not know</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Ntle le wena, na ho na le e mong ya nkang di-ARV? / Besides you, is there anyone else taking ARV?</td>
<td>( ) 1. Ee / Yes ; ( ) 2. Tjie / No / flela nomorong ya 16 / go to number 16 ; ( ) 3. Ha o tsebe / Do not know</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Na o se o tsebitise batho ba bang ka boemo ba hao ba HIV? / Have you disclosed your HIV status to other people?</td>
<td>( ) 1. Ee / Yes ; ( ) 2. Tjie / No / flela nomorong ya 16 / go to number 16</td>
<td></td>
</tr>
</tbody>
</table>
Appendix 4.

17. Haebé ke EE, na o bolelletse mang ka boemo ba hao? (lekola tsohle tse amehehang) /IF YES, who did you disclose your status to? (check all that apply)

( ) 1. Mme/trate /Mother/father; ( ) 2. Matsale/Ratsale /Mother-in-law/Father-in-law
( ) 3. Dikgaitsedi tsa hao /Sibling(s); ( ) 4. Molekane /Partner; ( ) 5. Motswalle /Friend; ( ) 6. Bana /Children
( ) 7. Setho se seng le leloko/lelapa /Other family/household member; ( ) 8. Ramosebetsi /Employer; ( ) 9. E mong /Other

18. Na boemo ba hao mosebetsing ke bofe? /What is your employment status?
( ) 1. Ha o sebetsa jwang; ( ) 2. O pensheneng /Retired  ꜙ fetela nomorong ya 20 /go to number 20
( ) 3. O a sebetsa /Employed; ( ) 4. O sebetsa mosebetsi oo o kenang ke letatsi /Work on daily labour basis

19. Na o sebetsa jwang? /How do you work?
( ) 1. Dinako tsohle /Full-time; ( ) 2. Nakwana /Past-time; ( ) 3. O a itshebetsa /Self-employed; ( ) 4. Ho hong /Other

20. Na mehlodi ya hao ya tjhelete ke efe? (kgetho tse fetang e le ngwe di amobelele) /What are your sources of income? (check all that apply)

( ) 1. Ka bowena /Self  ꜙ fetela nomorong ya 22 /go to number 22
( ) 2. Molekane /Partner  ꜙ fetela nomorong ya 22 /go to number 22
( ) 3. Kgaitsele /Sibling  ꜙ fetela nomorong ya 22 /go to number 22
( ) 4. Thuso ya penshene /Pension grant  ꜙ fetela nomorong ya 22 /go to number 22
( ) 5. Thuso ya kgolofalo /Disability grant  ꜙ fetela nomorong ya 21 /go to number 21
( ) 6. Thuso ya sapato ya bana /Child support grant  ꜙ fetela nomorong ya 22 /go to number 22
( ) 7. Motswadi /Parent  ꜙ fetela nomorong ya 22 /go to number 22
( ) 8. Ho hong /Other; ( ) 9. Letho /None  ꜙ fetela nomorong ya 22 /go to number 22

21. Haebé ke Ee, ke nako e ka e o fumana ha hao ba HIV? /Did you go into a readiness program before starting your ARVs?
( ) 1. Ee /Yes; ( ) 2. Tjhe /No

22. Na paloyohle ya moputso wa hao wa kgwedi e fetileleng e bile efe? /What was your own total income for last month?

23. Ke nako e ka e, pele ha ho ba o qale ka di-ARV tsa hao, moo o neng o tsbea ka boemo ba hao ba HIV? /How long, before starting your ARVs, did you know about your HIV status?

( ) 1. Dibeke tse 4 /Weeks; ( ) 2. Dikgwedi tse 1-5 /1-5 months; ( ) 3. Dikgwedi tse 6-12 /6-12 months
( ) 4. Dilemo tse 1-2 /1-2 years; ( ) 5. Dilemo tse 2-5 /2-5 years; ( ) 6. Ka hodimo ho dilemo tse 5 /More than 5 years

24. Na o ile ya kena lenaneong la ho itokisetsa pele o qala ho nka di-ARV tsa hao? /Did you go into a readiness program before starting to take your ARVs?
( ) 1. Ee /Yes; ( ) 2. Tjhe /No

25. (Bakeng sa basadi), na o moima ha jwale /For women, are you currently pregnant?
( ) 1. Ee /Yes; ( ) 2. Tjhe /No; ( ) 3. Ha o tsbea /Don't know; ( ) 9. Ha e hlokehe /Not applicable (if men)

26. Na o fumana kalao ya TB ha jwale? /Have you visited a private doctor, a pharmacist/chemist/other public hospital/clinic or a traditional healer, since you started ARVs at this clinic? (check all that apply)

( ) 1. Ee /Yes; ( ) 2. Tjhe /No

27. Na o kile ya alashwa bakeng sa TB pejana? /Have you been treated for TB before?
( ) 1. Ee /Yes  ꜙ fetela nomorong ya 29 /go to number 29; ( ) 2. Tjhe /No

28. Na o sa o tswa hlahlojiwa bakeng sa TB? /Have you been recently tested for TB?
( ) 1. Ee /Yes; ( ) 2. Tjhe /No

29. Na o kile ya etela ngaka ya porae, pharmacist/chemist sepetlele /tiliini ya mmoso ka ngaka ya setso, haesale ho ba o qale ka di-ARV tillinliking ena? (lekola tsohle tse tshwaneseng) /Have you visited a private doctor, a pharmacist/chemist/other public hospital/clinic or a traditional healer, since you started ARVs at this clinic? (check all that apply)

( ) 1. Dingaka tsa porae /Private doctor; ( ) 2. Pharmacist /chemist; ( ) 3. Ngaka ya setso /Traditional healer
( ) 4. Sepetlele/tiliini ya mmoso /Public hospital/clinic; ( ) 5. Tjhe, ho hang /No, not at all
**Ditaelo bakeng sa ya tsamaisang puisano: balla mokudi hona butle hape ka ho hlaka, hore o tle o etse hore a be le tshepo**

Instructions for interviewer: read this slowly and clearly to the patient, to build trust with him/her...

"Ke rata ho o botsa dipotso tse itseng mabapi le ka moo o nwang di-ARV tsa hao. Ke batla ho utlwisisa hore ke eng seo hantlentle batho ba se etsang ka dipilisi tsa bona. O se ke wa tshwenyeha ka ho mopolela ha e o nke dipilisi tsa hao tsohle. Batho ba bang ba nwa dipilisi tsa bona tsohle jwalo ka ha ba laetswe ha ba bang bona ba sa e nke dipilisi tsa bona tsohle. Ke batla ho tsela hore hantlentle ho etsahalang, e seng seo wena o nahanang hore ke "batla ho se ulwa." I would like to ask you some questions about the way you have been taking your ARVs. I need to understand what people are really doing with their medicines. Do not worry about telling me that you don’t take all your pills. Some people take all of their medications as they are prescribed while others may not take all of their medications. I need to know what is really happening, not what you think I “want to hear.”"

30. Ke kopa hore o ntshupele ho cartoon board ena, hore ke meriana efe eo o e nwang ha jwale /Please point to me on this cartoon board, which ARVs you are currently taking

<table>
<thead>
<tr>
<th>Lebitso la dipilisi /Name of drug</th>
<th>Polo ya DIPILISI tseo o laetsweng ho dinwa nako le nako (dipilisi ka ho ya ka tekanyo) /Number of PILLS prescribed to be taken each time (pills per dose)</th>
<th>Polo ya DINAKO tseo o laetsweng homka ka tsona ka letsatsi (ditekanyo ka letsatsi) /Number of TIMES prescribed to be taken per day (doses per day)</th>
</tr>
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</table>

31. Dipilisi tseo o sa di nwang /Missed pills

<table>
<thead>
<tr>
<th>Lebitso la dipilisi tsa HIV /Name of HIV drugs</th>
<th>Na o ile wa fetwa ke dipilisi tse kae /how many pills did you miss</th>
</tr>
</thead>
<tbody>
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<td>•</td>
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</tr>
</tbody>
</table>

32. Dipilisi tseo o ileng wa di nwa ka nako /Pills taken on time

<table>
<thead>
<tr>
<th>Lebitso la dipilisi tsa HIV /Name of HIV drugs</th>
<th>Ke moriana wa dipilisi tse kae tseo o di nweleng ka nako (ke hore, nakong ya hora (+/- 1 hour e belweng) /How many drug pills did you take on time (i.e. within +/- 1 hour of the prescribed time)</th>
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</table>

33. Na dife kapa dife tsa di-ARV tsa hao di na le ditaelo tse igkethang, tse jwalo ka “di nowa le dijo” kapa “di nowa o sa ja letho”? /Do any of your ARVs have special instructions, such as “take with food” or “take on an empty stomach” or “take with plenty of fluids”?  
( ) 1. Ee /Yes ; ( ) 2. Tjhe /No  

34. Haebe ke Ee potsong ya 33, ke ha ka moo o ileng wa latela ditaelo tseo tse igkethang matsatsang a mane a fetileng? /If Yes to question 33, how often did you follow those special instructions over the last four days?  
( ) 1. Ho hang /Never ; ( ) 2. Ka nako e nngwe /Sometimes ; ( ) 3. Halfo ya nako /Half of the time ; ( ) 4. Boholong ba nako /Most of the time ; ( ) 5. Ka nako yohle /All of the time

35. Batho ba bang ba fumana hore ba lebala ho nwa dipilisi tsa bona mafelong a beke. Na o ile wa fetwa ke ho nwa di-ARV tsa hao mafelong a beke a sa tswa feta? /Some people find that they forget to take their pills over the weekend. Did you miss any of your ARVs last weekend?  
( ) 1. Bekeng e fetileng /Within the past week  
( ) 2. Dibeko tse 1-2 tse fetileng /1-2 weeks ago  
( ) 3. Dibeko tse 2-4 tse fetileng /2-4 weeks ago  
( ) 4. Dikgwedi tse 1-3 tse fetileng /1-3 months ago  
( ) 5. Ka hodimo ho dikgwedi tse 3 tse fetileng /More than 3 months ago  
( ) 6. Ha nke le tlae nako ya ho nwa meriana /Never skip medications

36. Ke lefe kgetlo la ho getela moo o ileng wa fetwa ke ho nwa dife kapa dife tsa di-ARV tsa hao ho hang? /When was the last time you missed taking any of your ARVs at all?  
( ) 1. Bekeng e fetileng /Within the past week  
( ) 2. Dibeko tse 1-2 tse fetileng /1-2 weeks ago  
( ) 3. Dibeko tse 2-4 tse fetileng /2-4 weeks ago  
( ) 4. Dikgwedi tse 1-3 tse fetileng /1-3 months ago  
( ) 5. Ka hodimo ho dikgwedi tse 3 tse fetileng /More than 3 months ago  
( ) 6. Ha nke le tlae nako ya ho nwa meriana /Never skip medications
37. Batho ba fetwa ke ho nka dipilisi tsa bona tlasa mabaka a mangata a fapaneng. Ke lena lenane la mabaka a ka hlahang a etsang hore motho a fetwe ke ho nka dipilisi tsa hae. Na ke hakae moo o ileng wa fetwa ke ho naka dipilisi tsa hae lobane o o e (etsa sedikakidikwe karabong e le ngwe bakeng sa potso ka ngwe/lebaka leho ho fanweng ka lona) /People miss taking their medications for various reasons. Here is a list of possible reasons why you may miss taking your medications. How often have you missed taking your medications because you (circle one response for each question/reason given)

1. O ne o se lapeng? /Were away from home? (  )1 (  )2 (  )3 (  )4 (  )9
2. O ne o sebetsa ka dintho tse ding? /Were busy with other things? (  )1 (  )2 (  )3 (  )4 (  )9
3. O ile wa mpa wa lebala? /Simply forgot? (  )1 (  )2 (  )3 (  )4 (  )9
4. O ne o na le dipilisi tse ngata tseo o lokelang ho di nwa? /Had too many pills to take? (  )1 (  )2 (  )3 (  )4 (  )9
5. O ne o ba bala ho qoba ditlamorao? /Wanted to avoid side effects? (  )1 (  )2 (  )3 (  )4 (  )9
6. O ne o sa batle hore ba bang ba bone hore o nwa dipilisi? /Did not want others to notice you taking medication? (  )1 (  )2 (  )3 (  )4 (  )9
7. O fetotse nako ya tlwaelo ya ho nka dipilisi leetsatsi leletsatsi? /Had a change in daily routine? (  )1 (  )2 (  )3 (  )4 (  )9
8. O utlwile e ka dipilisi tse di kotsi? /Felt like the drug was toxic/harmful? (  )1 (  )2 (  )3 (  )4 (  )9
9. O ile wa fellwa ke dipilisi? /Ran out of pills? (  )1 (  )2 (  )3 (  )4 (  )9
10. O ile wa ikutlwa o kula? /Felt sick or ill? (  )1 (  )2 (  )3 (  )4 (  )9
11. O ile wa ikutlwa o tepeletse/ho sithabela? /Felt depressed/overwhelmed? (  )1 (  )2 (  )3 (  )4 (  )9
12. O ne o sa batle ho hw na dipilisi o sa ja? /You did not want to take the pills on an empty stomach? (  )1 (  )2 (  )3 (  )4 (  )9
13. O ile wa fellwa ke dipilisi? /Ran out of pills? (  )1 (  )2 (  )3 (  )4 (  )9
14. O ile wa ikutlwa o phetsi hantle/betere? /Felt good/better? (  )1 (  )2 (  )3 (  )4 (  )9
15. O ne o ba bala ho boloka dipilisi tse ding? /Wanted to save extra pills? (  )1 (  )2 (  )3 (  )4 (  )9
16. O nwa meriana ya setso ha o sa nke dipilisi tsa HIV? /Taking traditional medicine when not taking HIV medications? (  )1 (  )2 (  )3 (  )4 (  )9
17. O ile wa fellwa ke dipilisi? /Ran out of pills? (  )1 (  )2 (  )3 (  )4 (  )9
18. O ile wa ikutlwa o kula? /Felt sick or ill? (  )1 (  )2 (  )3 (  )4 (  )9
19. O ile wa ikutlwa o tepeletse/ho sithabela? /Felt depressed/overwhelmed? (  )1 (  )2 (  )3 (  )4 (  )9
20. Ho hong? (bolela lebaka le le leng le leholo) /Other? (state one main reason) (  )1 (  )2 (  )3 (  )4 (  )9

38. Na o ile wa hopola jwang ho tla apoyentementeng ya hao kajeno (etsa sefapano ho tshohle tse tshwanetseng) /How did you remember to come to your appointment today (cross all that apply)
( ) 1. Letsatsi la apoyentemente le ngotswe karateng ya rejistara ya tliliniking /Appointment date written on clinic register card
( ) 2. O e ngola dayaring/bukeng ya diapoyentemente /You write it down in a diary/appointment book
( ) 3. O leka ho hopola, ke hore o tshepetse mohopolong wa hao /You try to remember, i.e. you rely on your memory
( ) 4. Sebedisa selfounu ya hao /You use your mobile phone
( ) 5. Motswalle e moholo/wa leloko ya haufi le wena wa o hopotsa /A close friend/close relative reminds you
( ) 6. Molekane wa hao wa o hopotsa /Your partner reminds you
( ) 7. Motswalle wa hao mosebetsing wa o hopotsa /Your friend at work reminds you
( ) 8. Ho hong /Other (  )1 (  )2 (  )3 (  )4 (  )9

39. Dikgweding tse 6 tse fetileng, na o kile wa fetwa ke apoyentemente ya tliliniking (ke hore, ho bona nga ka hao kapa mooki) /During the last 6 months, have you ever missed a clinic appointment (i.e. to see your doctor or nurse)?
( ) 1. Ee /Yes
( ) 2. Tjhe /No
( ) 3. Ha o sa hopola /Don't remember
( ) 4. Ha e hlokehe /Not applicable

40. E ka ba makgetlo a makae moo o ileng wa fetwa ke apoyentemente ya hao ya tliniking dikgweding tse 6 tse fetileng? /Approximately, how many times have you missed your clinic appointment in the last 6 months? Palo /Number _______
41. Haeb e o ile wa fetwa ke apoyentemente, na ho na le motho e mong wa tiliniking kapa khemising ya ileng a iteanya le wena ho o bolella hore o fetiowe ke apoyentemente ya ha? /If you have ever missed an appointment, did someone from the clinic or the pharmacy contact you to tell you that you missed your appointment?  
(  ) 1. Ee /Yes ; (  ) 2. Tjhe /No ; (  ) 9. Ha e hlokehe /Not applicable

42. Ke lefe lebaka le entseng hore o se ke wa tla apoyentementeng e hlophisitsweng? /What has been your reason for not coming to the scheduled appointment?

<table>
<thead>
<tr>
<th>No</th>
<th>Reason</th>
<th>Ho bang /Never (1)</th>
<th>Ka sevelo /Rarely (2)</th>
<th>Ka dinko tse drug /Sometimes (3)</th>
<th>Hangana /Other (4)</th>
<th>Ha e hlokehe /Not applicable (9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>O ne o sa kgone ditjeo tsa transporoto / You could not afford the cost of transportation</td>
<td>(  ) 1 (  ) 2 (  ) 3 (  ) 4 (  ) 9</td>
<td></td>
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<tr>
<td>2.</td>
<td>Ke hole ho tla mona moo mme o ne o se na nako ya hoo / It is long distance to get here and you did not have time for it</td>
<td>(  ) 1 (  ) 2 (  ) 3 (  ) 4 (  ) 9</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>3.</td>
<td>Molekane wa hao o ho hanetse ho tla mona / Your partner refused you to come here</td>
<td>(  ) 1 (  ) 2 (  ) 3 (  ) 4 (  ) 9</td>
<td></td>
<td></td>
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<tr>
<td>4.</td>
<td>O ile wa mpa wa lebala / You simply forget</td>
<td>(  ) 1 (  ) 2 (  ) 3 (  ) 4 (  ) 9</td>
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</tr>
<tr>
<td>5.</td>
<td>O ne o sa ikutlwe hantle mme o hloka ho phomola lapeng / You were not feeling well and needed to rest at home</td>
<td>(  ) 1 (  ) 2 (  ) 3 (  ) 4 (  ) 9</td>
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<tr>
<td>6.</td>
<td>O ile wa ikutlwa hantle/o phetse mme wa bona hore ha o hloke dipilisi / You felt fine/healthy and felt you did not need the medications</td>
<td>(  ) 1 (  ) 2 (  ) 3 (  ) 4 (  ) 9</td>
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<tr>
<td>7.</td>
<td>O ne o tsamale ka matsatsi a phomolo / You were away for holidays</td>
<td>(  ) 1 (  ) 2 (  ) 3 (  ) 4 (  ) 9</td>
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<tr>
<td>8.</td>
<td>O ne o tsamale ka mosebetsi / You were away for work</td>
<td>(  ) 1 (  ) 2 (  ) 3 (  ) 4 (  ) 9</td>
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<tr>
<td>9.</td>
<td>Khemisi e ile ya fellwa ke dipilisi ya ba o tswhanela ho kgutla ha morao / Pharmacy ran out of drugs and you had to come back later</td>
<td>(  ) 1 (  ) 2 (  ) 3 (  ) 4 (  ) 9</td>
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<tr>
<td>10.</td>
<td>O ne o sa dumele hore dipilisi di ka etsa hore o be betere / You did not believe the medicine can help you to get better</td>
<td>(  ) 1 (  ) 2 (  ) 3 (  ) 4 (  ) 9</td>
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<tr>
<td>11.</td>
<td>Ho hong /Other</td>
<td>(  ) 1 (  ) 2 (  ) 3 (  ) 4 (  ) 9</td>
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</tbody>
</table>

43. Na dihora tsa mosebetsi tsa tiliniki ena ha di o sitise? /Are the opening hours of this clinic convenient for you?
(  ) 1. Ee /Yes ; (  ) 2. Tjhe /No

44. Dikgweding tse 6 tse fetileng, na o kile wa fetwa ke ho lata di-ARV tsa hao tsa tlatsetso khemising/tiliniking? /During the last 6 months, have you ever missed your ARV refill at the pharmacy/clinic?
(  ) 1. Ee /Yes
(  ) 2. Tjhe /No → Fetela nomorong ya 49
(  ) 3. Ha o sa hopola / You don't remember
(  ) 9. Ha e sebetse /Not applicable

45. Na ke makgetlo a makae moo o ileng wa fetwa ke di-ARV tsa hao tsa tlatsetso khemising/tiliniking dikgweding tse 6 tse fetileng / Approximately, how many times have you missed your ARV refill at the pharmacy/clinic during the last 6 months
Palo /Number ________

46. Dikgweding tse 6 tse fetileng, ke hakae moo ileng wa nka nako e fetang letsatsi le le leng o sa fumane dipilisi ho hang lebokosong la hao la dipilisi? / During the last 6 months, how often you spent more than one day without any drugs at all in your pillbox?
(  ) 1. Ho hang /Never at all
(  ) 2. E batlile e ba letsatsi le le leng kgwedie e ningwe e e ningwe /Almost one day every month
(  ) 3. E batlile e ba hang kgwedie e ningwe le e ningwe ya bobedi /Almost once every 2nd month
(  ) 4. Ha ngwe feela /One time only
Appendix 4.

47. Re kopa hore o bolele mabaka a 3 a entseng hore o fetwe ke nako ya ho tla ho tilio tlatselletsa dipilisi tsa hao tsa HIV? 
//Please state up to 3 reasons why you missed coming on time to refill your ARVs?

Lebaka la 1 /Reason 1

Lebaka la 2 /Reason 2

Lebaka la 3 /Reason 3

48. Haebe o kile wa fetwa ke ho ya tlatselletsa dipilisi tsa hao, na ho na le motho wa kemising ya ileng a iteanya le wena ho o bolella hore o fetilwe ke apoyentemente ya hao /If you have ever missed medication refill, did someone from the pharmacy contact you to tell you that you missed your appointment? ( ) 1. Ee /Yes ; ( ) 2. Tjhe /No ; ( ) 9. Ha e hlokehe /Not applicable

49. Jwale re tla o botsa hore o hopote jwang ho tla tiliniking/khemisi ho tla lata dipilisi tsa hao dikgweding tse 6 tse fetileng (etsa sefapano ho tsohle tse tshwanetseng) /Now we will ask you how did you remember to come to the clinic/pharmacy to pick-up your pills during the past 6 months(cross all that apply)

( ) 1. O sebedisa lebokoso la dipilisi /You use a pill box
( ) 2. O ngola dayaring/bukeng ya apoyentemente /You write it down in a diary/appointment book
( ) 3. O leka ho hopola, ke hore, o tshepetse mohopolong wa hao /You try to remember, i.e. you rely on your memory
( ) 4. O sebedisa selfounu ya hao /You use your mobile phone
( ) 5. Motswalle e moholo /wa leloko ya haufi le wena wa o hopotsa /A close friend/close relative reminds you
( ) 6. Molekane wa hao wa o hopotsa /Your partner reminds you
( ) 7. Motswalle wa hao mosebetsing wa o hopotsa /Your friend at your work reminds you
( ) 8. Ho hong /Other

50. Ha e le ho hong (Re kopa hore o re bolelle, hore na ka tlwaelo o hopola jwang apoyentemente ya hao?) /Other (Please share with us, how you normally recall your appointment?)

51. Na dihora tsa ho bula tsa kemisi ena di lokile bakeng sa ho tlatselletsa dipilisi tsa hao? /Are the opening hours of this pharmacy convenient for your drug refill? ( ) 1. Ee /Yes ; ( ) 2. Tjhe /No

52. Jwale re tla o botsa ka moo o neng o hopola ho awa dipilisi tsa hao ka teng ka nako dibekeng tse 4 tse fetileng, ngola tsohle tse hlokeang /Now we will ask you how you used to remember to take your pills on time during the past 4 weeks (check all that apply)

( ) 1. O sebedisa lebokoso la dipilisi /You use a pill box
( ) 2. O ngola dayaring/bukeng ya apoyentemente /You write it down in a diary/appointment book
( ) 3. O leka ho hopola, ke hore, o tshepetse mohopolong wa hao /You try to remember, i.e. you rely on your memory
( ) 4. O sebedisa selfounu ya hao /You use your mobile phone
( ) 5. Motswalle e moholo /wa leloko ya haufi le wena wa o hopotsa /A close friend/close relative reminds you
( ) 6. Molekane wa hao wa o hopotsa /Your partner reminds you
( ) 7. Motswalle wa hao mosebetsing wa o hopotsa /Your friend at your work reminds you
( ) 8. Ho hong /Other

53. Ha e le ho hong (Re kopa hore o re bolelle, hore na ka tlwaelo o hopola jwang apoyentemente ya hao?) /Other (Please share with us, how you normally recall that you have an appointment?)
### Appendix 4.

54. Dipotso tse latelang di botsa ka **matshwao ao e ka bang o kile wa ba le ona dibekeng tse mme tse fetileng**. Re tla o kopa hore o lekanye bobe ba **matshwao** /The following questions ask about symptoms you might have had during the past four weeks. We will ask you to rate the severity of the symptoms.

<table>
<thead>
<tr>
<th>Mokgathala kapa ho lalehelwa ke eneji?</th>
<th>Ha le matshwao lena mme /I have this symptom</th>
<th>Ke na le leshwao lena mme</th>
<th>Ha le mme le mme /I do not have this symptom</th>
<th>Ha e hlokehe /Not applicable</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Fatigue or loss of energy?</td>
<td>( ) 1 ( ) 2 ( ) 3 ( ) 4 ( ) 5 ( ) 9</td>
<td></td>
<td></td>
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<tr>
<td>2. Motheso, mothasela kapa ho futulela?</td>
<td>( ) 1 ( ) 2 ( ) 3 ( ) 4 ( ) 5 ( ) 9</td>
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<tr>
<td>3. Ho tsekela kapa ho kopakopana hlooho?</td>
<td>( ) 1 ( ) 2 ( ) 3 ( ) 4 ( ) 5 ( ) 9</td>
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<td></td>
<td></td>
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<tr>
<td>4. Mahlaba, ho shwa bobatsu kapa ho hlohloha matshothing kapa matong?</td>
<td>( ) 1 ( ) 2 ( ) 3 ( ) 4 ( ) 5 ( ) 9</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>5. Bothata ba ho hopola?</td>
<td>( ) 1 ( ) 2 ( ) 3 ( ) 4 ( ) 5 ( ) 9</td>
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<tr>
<td>6. Ho feroha kapa ho hlatsa?</td>
<td>( ) 1 ( ) 2 ( ) 3 ( ) 4 ( ) 5 ( ) 9</td>
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<tr>
<td>7. Ho feroha kapa ho sebetswa ke mala?</td>
<td>( ) 1 ( ) 2 ( ) 3 ( ) 4 ( ) 5 ( ) 9</td>
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<td></td>
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<tr>
<td>8. Ho hlonama, moya o le fatshe kapa o tepeletse?</td>
<td>( ) 1 ( ) 2 ( ) 3 ( ) 4 ( ) 5 ( ) 9</td>
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<td></td>
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<tr>
<td>9. O kuitive o tshihile kapa o tshwenyehile?</td>
<td>( ) 1 ( ) 2 ( ) 3 ( ) 4 ( ) 5 ( ) 9</td>
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<tr>
<td>10. Botlha ba ho robala kapa ho kgone ho robala?</td>
<td>( ) 1 ( ) 2 ( ) 3 ( ) 4 ( ) 5 ( ) 9</td>
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<tr>
<td>11. Mathata a letlalo, jwalo ka lekgopho, ho oma kapa ho hlohloha?</td>
<td>( ) 1 ( ) 2 ( ) 3 ( ) 4 ( ) 5 ( ) 9</td>
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<tr>
<td>12. Ho kgholola kapa bothata ba ho hema?</td>
<td>( ) 1 ( ) 2 ( ) 3 ( ) 4 ( ) 5 ( ) 9</td>
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<tr>
<td>13. Hlooho e opang?</td>
<td>( ) 1 ( ) 2 ( ) 3 ( ) 4 ( ) 5 ( ) 9</td>
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<tr>
<td>14. Ho se be le takatso ya dijo kapa ho ba le tsecho e fetohieng ya dijo?</td>
<td>( ) 1 ( ) 2 ( ) 3 ( ) 4 ( ) 5 ( ) 9</td>
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<td>15. Ho ruruha, mahlaba kapa kgase ke mpeng ya hao?</td>
<td>( ) 1 ( ) 2 ( ) 3 ( ) 4 ( ) 5 ( ) 9</td>
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<tr>
<td>16. Mesifa e boholo kapa mahlaba manonyelestong?</td>
<td>( ) 1 ( ) 2 ( ) 3 ( ) 4 ( ) 5 ( ) 9</td>
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<tr>
<td>17. Mathata a ho ba le thobalano, jwalo ka ho fellwa ke kgahleho kapa ho se kgotsofale?</td>
<td>( ) 1 ( ) 2 ( ) 3 ( ) 4 ( ) 5 ( ) 9</td>
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<tr>
<td>18. Diphetoho tsa ka moo mmene wa hao o bohehang ka teng, jwalo ka mafura a mangata kapa boima ba mmene bo eketschileng?</td>
<td>( ) 1 ( ) 2 ( ) 3 ( ) 4 ( ) 5 ( ) 9</td>
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<tr>
<td>19. Mathata le ho theosa boima ba mmene kapa ho fokotsa matla le eneji ya mmene butle?</td>
<td>( ) 1 ( ) 2 ( ) 3 ( ) 4 ( ) 5 ( ) 9</td>
<td></td>
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<tr>
<td>20. Ho tswa moriri kapa diphetoho tsa tsele e o bohehang ka yona?</td>
<td>( ) 1 ( ) 2 ( ) 3 ( ) 4 ( ) 5 ( ) 9</td>
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<tr>
<td>21. Tshabo?</td>
<td>( ) 1 ( ) 2 ( ) 3 ( ) 4 ( ) 5 ( ) 9</td>
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<tr>
<td>22. Mehopollo ya ho ipolaya?</td>
<td>( ) 1 ( ) 2 ( ) 3 ( ) 4 ( ) 5 ( ) 9</td>
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</tr>
<tr>
<td>23. Ha o na matshwao ho hang?</td>
<td>( ) 1 ( ) 2 ( ) 3 ( ) 4 ( ) 5 ( ) 9</td>
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</tbody>
</table>
Jwale re tla o botsa dipotso tse mahapi le ho lata dipilisi le ho tla dispoyentementeng tsa tilliniking /Now we will ask you questions in relation to picking up the medicine and coming to clinic appointments

55. Kajeno, na o tle ka letsatsi le lebelletsengweng la ketelo ya hao? /Today, did you come on the expected date for your visit?
   ( ) 1. Ee /Yes
   ( ) 2. Tjhe, o tle pele ho letsatsi la apoyentemente /No, you came earlier than the appointed date
   ( ) 3. Tjhe, o tle ka mora lona /No, you came late
   ( ) 4. ha o sa hopola /you do not remember

56. Na o nkile nako e kae kajeno ho fihla tilliniking? /How much time did it take you today to come to the clinic?
   Hora : Metsotso /Hour:Minutes (     :     )

57. Na o tle jwang tilliniking kajeno? /How did you come today to the clinic?
   ( ) 1. Minitekesi /Minitaxi ; ( ) 2. Bese /Bus ; ( ) 3. Tekesi /Taxi ; ( ) 4. Koloi ya hao /My own car ; ( ) 5. Ka maoto /Walking
   ( ) 6. Ho hong /Other _____________________

58. Na o ile wa tla mehe ho se ye mosebetsing hore o kgone ho tla tilliniking? /Did you need to take time off from work to be able to come today to clinic?
   ( ) 1. Ee /Yes ; ( ) 2. Tjhe /No ; ( ) 3. Ha o sebetse /You are unemployed

59. Ka dinako tse ding batho ba emisa kapa ka sitiswa ho nka di-ARV tsa bona ke mabaka a fapaneng. Na o kile wa emisa kapa wa sitiswa ho nka di-ARV tsa hao hobane ngaka ya hao e ne e bata hore o etse jwalo, kapa hobane o entse qeto e jwalo ka bowena? /Sometimes people stop or interrupt taking their ARVs for different reasons. Did you ever stop or interrupt taking your ARVs because your doctor wanted you to, or because you decided yourself?
   ( ) 1. Ha wa ka wa emisa ho hang /Never stopped at all ; ( ) 2. Ngaka /Doctor ; ( ) 3. Ka bowena /Self

Re a leboha! /Thank you!

Nako eo puisano e fedileng ka yona (hora le metsotso)
Time interview ended (hh:min) : __________   nako yohle ya puisano (min) Time interview ended (hh:min) __________

Ya tsamaisang puisano Interviewer: ____________________ Tshaeno / Signed: ____________________
Appendix 5. Screening informed consent form (Article II)

Screening Informed Consent

**Study Name:** South African Virologic Evaluation (SAVE) study

**Investigators:** Mr. Ziad El-Khatib, Dr. Lerato Mohapi, Dr. Alan Karstaedt and Prof. Lynn Morris

**Sponsor:** National Institute for Communicable Diseases (NICD), Johannesburg, President Emergency Plan for AIDS Relief (PEPFAR), USA, Swedish international development agency (Sida) and the Karolinska Institutet Faculty funds, Stockholm.

**INTRODUCTION:** Good day, my name is ________________________________ I am a research assistant at the National Institute for Communicable Diseases (NICD)-Sandringham, Johannesburg.

I would like to invite you to consider participating in a study entitled South African Virologic Evaluation (SAVE)

We are asking you to volunteer to provide a 10 ml blood sample (approximately 2 teaspoons) for HIV drug resistance research, which will be taken at the same time as your routine checks. The HIV in this specimen will be analyzed in the laboratory and the information will not be passed on to your Doctor. You will also be asked a number of questions by an interviewer relating to your geographical, adherence to treatment and household conditions. In addition we would like to review your medical records since your start of ARV treatment.

The study is for patients who have been on HIV treatment for more than 12 months.

The study is sponsored by the National Institute for Communicable Diseases (NICD), Johannesburg, President Emergency Plan for AIDS Relief (PEPFAR), USA, the Swedish international development agency (Sida), Sweden and the Karolinska Institutet Faculty funds.

**YOUR PARTICIPATION IS VOLUNTARY:** This consent form gives information about the study. We will discuss the process with you. Once you understand the study and if you agree to take part, we will ask you to sign your name or make your mark on this form. We will offer you a copy to keep.

Before you learn about the study process, it is important that you know the following:

- You do not have to be in this study if you do not want to.
- You may decide not to have the screening tests, or to stop the screening tests at any time, without losing your regular medical care.
- If you decide not to be enrolled in this study, you can still join other research studies later, if available and you qualify.
- You will receive the results by publishing it in a poster in the clinic.

**PROCEDURES:** If you agree to participate, we will ask you to give 10 ml of blood (approximately 2 teaspoons) and to answer questions in relation to your adherence to HIV drugs and to if you had problems to come to the clinic.

If you are ready to give blood, the study staff will collect blood either from your arm or your finger with a sterile instrument (needle). They send your blood to be stored in safe place. It will take some months before your blood will be analysed to see if the HIV virus has changed its structure or not. The results can not be told to you or to your physician immediately because we have to wait until the end of the study.

**RISKS AND/OR DISCOMFORTS:** You may feel minimal discomfort or pain when your blood is drawn. You may feel dizzy or faint. You may have a bruise where the sterile instrument (needle) goes into your arm.

**BENEFITS:** You may get no direct benefit from the study. You will not receive anti-HIV drugs as part of this research project. However, by taking part of this study you are contributing to improve the HIV service in South Africa.

**REASONS WHY YOU MAY BE WITHDRAWN FROM THE STUDY:** You may be removed from the screening tests without your consent for the following reasons:

- The research study is stopped or canceled.
- You asked to be withdrawn

**COSTS TO YOU:** There is no cost to you for the blood sample genotyping tests.

**REIMBURSEMENT:** You will be reimbursed for amount of 50ZAR for transport costs.

**CONFIDENTIALITY:** Efforts will be made to keep your personal information confidential. However absolute confidentiality cannot be guaranteed. Your personal information may be disclosed if required by law. Any sample from you or information about you will be identified by code. The link between your name and code will be kept in a secure location at the clinic only. Any publication of this study will not use your name or identify you personally.

The records of your screening tests may be reviewed by study staff and representatives of:

- Sponsor
- Health Research Ethics Committee, University of the Witwatersrand

**RESEARCH-RELATED INJURY:** It is unlikely that you will be injured as a result of giving small blood sample (equivalent of 2 teaspoons). If you are injured as a result of giving small blood sample, the study staff will give you immediate necessary treatment for your injuries, free of charge. The study staff also will tell you where you can get additional treatment for your injuries, if needed. There is no program for monetary compensation or other forms of compensation for such injuries. You do not give up any legal rights by signing this consent form.

**PROBLEMS OR QUESTIONS:** If you ever have any questions about the screening tests, or if you have a research-related injury, you should contact Mr. Ziad El-Khatib at 072-523 97 16. If you have questions about your rights as a research participant, you should contact Professor Cleaton-Jones, Chairperson of the University of the Witwatersrand Human Research Ethics Committee (HREC), which is an independent committee, established to help protect the rights of research participants, at (011) 717 2229.