RESEARCH PROTOCOL

REVAMP:
Resistance Testing Versus Adherence Support for Management of Patients with Virologic Failure on First-Line Antiretroviral Therapy in sub-Saharan Africa

An open-label randomized controlled trial

Harvard Medical School
Massachusetts General Hospital
Mbarara University of Science and Technology
University of KwaZulu-Natal
Emory University
University of Rochester
Africa Health Research Institute
University of California San Francisco

TABLE OF CONTENTS

ABBREVIATIONS

ART	Antiretroviral therapy
DALY	Disability adjusted life year
DBS	Dried blood spot
EDTA	Ethylenediaminetetraacetic acid
B-HCG	β-human chorionic gonadotropin
II	Integrase inhibitor
ISPOR	International Society of Pharmacoeconomics Outcomes Research
ISS	Immune suppression syndrome
JCRC	Joint Clinical Research Centre
ml	Milliliter
MRRH	Mbarara Regional Referral Hospital
NHLS	National Health Laboratory Services
NNRTI	Non-nucleoside reverse transcriptase inhibitor
PEPFAR	President's Emergency Plan for AIDS Relief
PI	Protease inhibitor
SOC	Standard of care
RT	Resistance testing
UKZN	University of KwaZulu-Natal
UNAIDS	Joint United Nations Programme on HIV/AIDS
VF	Virologic failure
WHO	World Health Organization

I. INVESTIGATORS AND INSTITUTIONS

A. Massachusetts General Hospital / Harvard Medical School

Mark J. Siedner, MD MPH: Co-Principal Investigator
Tel: +1-617-726-4686; Email: msiedner@mgh.harvard.edu
Rajesh Gandhi, MD: Co-Investigator, Protocol HIV Clinician
Email: rgandhi@mgh.harvard.edu
Kevin Ard, MD MPH: Co-Investigator, Protocol HIV Clinician
Email: kard@partners.org
Suzanne McCluskey, MD: Co-Investigator
Email: smccluskey@mgh.harvard.edu
Jonathan Li, MD PhD: Co-Investigator, Sub-Study Virologist
Email: jli@bwh.harvard.edu
Bethany Hedt-Gauthier: Co-Investigator, Sub-Study Biostatistician
Email: Bethany_Gauthier@hms.harvard.edu

B. Mbarara University of Science and Technology

Winnie Muyindike, MBChB MMed: Co-Principal Investigator
Tel: +256-772-521-619; Email: wmuyindike@gmail.com

Mwebesa Bosco Bwana, MBChB MPH: Co-Investigator (posthumous)

C. University of KwaZulu-Natal

Yunus Moosa, MBChb, PhD: Site Principal Investigator
Tel: +27-31-260-4368; Email: moosay@ukzn.ac.za
Pravikrishnen Moodley, MBChB: Co-Investigator, Protocol Clinical Virologist
Email: moodleyp36@ukzn.ac.za
Jaysingh Brijkumar, MBBS: Co-Investigator, Protocol South African HIV Clinician
Email: jaysingh.brijkumar@kznhealth.gov.za
Gavin George, PhD: Co-Investigator, Protocol Economist
Email: georgeg@ukzn.ac.za

D. Emory University

Vincent Marconi, MD: Co-Investigator, Protocol HIV Clinician and Epidemiologist
Email: vcmarco@emory.edu

E. University of Rochester

Brent Johnson, PhD: Co-Investigator, Protocol Statistician
Email: brent_johnson@urmc.rochester.edu

F. Griffith University

Tamlyn Rautenberg, PhD: Co-Investigator, Health Economics

Email: t.rautenberg@griffith.edu.au

G. Africa Health Research Institute

John Adamson, PhD: Co-Investigator, Therapeutic Drug Monitoring
Email: john.adamson@ahri.org

H. University of California San Francisco

Monica Gandhi, MD MPH: Co-Investigator, Therapeutic Drug Monitoring
Email: Monica.Gandhi@ucsf.edu

II. PROJECT SUMMARY AND SPECIFIC AIMS

In an effort to optimize the global HIV care continuum and foster sustained control of the HIV epidemic, the Joint United Nations Programme on HIV/AIDS (UNAIDS) has set a "90-90-90" treatment target for 2020. Achievement of this goal would mean, in part, that 90% of those receiving antiretroviral therapy (ART) sustain an undetectable HIV viral load. In sub-Saharan Africa, however, rates of sustained virologic suppression remain well below that goal, with 1 in 3 developing virologic failure during the first two years of therapy. Patients with virologic failure have higher rates of poor clinical outcomes, increased diagnostic and therapeutic costs, and could potentially thwart treatment as prevention strategies. Management of virologic failure must be optimized to attain the goal of maintaining 90% on the continuum of care. Current WHO guidelines recommend use of viral load testing alone to guide management. This strategy possibly promotes unnecessary switching of patients with wild-type virus to second-line therapy, while continuing other patients on first-line therapy despite the presence of unidentified drug resistance. Yet, whether addition of resistance testing to routine management of virologic failure improves outcomes or is cost-effective is unknown. As such, a data-driven approach to management of virologic failure, incorporating both efficacy and cost, is needed to address this critical gap in knowledge.

The goal of this study is to determine whether addition of routine resistance testing, to guide management of virologic failure and sustain the successful completion of the HIV continuum of care, improves clinical outcomes and reduces costs for patients with virologic failure on first-line therapy in sub-Saharan Africa. We will enroll patients in care at public HIV clinics in Uganda and South Africa who have virologic failure on a first-line ART regimen, into a randomized controlled trial to complete the following aims:

1) **Aim 1: Estimate the effectiveness of resistance test-based management of virologic failure (resistance testing arm), versus viral-load based management per WHO guidelines (standard of care arm)**. We hypothesize that resistance testing-based management will be superior to standard of care management as measured by the proportion of patients with undetectable viral load 9 months after detection of virologic failure.

2) **Aim 2: Evaluate the cost effectiveness of resistance-test versus viral load-based management of virologic failure in sub-Saharan Africa**. We hypothesize that resistance testing will be cost effective, because the cost of the assay will be offset by downstream cost savings through reduced use of second line therapy.

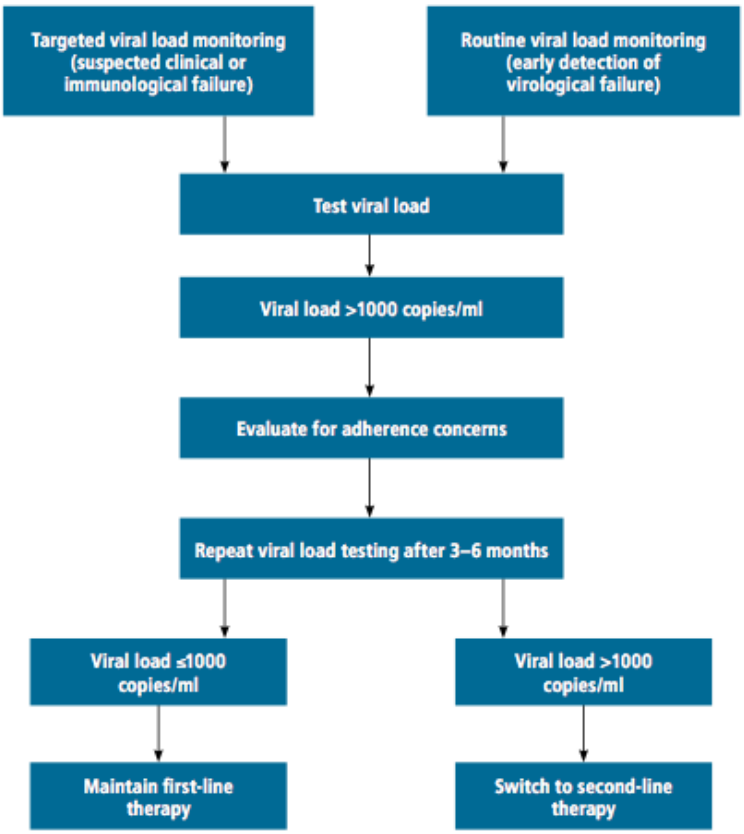
We intend that results of this study will be a useful resource for public health programmers, ministries of health and multinational partners to optimize the clinical management of virologic failure in the region.

III. BACKGROUND AND SIGNIFICANCE

In an effort to optimize the global HIV care continuum and foster sustained control of the HIV epidemic, the Joint United Nations Programme on HIV/AIDS (UNAIDS) has set a "90-90-90" treatment target for 2020 [2]. Achievement of this goal would mean, in part, that 90% of those receiving antiretroviral therapy (ART) sustain an undetectable HIV RNA viral load (viral load). At the epicenter of the epidemic in sub-Saharan Africa, however, rates of virologic suppression remain well below that goal; indeed, a systematic review of virologic suppression in the sub-Saharan African region found that only 2 in 3 patients maintain virologic suppression over two years of observation [3]. To maintain the goal of achieving 90% sustained virologic suppression, management of virologic failure must be optimized.

Yet, the optimal strategy for management of virologic failure in sub-Saharan Africa, in terms of both efficacy and cost, is unknown. The 2013 World Health Organization (WHO) HIV treatment guidelines recommend monitoring patients in resource-limited settings with viral load measurements at 6 months after ART initiation and annually thereafter [1]. Virologic failure is defined as two consecutive viral load measurements greater than 1,000 copies/mL, three-six months apart, with adherence reinforcement in the interim (**Figure 1**). The WHO guidelines stipulate that patients meeting this definition of virologic failure should switch to second-line ART. These recommendations are likely to have important ramifications on management of virologic failure in sub-Saharan Africa as viral load testing becomes more widespread through cost reductions [4]. Yet, while viral load testing represents an important advance for HIV care in the region [5], it also raises critical questions about the optimal management of patients who meet the WHO definition of treatment failure. In particular, the use of viral load measurements alone to define treatment failure may result in unnecessary switches to costlier second-line therapy, potentially imperiling the financial sustainability of HIV treatment programs.

Figure 1. World Health Organization schema for monitoring and management of treatment failure [1]



Rigorous data are lacking to support key aspects of WHO guidelines. While some studies have found high rates of resistance among those with a viral load >1,000 copies/mL on first-line therapy [6, 7], others have demonstrated high rates of re-suppression without regimen switches [2, 8, 9]. Although the recommendation to conduct adherence counseling and repeat viral load testing prior to switching therapy is meant to increase the specificity for detection of resistance, this strategy is also unproven. For example, a recent study in Swaziland demonstrated no additional benefit to adherence counseling on re-suppression of viremia for those with treatment failure [10]. Finally, the requirement for two consecutive viral load tests, and the additional clinical encounters required to report results and conduct clinical decision making, may pose

substantial financial and logistical challenges for patients [11-14].
Consequently, the current guidelines may introduce substantial delays between detection of virologic failure and regimen change, place vulnerable patients at risk for loss to follow-up, and conceivably increase the risk of HIV transmission and transmitted drug resistance. An alternate strategy would be to perform drug resistance testing as soon as possible after the initial detectable viral load to more precisely and promptly guide ART treatment decisions. A group of clinical trials conducted soon after ART became standard of care in the United States found that resistance testing improves virologic control and/or CD4 count response [15-18]. Notably, these studies were conducted solely in resource-rich settings and did not consider costs in their assessments. Similar data for the sub-Saharan Africa region are unavailable.

Without resistance testing available, the current guidelines have the potential to result in switching most patients with virologic failure to second-line therapy and could confer both patient- and population-level risks. For example, most of the region lacks a third-line option for patients who are unable to tolerate PI-based regimens or are affected by drug-drug interactions with rifampin or other medications. Additionally, patients on PI-based regimens require additional clinical monitoring due to the heightened risk of metabolic complications [19]. Most important, the financial sustainability of switching large populations of patients to second-line therapy is uncertain. If one in three patients initiating ART continues to suffer virologic failure within two years [3], treatment programs in the region would be charged with supplying second line regimens to over 5 million individuals.

As the President's Emergency Plan for AIDS Relief (PEPFAR) embarks on its third phase of implementation with a focus on sustainability, it is critical to develop a data-driven approach to management of virologic failure in sub-Saharan Africa. Although prices for ART have decreased with the availability of generic, fixed-dose combination regimens, PI-based second line therapy remains approximately 2-3 times as costly as first-line regimens in resource-limited settings (mean regimen costs $816 versus $305/year [20]). One report estimated that switching to second-line therapy in Africa was unnecessary in approximately one third of cases (i.e., done in the presence of wild-type virus) [21]. While preventing these unnecessary regimen switches by routine resistance testing could save funds, this strategy would need to be weighed against the cost of resistance testing.

Studies assessing cost-effectiveness of resistance testing in resource-limited settings have demonstrated contrasting results, partially due to differing assumptions about its efficacy (**Table 1**) For example, one study demonstrated that the addition of resistance testing was highly cost-effective when wild-type virus at failure was relatively common (>12%), and in fact became cost saving when the resistance testing was less than $100 per test [22]. A second study demonstrated a cost-neutral effect for the addition of resistance testing, assuming a cost of $242 per test, but with resulting clinical benefit to patients [23]. In contrast, a third study found no additional benefit to resistance testing over viral load testing alone, even with resistance testing costs as little as $30 per test [24]. The variation in results supports the need for a well-designed clinical trial with primary data to enable modeling of the economic impact of resistance testing in sub-Saharan Africa.

In summary, the increased availability of viral load testing will likely result in sizeable increases in identification of virologic failure in sub-Saharan Africa. Without resistance testing, many patients with treatment failure, the optimal management of virologic failure remains unknown. The current guidelines raise the possibility of frequent switching of patients to second-line therapy with unclear – and potentially deleterious – consequences for patients and health systems.

As PEPFAR shifts its focus to sustainable control of the HIV epidemic, it is crucial to determine the efficacy and costs of the current standard of care compared to a resistance-based testing approach for management of treatment failure. We designed the current study with the goal of identifying the optimal clinical management and allocation of resources for patients with virologic failure to help guide ministries of health and multinational partners in the region.

Table 1: Economic Studies Evaluating Resistance Testing in sub-Saharan Africa

Analysis Model	Population	Perspective	Time Horizon	Outcome	Sensitivity Analysis	Primary Result
Cost-Effectiveness of Preventing AIDS Complications state-transition model [22]	South Africa	Modified societal	Lifetime	Cost/year of life saved	Univariate and multiway	**Very cost effective**
Cost minimization model [23]	South Africa	Presumed Payer	5 years	Cost per strategy	Deterministic/ Probabilistic	**Cost Neutral**
HIV synthesis transmission individual-based stochastic model [24]	Zimbabwe	Unstated	10 years	Cost/ disability adjusted life year (DALY) averted	Several one way sensitivity analyses	**Not cost-effective**

IV. STUDY DESIGN AND SUBJECT SELECTION

A. Overview

The study design is an open-label, randomized controlled trial. The study will be conducted at study sites in Uganda and South Africa. The study population will include HIV-infected patients on first-line antiretroviral therapy with a recent viral load >1,000 copies/milliliter (mL). Eligible participants will be randomized to the WHO-based standard of care for management of virologic failure or immediate resistance testing with use of results to guide ART regimen decisions. The study will add the use of a diagnostic test, HIV-1 RNA resistance testing to clinical management of participants in the resistance testing arm, but all other clinical care including provision of ART therapy will be done by clinic sites in keeping with local HIV guidelines. The primary outcome of interest will be viral suppression (<200 copies/mL) at 9 months after study enrollment, and will be assessed using an intention to treat analysis, where missing or absent results will be considered failures. Secondary outcomes of interest will be viral suppression below the limit of assay detection, viral suppression on continuation of first-line (non-nucleoside reverse transcriptase inhibitor [NNRTI]-based) therapy, drug resistance at study conclusion, and mortality, among others. We will analyze primary data collected during the trial to conduct an economic analysis with the goal of estimating the cost-effectiveness of resistance testing versus standard or care.

B. Study Sites

1. Uganda

The Immune Suppression Syndrome (ISS) clinic at the Mbarara Regional Referral Hospital (MRRH) and its partner clinic across the street, the **Mbarara Municipal Clinic**, are PEPFAR-supported clinics, which, in combination, follow more than 20,000 patients with HIV/AIDS and have an established clinical research infrastructure. The clinics employee over 50 clinical care staff including nurses, clinicians, pharmacists, counselors, phlebotomists, patient trackers, and administrative staff. The clinics serve as the primary research sites for the MGH-MUST Global Health Collaborative, which employs an additional 60 research staff with expertise in recruitment, enrollment, participant monitoring, informed consent, data collection, phlebotomy, and laboratory storage and testing.

2. South Africa

In Durban, participants will be recruited from the **Addington Hospital Sinathando HIV Clinic, Clairwood Hospital HIV Clinic, Wentworth Clinic, and King Dinizulu Hospital.** The four clinics together manage an active caseload of over 10,000 HIV patients. The study will be managed centrally from the University of KwaZulu-Natal (UKZN), which operates numerous HIV clinical research projects and employs research staff with experience in recruitment, enrollment, participant monitoring, informed consent, data collection, phlebotomy, and laboratory processing, storage, and testing.

C. Eligibility Criteria

We will recruit persons with HIV-infection who are in care at any of the five above clinics who meet the following inclusion criteria:

1. Inclusion criteria:

a. In care at a public HIV clinic within a PEPFAR-focus sub-Saharan African country (South Africa or Uganda) and living within 100 kilometers of the clinic
b. Age ≥ 18 years at the time of enrollment
c. Currently prescribed first-line (non-nucleoside reverse transcriptase inhibitor [NNRTI]-based) ART for at least 5 months. Switches within first line regimens, including NNRTI and nucleos(t)ide backbone changes are allowed.
d. Detectable plasma viral load > 1,000 copies/mL and/or dried blood spot viral load > 1,000 copies/mL within 90 days of enrollment

2. Exclusion criteria:

a. Known prior drug resistance
b. Prior exposure to PI-based ART
c. Current clinical indication to start PI-based ART
d. Not planning to remain in the clinic catchment area for the next nine months

3. Eligibility criteria rationale

The study aims to enroll HIV-infected adults on first-line ART with a newly identified instance of a viral load >1,000 copies/mL. Patients attending study clinics implementing dried blood spots (DBS) for

virologic testing will be eligible, provided they have a DBS viral load >1,000 copies/mL [25, 26], or if they undergo repeat plasma viral load testing to confirm a viral load >1,000 copies/mL. We aim to exclude those with known prior resistance, those on second line therapy, and those at high risk for default from study because of immigration out of the clinic catchment area.

D. Recruitment and Enrollment

We will conduct both passive and active enrollment. For active enrollment, study staff will work in partnership with clinic staff to identify potential participants through review of clinic viral load result registers for detectable viral loads. In accordance with guidelines in both Uganda and South Africa, patients with detectable viral loads will be contacted to return to clinic for immediate care. Potential participants will be pre-screened for eligibility criteria and referred to study research assistants who will use the study screening form to assess potentially eligible patients for the remainder of eligibility criteria. For passive recruitment, study clinicians will contact study staff directly for any patient who them deem might meet study criteria.

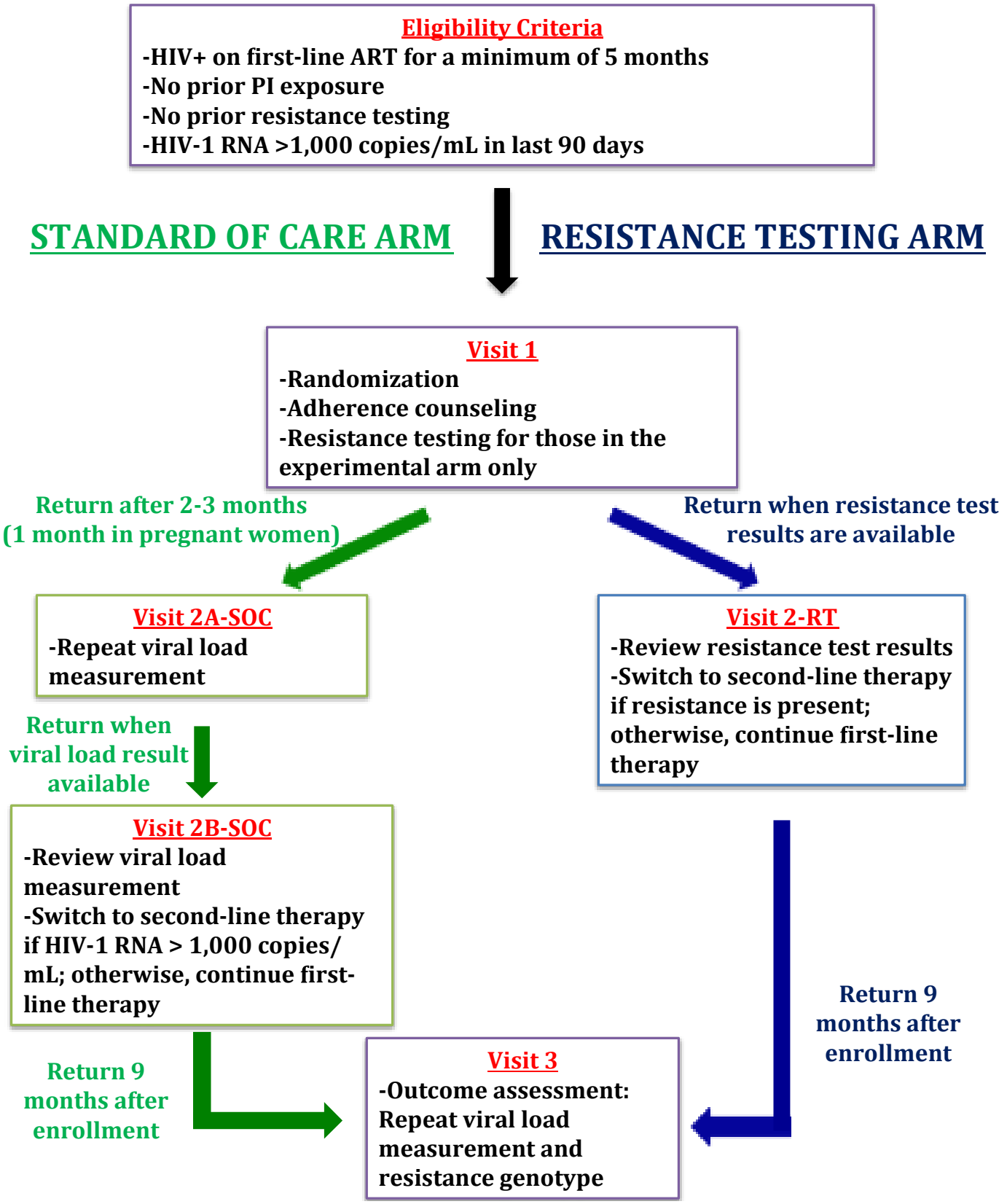
E. Informed Consent

If eligible, potential participants will be taken to a research office to complete the informed consent process. Informed consent forms have been translated into local languages, and will be conducted in English, Runyankole, or IsiZulu, depending on the preference of the participant, by a research assistant trained in ethical conduct of human research. There will be two separate informed consent forms: one for study procedures, and a second for permission to store laboratory specimens. During the consenting process, the study purpose, a protocol summary, and risks and benefits of study activities will be explained in detail. It will be clearly explained that choosing not to enroll will have no effect on clinical care. After completion of the consent forms, the potential participant will be given time to ask questions and consider their interest in joining before signing. Those who decline participation will be referred back to the clinic and continue with standard clinic practice, but will be eligible to enroll in the study at a later time if they remain eligible.

F. Enrollment of Pregnant Women

Pregnant women in case at HIV clinics that also offer antenatal HIV services will be eligible for enrollment in this study. Their inclusion will be critical to ensure that study results are generalizable to this population, who stand to gain as much if not more from studies intended to elucidate optimal strategies of HIV care. To ensure safeguarding of pregnant women and their fetuses, procedures will be slightly altered for this population. First, to confirm pregnancy status, women under 50 years of age will undergo urine β-human chorionic gonadotropin (HCG) testing at the enrollment visit. Those who test positive will be referred to antenatal care in addition to the remainder of study procedures. Additionally, pregnant women in the standard of care arm will have a slightly altered visit schedule, such that they will return in 1 month after study enrollment for a repeat viral load test (as opposed to 2-3 months). This recommendation is in keeping with South African Prevention of Mother-to-Child Transmission Guidelines [27], and is intended to maximize chances of viral re-suppression prior to delivery.

Figure 2. Study Schema



G. Randomization

Consenting participants will be randomized by the REDCap randomization module to the standard of care (SOC) or the resistance-testing based (RT) arm (Figure 2). In brief, after screening has been completed, eligibility confirmed, and informed consent forms signed, a study participant is given a unique consecutive identification number in REDCap. The randomization field is selected, and the research assistant is informed which arm the participant has been selected for by the application. Randomization will be in blocks of 4 and stratified by study site, pregnancy, and duration of time since ART initiation (less than versus greater than one year). Randomization will be stratified by ART duration because of the potential heterogeneity in resistance rates between those with early treatment failure from primary drug resistance versus late failure due to acquired drug resistance. Strategy allocation will be unblinded to participants or clinic staff.

V. STUDY PROCEDURES FOR PARTICIPANTS RANDOMIZED TO STANDARD OF CARE ARM

A. Visit 1-SOC: Baseline Visit for Standard of Care Participants

At Study Visit 1, participants randomized to the SOC will complete the baseline questionnaire to collect sociodemographic, HIV clinical and treatment history, self-reported ART medication adherence [28], quality of life [29-31], and resource allocation data. Study staff will review participant records to collect data on clinic initiation start date, opportunistic infection history, ART initiation date, ART regimen history, CD4 count and viral load result histories. A single 10cc blood specimen will be drawn for storage for future testing for viral load, resistance testing, and drug therapeutic monitoring (See Table 3 – Page 18). A voided urine specimen will also be collected for future testing for therapeutic drug monitoring. Participants will be referred to clinic counselors who will conduct adherence support counseling as per standard clinical procedures. A follow-up study visit will be scheduled 2-3 months from the baseline date (depending on the local clinic policy), with the exception of pregnant women, who will be asked to return in approximately 1 month. Any interim clinical visits that are indicated by the clinic staff will be maintained. The participant will be instructed to continue their current ART regimen until at least the next clinical visit.

B. Visit 2A-SOC: Repeat Viral Load Testing Visit

At Study Visit 2A, participants in the SOC arm will undergo blood collection for viral load testing in keeping with WHO guidelines. An additional tube 10cc tube will be drawn for storage for future testing for viral load, resistance testing, and drug therapeutic monitoring. A voided urine specimen will also be collected for future testing for therapeutic drug monitoring A study questionnaire will be administered to assess self-reported ART medication adherence. No other procedures are scheduled at this visit. Participants will be notified that study staff will contact them as soon as their results are available, to request return to clinic for further management. The participant will be instructed to continue their current ART regimen until at least the next clinical visit. As soon as the viral load result is available, study participants will be contacted by phone and requested to return to clinic for review. If the viral load is indeterminate or not completed for any reason, study staff will request that the participant return for a repeat viral load test.

Table 2. Summary of study procedures

Study Arm	Visit	Blood Collection	Study Forms	Other Procedures/Forms
Standard of Care Arm	Visit 1-SOC	10 cc EDTA	-Baseline questionnaire -Tracking form -Adherence questionnaire -Quality of life (EQ5D) -Resource use questionnaire -Staff resource use -ART and laboratory history	-Adherence counseling -Collection of a voided urine specimen
	Visit 2A-SOC	4 cc EDTA + 10 cc EDTA	-Staff resource use -Adherence questionnaire	-Viral load test -Collection of a voided urine specimen
	Visit 2B-SOC	None	-Staff resource use	-Review viral load results -Adherence counseling
	Visit 3	4cc EDTA + 10cc EDTA	-Adherence questionnaire -Quality of life (EQ5D) -Resource use follow-up -Staff resource use -Interim ART and laboratory history	-Viral load test, with reflex resistance testing if detectable -Collection of a voided urine specimen
Resistance Testing Arm	Visit 1-RT	4cc EDTA + 10cc EDTA	-Baseline questionnaire -Tracking form -Adherence questionnaire -Quality of life (EQ5D) -Resource use questionnaire -Staff resource use -ART and laboratory history	-Adherence counseling -Resistance test -Collection of a voided urine specimen
	Visit 2-RT	None	-Staff resource use	-Review resistance test results -Adherence counseling
	Visit 3	4cc EDTA + 10cc EDTA	-Adherence questionnaire -Quality of life (EQ5D) -Resource use follow-up -Staff resource use -Interim ART and laboratory history	-Viral load test, with reflex resistance testing if detectable -Collection of a voided urine specimen

C. Visit 2B-SOC: Viral Load Testing Results and Therapeutic Management

At Study Visit 2B, study clinicians will review the viral load result. Participants with a viral load ≤ 1,000 copies/mL will continue their first-line (NNRTI-based) ART regimen without change. Participants with a viral load >1,000 copies/mL will change ART regimen to a second-line, protease inhibitor (PI)-based or, if available, integrase inhibitor (II)-based therapy. Clinicians will also be encouraged to change the nucleos(t)ide reverse transcriptase component of the regimen (for example, changing from zidovudine to tenofovir), based on prior exposures, as well as WHO and national guidelines. All regimen decisions will be made by the study clinician, in cooperation with clinic staff at the study sites. In the case of complex

management issues, the site principal investigators (Dr. Winnie Muyindike in Mbarara and Dr. Yunus Moosa in Durban) will be contacted to offer input. At the conclusion of Visit 2B-SOC, participants in the SOC arm will be scheduled for a final study visit approximately 9 months after enrollment. A final visit at this time is chosen to allow ample time for drug suppression for participants with detectable viral load, and to ensure balance in observation time between study arms. Non-study clinical visits for routine clinical care and adherence counseling recommendations will continue in the interim as determined and scheduled by clinic staff.

D. Visit 3: Outcome Assessment

At Study Visit 3, participants will undergo repeat blood testing for plasma viral load and, if the viral load is detectable, reflex resistance testing will be performed. An additional 10cc tube will be drawn for storage for future testing for viral load, resistance testing, and drug therapeutic monitoring. A voided urine specimen will also be collected for future testing for therapeutic drug monitoring. A study questionnaire will be administered to assess resource allocation, ART medication adherence, and quality of life. Study staff will review participant records to update interim CD4 count, viral load, and ART regimen data. Results of viral load and resistance testing from this visit will be immediately made available to clinic staff for further patient management. At the conclusion of Visit 3, study procedures will be complete.

E. Missing and Late Appointments

If study participants do not present for study visits, study staff will call them to encourage return to clinic for continuation or completion of procedures. For participants who do not return within 7 days of a scheduled visit and unreachable by phone, a study staff member will attempt to track them at home using a standardized lost-to-follow-up form and procedures developed and used successfully both for program and clinical care in Mbarara for over 10 years [32, 33]. If participants are located, study staff will encourage them to return to clinic to complete procedures and/or conduct the blood draw and questionnaire in in the field if the participant agrees.

During the COVID-19 pandemic, participants who do not present for regularly-scheduled HIV clinic appointments will be phoned when contact information is available. Rather than tracking at home for participants that cannot be reached, tracking will be delayed until the COVID-19 pandemic subsides and regularly scheduled study procedures can resume.

VI. STUDY PROCEDURES FOR PARTICIPANTS RANDOMIZED TO RESISTANCE TESTING ARM

A. Visit 1-RT: Baseline Visit for Resistance Testing Participants

At Study Visit 1, participants randomized to the RT will complete the baseline questionnaire to collect sociodemographic, HIV clinical and treatment history, self-reported ART medication adherence, quality of life, and resource allocation data. Study staff will review participant records to collect data on clinic initiation start date, opportunistic infection history, ART initiation date, ART regimen history, CD4 count and viral load result histories. Upon completion of the baseline questionnaire, participants will undergo phlebotomy for resistance testing. Participants will be notified that study staff will contact them as soon as their results are available, to request return to clinic for further management. A voided urine specimen will also be collected for future testing for therapeutic drug monitoring. Upon completion of the study procedures, participants will be referred to clinic counselors who will conduct adherence support

counseling as per standard clinical procedures. The participant will be instructed to continue their current ART regimen until at least the next clinical visit. When the resistance test result is available, study participants will be contacted by phone and requested to return to clinic for review.

B. Visit 2-RT: Resistance Testing Results and Therapeutic Management

At Study Visit 2-RT, study clinicians will review the resistance testing result. A study HIV-1 RNA drug resistance interpretation guide will be used to help guide decision-making. Participants without significant drug resistance, as determined by the study clinician in consultation with the resistance interpretation guide and, if needed, study investigators will continue their first-line (NNRTI-based) ART regimen without change. Participants will be referred to clinic counselors for adherence support. Participants with meaningful therapeutic drug resistance will change ART regimen to a second-line, protease inhibitor (PI)-based or, if available, integrase inhibitor (II)-based therapy. Clinicians will also be encouraged to change the nucleos(t)ide reverse transcriptase component of the regimen (for example, changing from zidovudine to tenofovir). All regimen decisions will be made by the study clinician, in cooperation with clinic staff at the study sites. In the case of complex management issues, the site principal investigators (Dr. Winnie Muyindike in Mbarara and Dr. Yunus Moosa in Durban) will be contacted to offer input. At the conclusion of Visit 2-RT, participants will be scheduled for a final study visit approximately 9 months later. A final visit 9 months later is chosen to match the approximate 9-month study duration for participants in the SOC arm. Non-study clinical visits for routine clinical care will continue in the interim as determined and scheduled by clinic staff.

C. Visit 3: Outcome Assessment

At Study Visit 3, participants will undergo repeat blood testing for plasma viral load and, if the viral load is detectable, reflex resistance testing will be performed. An additional 10cc tube will be drawn for storage for future testing for viral load, resistance testing, and drug therapeutic monitoring. A voided urine specimen will also be collected for future testing for therapeutic drug monitoring. A study questionnaire will be administered to assess resource allocation and quality of life. Study staff will review participant records to update interim CD4 count, viral load, and ART regimen data. Results of viral load and resistance testing from this visit will be immediately made available to clinic staff for further patient management. At the conclusion of Visit 3, study procedures will be complete.

For participants whose visits are affected by the COVID-19 pandemic, study staff will administer study questionnaires via phone call where participant phone contact information is available, rather than in-person study visits. In these cases, instead of collecting study specimens (urine and blood) during the study visit, alternative arrangements will be made that do not put study staff at risk. REVAMP study staff will work with HIV clinic staff who are already in the clinic to collect these specimens during the participants' regularly scheduled HIV clinic visits if they occur during the Visit 3 follow-up window. No participants will be asked to come to the study site on days that they are not already scheduled to come to the clinic for routine appointments. For participants who cannot be reached by phone and/or if they do not attend clinic during the period of the COVID-19 pandemic, their study visits may fall outside of the Visit 3 timeline, and a study closure visit will be conducted as soon as possible once the COVID-19 pandemic subsides.

D. Missing and Late Appointments

If study participants do not present for study visits, study staff will call them to encourage return to clinic for continuation or completion of procedures. For participants who do not return within 7 days of a scheduled visit and unreachable by phone, a study staff member will attempt to track them at home using a standardized lost-to-follow-up form and procedures developed and used successfully both for program and clinical care in Mbarara for over 10 years [32, 33]. If participants are located, study staff will encourage them to return to clinic to complete procedures and/or conduct the blood draw and questionnaire in in the field if the participant agrees.

During the COVID-19 pandemic, participants who do not present for regularly-scheduled HIV clinic appointments will be phoned when contact information is available. Rather than tracking at home for participants that cannot be reached, tracking will be delayed until the COVID-19 pandemic subsides and regularly scheduled study procedures can resume.

VII. COVID Study Amendment

We made the following provisions to account for the COVID-19 epidemic period. Study enrollment is complete and no new participants will be enrolled. Additionally, all study-specific visits have been cancelled in both Uganda and South Africa during this period, until such time that local regulations and authorities recommend for the resumption of non-essential activities. In the interim we will take the following steps in each country :

1. In Uganda, REVAMP study staff will work with HIV clinic staff who are already in the clinic to collect these specimens during the participants' regularly scheduled HIV clinic visits if they occur during the Visit 3 follow-up window. If participants do present to clinic for routine services, study staff will call them to administer study questionnaires via phone call where participant phone contact information is available, rather than in-person study visits.

2. In South Africa, we have discussed study procedures with both clinic leadership and the local IRB. Both recommended continuing of procedures under the following conditions:

a. Participants will be seen when attending for routine clinical care and or coming in for treatment collection and will not be asked to attend for study purposes.
b. The study is reviewing participants that are potentially failing treatment and require a viral load test. The study will provide both viral load testing and resistance testing to those who have a detectable viral load per study protocol, which will advise further management of the participants.
c. On presentation at the clinic patients will undergo routine screening for COVID 19 by clinic staff. Participants who screen positive will not be seen by research staff. Those that screen negative will have blood drawn by study nurses.
d. Study funding will provide private transport to and from clinics for all study staff. No public transport will be used
e. Study nurses are expected not to spend more than 10 to 15 minutes with participants during the study visit and will remain 2 metres away for all procedures aside from blood collection
f. All study nurses have been trained on proper use of personal protective equipment and supplied with masks, gloves, face shields, and hand sanitizer which they will strictly adhere to for all participant encounters

3. In both countries, For participants who cannot be reached by phone and/or if they do not attend clinic during the period of the COVID-19 pandemic, their study visits may fall outside of the Visit 3 timeline, and a study closure visit will be conducted as soon as possible once the COVID-19 pandemic subsides.

VIII. DATA COLLECTION AND MANAGEMENT

A. Sources of Data

1. Screening Form. Screening forms will be completed by research assistants prior to enrollment in order to confirm study eligibility.

2. Study questionnaires administered directly to study participants

a. Sociodemographics Questionnaire at enrollment visit to collect basic age, sex, and socioeconomic data.
b. EQ5D Quality of Life Questionnaire. EQ5D is a validated quality of life survey, that has been used both in populations with HIV infection and in sub-Saharan Africa [29-31]. This questionnaire will be administered at enrollment (Visit 1-SOC and Visit 1-RT) and again at Visit 3. The questionnaire will be instrumental in estimating cost-effectiveness of the intervention.
c. Self-reported ART medication adherence. We will use a standardized ART medication adherence questionnaire, consisting of three questions about recent adherence. The questionnaire has been used and validated in sub-Saharan Africa, most notably in the Partners PREP study [28].
d. Tracking form. A form will be completed that records participants name, clinic ID number, phone number(s), treatment supporter, and address information. This form along with the signed consent form will be retained in a locked file cabinet, but data from this form will not be entered into the study database, in an effort to optimize data confidentiality. The form will be used to locate missing study participants who do not present for follow-up study visits.

3. Resource Allocation Questionnaire collected from study clinician and study participant. At Visits 1 and 3, research assistants will complete data on resource allocation from the participant and study staff. This will include costs of medical care and clinic resources. This data will be used for the Budget Impact and Cost-Effectiveness Analyses.

4. Clinical records. Study staff will request clinical records of study participants. This will include participant charts and clinical, pharmacy, and/or clinical database data.

a. HIV clinical history, including clinic enrollment date, ART start date, and ART regimen history
b. Laboratory testing history including results of CD4 count, viral load testing results, and prior resistance testing results
c. Data will be collected at study enrollment and at study conclusion to collect data both prior to and during study observation period.

5. Laboratory results. Laboratory results ordered as part of the study, including viral load and resistance testing results will be collected from partner laboratories and entered into the study database. Results of

these tests will also be provided to study participants. Because other tests of stored specimens, such as for drug therapeutic monitoring and next generation sequencing will be done at a later time, these results will not be available to participants.

B. Specimen Collection, Process, Storage, and Laboratory Testing

1. Laboratory Testing Summary

A voided urine specimen will be collected at the visit 1, visit 2A-SOC, and visit 3. Study tests will be divided into major categories:

a. All women under 50 will under go a urine β-HCG test at enrollment to determine pregnancy status and, if pregnant, determine the visit schedule for those in the SOC arm.
b. Antiretroviral therapy drug levels

Blood will be drawn by study or clinic phlebotomists at each visit. A single blood draw will occur at each visit. Study tests will be divided into three major categories:

a. Viral load tests (plasma from processed whole blood collected into EDTA tubes)
b. Resistance tests (plasma from processed whole blood collected into EDTA tubes)
c. Antiretroviral therapy drug levels (DBS from unprocessed whole blood collected into EDTA tubes)

Specimens will be divided into 1) those for immediate testing for protocol treatment decisions/outcome measurements, and 2) those to be stored for future testing. Specific processing, transport, and storage procedures will vary by country, based on logistics and laboratories partners in each country.

2. Laboratory Partners

a. Uganda: Joint Clinical Research Center. Joint Clinical Research Center (JCRC) serves as the primary HIV clinical laboratory for the country and has partnered with both the NIH and AIDS Clinical Trials Group to conduct laboratory testing for over 20 clinical studies. JCRC will conduct all viral load and resistance testing for study participants in Uganda. Partners for this work include Dr. Henry Mugerwa, Dr. Immaculate Nankya, and Dr. Abbas Lugemwa.

b. South Africa: Laboratory testing in South African will be conducted at the Department of Virology based at Inkosi Albert Luthuli Central Hospital, which operates as an academic partnership between UKZN and the National Health Laboratory Services (NHLS). The laboratory employs a staff of technicians who conduct approximately 20,000 viral load tests each month and HIV drug resistance testing for clinical care and research purposes. The primary partner for this work will be Dr. Pravi Moodley, director of the Virology Laboratory. In addition, we will partner with Africa Health Research Institute (ARHI) Pharmacology Core in Durban, South Africa for a portion of the therapeutic drug monitoring tests. Urine specimens from the study will undergo quantification of tenofovir levels by liquid chromatography with tandem mass spectrometry. The primary partner for this work is Dr. John Adamson, director of the Pharmacology core. Specimens sent will not contain any identifiers to link outside collaborators to individual subjects.

c. United States: While we will attempt to conduct all testing in country, additional testing of stored specimens for low copy viral load assays and drug levels may be conducted in the United States if availability for quality controlled laboratories are not identified. Partner laboratories in the United States are at Harvard Medical School (Dr. Athe Tsibris and Dr. Jonathan Li, low copy viral load and high sensitivity resistance testing), the University of Colorado (Dr. Peter Anderson, pharmacology), the Unviersity of California San Francisco (Dr. Monica Gandhi, urine therapeutic drug monitoring),and UrSure, Inc (Giffin Daughtridge, urine therapeutic drug monitoring).[34] Study participants will undergo a separate informed consent process to agree to storage and future testing of their specimens and Materials Transfer Agreements will be reviewed and approved before transfer of any specimens out of country.

3. Specimen Collection and Processing

Please see the phlebotomy and specimen collection standard operating procedures for full details of specimen collection and processing procedures. In brief, a single blood draw will be performed at relevant study visit to collect whole blood into EDTA tubes (depending on the visit, with a maximum of 15 cc per visit). Tubes will be labeled with pertinent study identifiers, refrigerated until transfer, and/or immediately transferred to the relevant laboratory (based on country) on the day of collection. Approximately 300uL of whole blood will first be removed from EDTA tubes to prepare 5 dried blood spots (~50uL/each) for future drug level testing. Dry blood spot cards will remain at room temperature overnight (minimum 4 hours) to allow drying, and then frozen for future testing. The remainder of the blood will be centrifuged to allow separation of plasma, which will be aliquoted into 1cc aliquots. Specimens will be divided for immediate testing or storage at -80^{o}C.

In addition, voided urine specimens will be collected at the enrollment visit, visit 2A-SOC, and visit 3. For women of under age 50, the urine specimen at the enrollment visit will be used for the urine β-HCG test. Otherwise, and for all other participants, specimen containers will be labeled with pertinent study identifiers, refrigerated until transfer, and/or immediately transferred to the relevant laboratory (based on country) on the day of collection. 1cc of the urine specimen will be aliquoted and stored at -80^{o}C for future testing. Therapeutic drug monitoring will be done in South Africa by Africa Health Research Institute and in the United States by University of California San Francisco and UrSure, Inc.

4. Phlebotomy Schedule

Table 3. Phlebotomy and Urine Collection Schedule

Study Arm	Visit	Tube and Whole Blood Volume	Specimens	Purpose
Standard of Care Arm	Visit 1-SOC	10 cc EDTA	5 X 1cc plasma*	Storage for future single copy viral load and minority resistance testing, and bulk (Sanger) resistance testing
			Dried Blood Spots^	Storage for future therapeutic drug monitoring
		Urine collection	1cc voided urine	Storage for future drug monitoring
	Visit 2A-SOC	**4 cc EDTA#**	**2 X 1cc plasma**	**Viral load testing for ART treatment allocation**
		10 cc EDTA	5 X 1cc plasma*	Storage for future single copy viral load and minority resistance testing, and bulk (Sanger) resistance testing
			Dried Blood Spots^	Storage for future therapeutic drug monitoring
		Urine collection	1cc voided urine	Storage for future drug monitoring
	Visit 2B-SOC	None	No blood draw	
	Visit 3	**4cc EDTA#**	**2 X 1cc plasma**	**Viral load and resistance testing for outcome assessment**
		10cc EDTA	5 X 1cc plasma*	Storage for future single copy viral load and minority resistance testing, and bulk (Sanger) resistance testing
			Dried Blood Spots^	Storage for future therapeutic drug monitoring
		Urine collection	1cc voided urine	Storage for future drug monitoring
Resistance Testing Arm	Visit 1-RT	**4cc EDTA#**	**2 X 1cc plasma**	**Resistance testing for ART treatment allocation**
		10cc EDTA	5 X 1cc plasma*	Storage for future single copy viral load and minority resistance testing, and bulk (Sanger) resistance testing
			Dried Blood Spots^	Storage for future therapeutic drug monitoring
		Urine collection	1cc voided urine	Storage for future drug monitoring

Visti 2-RT	None	No blood draw	
Visit 3	**4cc EDTA#**	**2 X 1cc plasma**	**Viral load and resistance testing for outcome assessment**
	10cc EDTA	5 X 1cc plasma*	Storage for future single copy viral load and minority resistance testing, and bulk (Sanger) resistance testing
		Dried Blood Spots^	Storage for future therapeutic drug monitoring
	Urine collection	1cc voided urine	Storage for future drug monitoring

#Specimens in bold-faced font will be processed and tested immediately for study purposes. Other specimens will be stored for future testing.
*10cc of whole blood will be centrifuged to prepare approximately 5cc plasma that will be stored as 5 X 1cc aliquots. 3cc of stored plasma will be tested for low copy viral load assay and the remaining 2cc will be used for low copy resistance testing assays. These tests will be done at Harvard Medical School
^Dried blood spots will be tested for antiretroviral therapy drug levels to assay for treatment adherence

C. Data Management and Storage

1. Paper Forms. There will be two paper forms used in the study: the Informed Consent Forms and the Tracking Form. Both of these two forms will be stored in locked file cabinets, in locked research offices.

2. Electronic Database. The remainder of study data will be collected and uploaded into the study REDCap Database. REDCap is a password protected, online database application that allows for storage of study data on encrypted servers in the United States. The application includes features for field limits, data quality control, study scheduling, randomization, and real-time study monitoring. Questionnaires and clinical records will be completed in real-time and entered by study staff using encrypted study mobile tablets. The REDCap application allows for both online (real-time) data entry or, when internet service is not available, offline entry with syncing to the server when internet becomes available again.

3. Clinical Records. Records from clinic databases may be entered directly into the database form participant charts or transferred electronically to the study project coordinator for upload into the study database. All data transfers will be done using password-protected, encrypted files, transferred using secure email or encrypted hard drives, and be free of specific patient identifiers including name, dates of birth, or address.

D. Data Protection and Confidentiality

Paper forms that include participant protected health information will be stored on the day of enrollment in participant specific folders within locked file cabinets in locked research offices. Only study staff will have access to the file cabinets. All other study data will be free of identifying information. Data will be stored on an encrypted REDCap Server hosted by Massachusetts General Hospital Research Management in Boston, Massachusetts. Access to the study database both on site and remotely is restricted to study staff and password protected. All study mobile tablets will be password protected and encrypted prior to

study initiation. Data collected on mobile tablets will be uploaded to the server in real-time during data entry, or synced to the server as soon as data/Internet access becomes available if it is not during data entry, using the REDCap Offline Application.

IX. STUDY MONITORING AND QUALITY ASSURANCE

A. Staff Trainings

1. Research Staff

All research assistants will complete training through the CITI biomedical human subjects research course or a refresher course, as indicated. Only research staff fluent in the local languages (Runyankole or IsiZulu) will conduct informed consent and survey procedures. Study staff certified in phlebotomy will complete any blood collection procedures. Study staff will maintain active research ethics certification and attend any annual research ethics conferences as mandated by local policy at their institutions.

2. Clinical Study Staff

Prior to study initiation, clinical study staff will complete a training in the interpretation of HIV-1 resistance testing results and management of virologic failure. The course will be designed and directed by study Co-Investigators Drs. Rajesh Gandhi, Vincent Marconi, and/or Kevin Ard and conducted at the study sites. The course will include a review of major non-nucleoside and nucleos(t)ide reverse transcriptase inhibitor mutations, second-line regimen options, drug-drug interactions, common drug toxicities, and a case-based learning session.

B. Data Monitoring

After review of the study protocol with National Institutes of Health officials, the proposed study is considered to present minimal risk to participants given that subjects will complete questionnaires and undergo routine HIV monitoring with viral load and resistance testing per standard guidelines, and no investigational agents or tests will be used. Therefore, we will not establish a formal Data and Safety Monitoring Board. The study statistician and co-investigators will meet annually to perform the following activities: a) review the research protocol and plans for data and safety monitoring; b) review progress of the trial, including analysis of data quality and timeliness; subject recruitment, randomization and retention; subject risk versus benefit; and other factors that may affect outcome. c) review serious and unexpected adverse event reports, provide commentary, and provide oversight to ensure that reports are relayed to individual IRBs; d) review proposed modifications to the study prior to their implementation. Meetings will be held every twelve months beginning in Year 2 of the study. We will also have an independent data monitor who will annually review blinded summaries of outcome and adverse event data and provide a report to study investigators on recommendations amending the study.

C. Adverse and Unexpected Event Reporting

To identify adverse events: (1) study clinicians will perform a clinical review at each study visit and (2) study staff will instruct participants to report any unexpected or severe adverse events to their assigned research assistant. Such a report will immediately be brought to the attention of study principal investigators. Any adverse events that are unanticipated (i.e. not related to standard HIV clinical care or thought to be directly related to study procedures) or severe (e.g. death) will be brought to the attention

of the principal Investigator and reported to participating ethical review committees within 7 days. The relevant committee will determine whether it is appropriate to pause the study or alter the study protocol, and will provide suggestions/modifications to the study procedures as necessary. Possible modifications include adding adverse events to the consent form and re-consenting all study participants. The site principal investigator will be responsible for monitoring participant safety at each site on a monthly basis at regularly scheduled research meetings. They will keep a written log of all events and ensure that the ethical review committee is contacted when necessary. They will also keep a log of the outcome of committee decisions regarding adverse events and apprise the research team of any changes that need to occur as a result of such decisions.

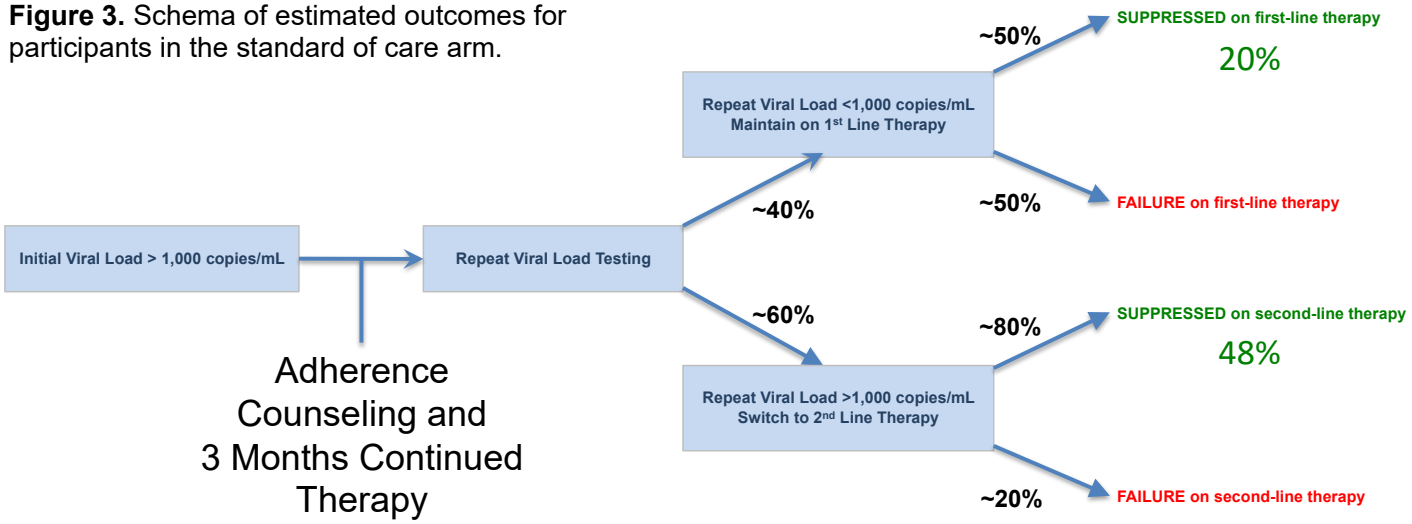
X. STATISTICAL CONSIDERATIONS AND ANALYSES

A. Sample Size

We propose to enroll and maintain a sample size of 840 patients, with approximately 420 randomized to each arm. This sample will enable adequate power to detect a clinically significant, 10% or greater rate of our primary outcome (viral load <200 copies/mL) at study conclusion in the RT arm (see below for power calculation). We have selected this threshold because we predict that a finding of this magnitude would be necessary to advocate for a change in current practice of virologic failure management.

To assess our sample size calculation, we first estimated predicted rates of viral suppression in the SOC arm (Figure 3). To do so, we combined estimated suppression rates for those who remain on first line therapy and those who are switched to second line therapy, due to a persistent elevation in viral load >1,000 copies/mL. Multiple studies have reported that approximately 40% of people (range 27% - 59%) with an initial detectable viral load on first-line therapy load will re-suppress while remaining on first line therapy in the interim [2, 8-10]. Among those who do remain on first-line therapy, we estimate that only approximately 50% will remain suppressed on first-line therapy, owing to both high rates of underlying resistance [8], and sub-optimal adherence. Among patients in the SOC arm with persistent virologic failure on repeat testing who are switched to second line therapy, we expect approximately 80% will re-suppress [35]. In summary, we predict that net re-suppression rates in the standard of care arm compiling those who remain on first-line therapy (20%) and those who switch to second line therapy (48%), will be approximately 68%. While comparative rates of re-suppression with resistance testing are unknown, we hypothesize that resistance testing has the potential to significantly improve viral suppression rates, particularly because rates of resistance at first-line failure are common in the region (65-90%) [7, 8, 36], and because those who are switched to a protease inhibitor-based combination regimen are highly likely to re-suppress [37-39].

Figure 3. Schema of estimated outcomes for participants in the standard of care arm.



Assuming a 68% viral suppression rate in the SOC arm, using a two-sample test of binomial proportions with a Type I error rate of 5%, and allowing for a 5% rate of invalid tests, we compute a 69%, 88%, and 97% power to detect a difference in suppression rates of 8, 10, and 12%, respectively (Table 3). Based on these estimates, we plan to enroll approximately 840 total participants into the study to allow sufficient power to detect a programmatically meaningful 10% superiority rate of viral suppression in the RT arm.

Table 3. Sample Size Estimates

Viral Suppression Rate in SOC Arm	Viral Suppression Rate in RT Arm	Difference in Outcome Rate	α	Sample Size (per group)	Invalid Test Rate	Power
68%	76%	8%	0.05	420	5%	0.69
68%	78%	10%	0.05	420	5%	0.88
68%	80%	12%	0.05	420	5%	0.97

B. Efficacy Analyses

1. Primary Outcome of Interest

Proportion of patients with a laboratory confirmed suppressed viral load, as defined by <200 copies/mL, at study conclusion (Visit 3).

2. Rationale for Primary Outcome of Interest

We have chosen this as our primary outcome to ensure a balanced outcome assessment 9 months after study initiation, standardization of viral load results across sites with different viral load testing platforms, and because it is an accepted international standard for other international studies of virologic suppression [39, 40].

3. Secondary Outcomes of Interest

a. Proportion of patients with an undetectable viral load (below limit of detection) at study conclusion
b. Proportion of patients with an undetectable viral load on first-line (NNRTI-based) therapy at study conclusion
c. Proportion of patients with International AIDS Society-defined drug resistance mutations to their current regimen [41]. As part of this analysis, we will also evaluate for minority drug resistance.
d. Total patient care costs, including diagnostic testing and ART costs for the study duration
e. Retention in HIV clinical care at study completion
f. 9-month mortality rate
g. Change in health-related quality of life from baseline to 9 months

4. Efficacy Analysis Plan

Study data coordinators will perform routine reviews of data quality to identify unusual values and missing data entries. Initial analyses will include common exploratory data analyses, table construction, and scatterplots to assist in data quality control, randomization balance, and exploring for possible outliers or leverage points. For our analysis of the primary outcome, we will use a two-sample test of binomial proportions to compare the proportions of participants in each arm who achieve virologic suppression at the study conclusion. For our primary analysis we will conduct an intention to treat analysis. For this analysis, any study participant without a confirmed viral load result at the 9-month study endpoint will be considered as having a detectable viral load (failure). In secondary analyses, we will conduct as-treated analyses for any study participants who do not complete resistance testing in the RT arm or participant who do receive resistance testing in the SOC arm, as documented by clinic records. We will also conduct a sensitivity analysis by censoring participants who are lost-to-follow-up during study procedures and do not have a recorded viral load at study conclusion. The unadjusted comparison of outcome rates will be followed by adjusted comparisons via logistic regression, adjusting for other confounding factors that may explain any heterogeneity or possibly confound assigned treatment and outcome, despite treatment randomization.

The primary analyses will be followed by similar statistical analyses of secondary endpoints including the proportions suppressed on first-line therapy, rates of resistance, rates of retention in care, and the 9-month mortality rate. We will compare health related quality of life measures within participants between baseline and study conclusion using paired t-tests.

C. Economic Analyses

1. Budget Impact Analysis

The goal of this analysis it to estimate total projected costs for treatment programs, comparing the WHO standard of care versus a resistance-testing based approach to management of virologic failure. We will follow International Society of Pharmacoeconomics Outcomes Research (ISPOR) guidelines to develop models to determine the budget impact of adopting resistance testing in South Africa and Uganda, respectively [42]. We will populate each model with data provided by the clinical trial on costs of care, and add country-specific HIV epidemiology, demographic and expenditure data to estimate the budget impact of adopting resistance testing over five years for each strategy. We will build the model such that country-specific fields will be flexible to enable analysis with other countries, to facilitate policy planning

and decision-making. Results of this analysis will advise HIV program partners on the net resources required in each country to implement routine resistance testing over a 5-year period.

2. Cost Effectiveness Analysis

If the resistance testing intervention is superior or similar to the standard of care strategy, we will proceed with a full cost-effectiveness analysis. This analysis seeks to build upon prior analyses that have considered the economics of resistance testing in sub-Saharan Africa [22-24]. We will achieve this by incorporating primary efficacy, quality of life, and cost data from the clinical trial, and by estimating the cost utility of resistance testing versus standard of care by selecting quality adjusted life years (QALY) as our primary outcome of interest.

To do so, we will develop a cost utility model in accordance with best practice recommendations for modeling from the ISPOR Good Research Practice Task Force [43] and will follow the guidelines set out by the National Institute For Health and Clinical Excellence Reference Case [44]. We will develop a decision tree cohort Markov Model using TreeAge (TreeAge Software, Inc., Williamston, MA) with methods similar to those previously described by Rosen *et al* in their analysis of the cost effectiveness of resistance testing-based management of virologic failure [23]. The outcome of interest will be the cost per QALY ($/QALY), presented as an incremental cost-effectiveness ratio. We will populate the model for the first 9 months with comparative efficacy, health-related quality of life (EQ5D questionnaire), and resource allocation data derived directly from the clinical trial. We will value health-related quality of life using the time trade off approach for a reference population in sub-Saharan Africa as adopted in similar HIV-related cost-effectiveness analyses [45]. Thereafter, we will use published data on the natural history of HIV disease to model clinical outcomes every 6 months after study conclusion over a patient's lifetime, including risk of opportunistic infections, hospitalization, quality of life and death [46-50].

We will vary the probability of each outcome by CD4 category, presence (or not) of detectable viral load and presence (or not) of resistance, to extrapolate cost utility (Table 7). To characterize uncertainty in the model, we will conduct a probabilistic sensitivity analysis (PSA), and present data as cost-effectiveness acceptability curves and PSA plots. Convergent validity will be undertaken to compare the model to similarly published models on the cost effectiveness of resistance testing. Whereas results will be immediately relevant for the South African and Ugandan populations, the proposed structure of the model will be designed such that it may be used for adaptation to countries with similar health care contexts. Results will be presented in US dollars to inform foreign stakeholders.

XI. HUMAN SUBJECTS CONSIDERATIONS

A. Study Risks and Discomforts

1. Risk 1: Unintended disclosure of HIV status or other confidential information. The study intends to minimize this risk through a number of protective measures. Participants will be enrolled directly from the HIV clinic and all study procedures will be performed in adjacent study offices. All interactions with study staff will be in the clinic or private rooms. Study sites have performed thousands of study visits in such a manner. If we visit participants at home to perform tracking of those lost from study, we will make every effort to plan the visit with the participant in advance (e.g. by cell phone), so he or she can make any arrangements necessary for maintaining privacy. We will also approach the home in an unmarked vehicle or on foot to avoid raising concerns. Study forms with identifiers (consent form and tracking form) will be kept in locked file cabinets, which are stored in locked research offices. To maintain data

confidentiality, participants will be assigned a unique identifier on study enrollment, and all study data will be entered on encrypted, password protected study tablets. The tablets will immediately sync study data to an encrypted, password protected Redcap Study Database, and only key study personnel will have access to the database.

2. Risk 2: Discomfort or complication from phlebotomy procedures. Participants will undergo phlebotomy at each study visit. A trained phlebotomist will perform all blood collection using standard precautions. No more than 15 cc (approximately 3 teaspoons) of blood will be drawn on a single day, and no more than 50 cc over the course of the study period.

3. Risk 3: Time Commitments, Transportation costs and other indirect costs of additional study visits. We will reimburse participants for the cost of transportation to study visits performed outside of standard clinical visits. We will also attempt to schedule study visits on the dates of previously scheduled clinical visits to minimize the burden of study procedures. We have attempted to minimize additional time required for study procedures. The longest study visit will be the enrollment visit, largely due to the informed consent procedures, which we estimate will take approximately 1 hour to complete, based on prior similar studies. Study questionnaires will take approximately 30 minutes to complete at the enrollment (Visit 1) and final (Visit 3) study visits.

B. Potential Benefits

1. Potential Benefits to Participants. Study participants will receive nominal incentives for completing the enrollment study visit (e.g. a meal, bar of soap and/or 1 kilogram pack of sugar, depending on the study site). They will also receive study incentives and/or transportation allocation consistent with local expectations, following the established standards with institutional review approval in each country. Details of these reimbursements are described in consent forms. All viral load and resistance testing performed as part of the study will be free for study participants – covered either as part of standard clinical care (as is done for viral load testing in South Africa), or if not, by the study itself. Study participants might benefit from additional viral load and resistance testing results performed as part of the study, or the input from study clinicians trained in management of virologic failure as part of the study protocol. All ART regimens and any other treatments or care will be provided by the partner HIV clinics. Finally, participants might also benefit from knowing that they are helping to improve our understanding of optimal management of virologic failure in sub-Saharan Africa.

2. Potential Benefits to Society and Others. In Uganda, where viral load testing is not always available, the study will provide additional viral load testing free of charge to the study clinics to enhance screening of potential participants, and non-participants might benefit from this service. Results of the study might be of benefit to HIV infected people in the sub-Saharan African region. Over 10 million people in sub-Saharan Africa have accessed ART over the past decade. Approximately 1 in 3 of them who remain on therapy will suffer virologic failure within two years of treatment initiation. Thus, understanding the optimal and most-cost effective method of virologic failure management will have a critical impact on both patients and treatment programs as viral load testing becomes more affordable and available in the region.

XII. REFERENCES

1. Haberer JE, Kiwanuka J, Nansera D, Ragland K, Mellins C, Bangsberg DR. **Multiple measures reveal antiretroviral adherence successes and challenges in HIV-infected Ugandan children**. *PLoS One* 2012; 7(5):e36737.
2. Gupta RK, Goodall RL, Ranopa M, Kityo C, Munderi P, Lyagoba F, et al. **High Rate of HIV Resuppression After Viral Failure on First-line Antiretroviral Therapy in the Absence of Switch to Second-line Therapy**. *Clin Infect Dis* 2014; 58(7):1023-1026.
3. Barth RE, van der Loeff MF, Schuurman R, Hoepelman AI, Wensing AM. **Virological follow-up of adult patients in antiretroviral treatment programmes in sub-Saharan Africa: a systematic review**. *Lancet Infect Dis* 2010; 10(3):155-166.
4. UNAIDS. **Landmark HIV diagnostic access program will save $150m and help acheive new global goals on HIV. Available at:** http://www.unaids.org/en/resources/presscentre/pressreleaseandstatementarchive/2014/september/20140925prviralload**; Accessed 13 Dec 2014.** In; 2014.
5. Mermin J, Ekwaru JP, Were W, Degerman R, Bunnell R, Kaharuza F, et al. **Utility of routine viral load, CD4 cell count, and clinical monitoring among adults with HIV receiving antiretroviral therapy in Uganda: randomised trial**. *Bmj* 2011; 343:d6792.
6. Marconi VC, Sunpath H, Lu Z, Gordon M, Koranteng-Apeagyei K, Hampton J, et al. **Prevalence of HIV-1 drug resistance after failure of a first highly active antiretroviral therapy regimen in KwaZulu Natal, South Africa**. *Clin Infect Dis* 2008; 46(10):1589-1597.
7. Hosseinipour MC, van Oosterhout JJ, Weigel R, Phiri S, Kamwendo D, Parkin N, et al. **The public health approach to identify antiretroviral therapy failure: high-level nucleoside reverse transcriptase inhibitor resistance among Malawians failing first-line antiretroviral therapy**. *AIDS* 2009; 23(9):1127-1134.
8. Hoffmann CJ, Charalambous S, Sim J, Ledwaba J, Schwikkard G, Chaisson RE, et al. **Viremia, resuppression, and time to resistance in human immunodeficiency virus (HIV) subtype C during first-line antiretroviral therapy in South Africa**. *Clin Infect Dis* 2009; 49(12):1928-1935.
9. El-Khatib Z, Delong AK, Katzenstein D, Ekstrom AM, Ledwaba J, Mohapi L, et al. **Drug resistance patterns and virus re-suppression among HIV-1 subtype C infected patients receiving non-nucleoside reverse transcriptase inhibitors in South Africa**. *J AIDS Clin Res* 2011; 2(117).
10. Jobanputra K, Parker LA, Azih C, Okello V, Maphalala G, Kershberger B, et al. **Factors associated with virological failure and suppression after enhanced adherence counselling, in children, adolescents and adults on antiretroviral therapy for HIV in Swaziland**. *PLoS One* 2015; 10(2):e0116144.
11. Tuller DM, Bangsberg DR, Senkungu J, Ware NC, Emenyonu N, Weiser SD. **Transportation costs impede sustained adherence and access to HAART in a clinic population in southwestern Uganda: a qualitative study**. *AIDS Behav* 2010; 14(4):778-784.
12. Siedner MJ, Lankowski A, Tsai AC, Muzoora C, Martin JN, Hunt PW, et al. **GPS-measured distance to clinic, but not self-reported transportation factors, are associated with missed HIV clinic visits in rural Uganda**. *AIDS* 2013; 27(9):1503-1508.
13. Lankowski AJ, Siedner MJ, Bangsberg DR, Tsai AC. **Impact of geographic and transportation-related barriers on HIV outcomes in sub-Saharan Africa: a systematic review**. *AIDS and behavior* 2014; 18(7):1199-1223.
14. Siedner MJ, Lankowski A, Haberer JE, Kembabazi A, Emenyonu N, Tsai AC, et al. **Rethinking the "pre" in pre-therapy counseling: no benefit of additional visits prior to therapy on adherence or viremia in Ugandans initiating ARVs**. *PloS one* 2012; 7(6):e39894.

15. Durant J, Clevenbergh P, Halfon P, Delgiudice P, Porsin S, Simonet P, et al. **Drug-resistance genotyping in HIV-1 therapy: the VIRADAPT randomised controlled trial**. *Lancet* 1999; 353(9171):2195-2199.
16. Cohen CJ, Hunt S, Sension M, Farthing C, Conant M, Jacobson S, et al. **A randomized trial assessing the impact of phenotypic resistance testing on antiretroviral therapy**. *AIDS* 2002; 16(4):579-588.
17. Tural C, Ruiz L, Holtzer C, Schapiro J, Viciana P, Gonzalez J, et al. **Clinical utility of HIV-1 genotyping and expert advice: the Havana trial**. *AIDS* 2002; 16(2):209-218.
18. Haubrich RH, Kemper CA, Hellmann NS, Keiser PH, Witt MD, Tilles JG, et al. **A randomized, prospective study of phenotype susceptibility testing versus standard of care to manage antiretroviral therapy: CCTG 575**. *AIDS* 2005; 19(3):295-302.
19. Friis-Moller N, Reiss P, Sabin CA, Weber R, Monforte A, El-Sadr W, et al. **Class of antiretroviral drugs and the risk of myocardial infarction**. *N Engl J Med* 2007; 356(17):1723-1735.
20. Haberer JE, Kahane J, Kigozi I, Emenyonu N, Hunt P, Martin J, et al. **Real-time adherence monitoring for HIV antiretroviral therapy**. *AIDS Behav* 2010; 14(6):1340-1346.
21. Boender ST, Hoenderboom BM, Sigaloff KC, Wellington M, Siwale M, Kityo CM, et al. **Pretreatment HIV Drug Resistance Increases Regimen Switch in sub-Saharan Africa. Presented at 2015 Conference on Retroviruses and Opportunistic Infections. 23-26 February, 2015. Available at:** http://www.croiconference.org/sessions/pretreatment-hiv-drug-resistance-increases-regimen-switch-sub-saharan-africa**. Accessed 1 March 2015.** 2015.
22. Levison JH, Wood R, Scott CA, Ciaranello AL, Martinson NA, Rusu C, et al. **The clinical and economic impact of genotype testing at first-line antiretroviral therapy failure for HIV-infected patients in South Africa**. *Clin Infect Dis* 2013; 56(4):587-597.
23. Rosen S, Long L, Sanne I, Stevens WS, Fox MP. **The net cost of incorporating resistance testing into HIV/AIDS treatment in South Africa: a Markov model with primary data**. *Journal of the International AIDS Society* 2011; 14:24.
24. Phillips A, Cambiano V, Nakagawa F, Magubu T, Miners A, Ford D, et al. **Cost-effectiveness of HIV drug resistance testing to inform switching to second line antiretroviral therapy in low income settings**. *PloS one* 2014; 9(10):e109148.
25. Inzaule SC, Hamers RL, Zeh CE, Rinke de Wit TF. **Stringent HIV Viral Load Threshold for Virological Failure Using Dried Blood Spots: Is the Perfect the Enemy of the Good?** *Journal of acquired immune deficiency syndromes* 2016; 71(1):e30-33.
26. Smit PW, Sollis KA, Fiscus S, Ford N, Vitoria M, Essajee S, et al. **Systematic review of the use of dried blood spots for monitoring HIV viral load and for early infant diagnosis**. *PLoS One* 2014; 9(3):e86461.
27. **National Consolidated Guidelines for the Prevention of Mother-to-Child Transmission of HIV (PMTCT) and the Management of HIV in Children, Adolescents and Adults. National Department of Health, South Africa. Available at:** http://www.sahivsoc.org/upload/documents/ART_Guidelines_15052015.pdf. 2015.
28. Musinguzi N, Muganzi CD, Boum Y, 2nd, Ronald A, Marzinke MA, Hendrix CW, et al. **Comparison of subjective and objective adherence measures for preexposure prophylaxis against HIV infection among serodiscordant couples in East Africa**. *AIDS* 2016; 30(7):1121-1129.
29. Hughes J, Jelsma J, Maclean E, Darder M, Tinise X. **The health-related quality of life of people living with HIV/AIDS**. *Disability and rehabilitation* 2004; 26(6):371-376.
30. Jelsma J, Maclean E, Hughes J, Tinise X, Darder M. **An investigation into the health-related quality of life of individuals living with HIV who are receiving HAART**. *AIDS care* 2005; 17(5):579-588.
31. Louwagie GM, Bachmann MO, Meyer K, Booysen Fle R, Fairall LR, Heunis C. **Highly active antiretroviral treatment and health related quality of life in South African adults with human immunodeficiency virus infection: A cross-sectional analytical study**. *BMC public health* 2007; 7:244.

32. Geng EH, Emenyonu N, Bwana MB, Glidden DV, Martin JN. **Sampling-based approach to determining outcomes of patients lost to follow-up in antiretroviral therapy scale-up programs in Africa**. *JAMA* 2008; 300(5):506-507.
33. Geng EH, Glidden DV, Emenyonu N, Musinguzi N, Bwana MB, Neilands TB, et al. **Tracking a sample of patients lost to follow-up has a major impact on understanding determinants of survival in HIV-infected patients on antiretroviral therapy in Africa**. *Trop Med Int Health* 2010; 15 Suppl 1:63-69.
34. Koenig HC, Mounzer K, Daughtridge GW, Sloan CE, Lalley-Chareczko L, Moorthy GS, et al. **Urine assay for tenofovir to monitor adherence in real time to tenofovir disoproxil fumarate/emtricitabine as pre-exposure prophylaxis**. *HIV Med* 2017; 18(6):412-418.
35. Gilks CF, Walker AS, Dunn DT, Gibb DM, Kikaire B, Reid A, et al. **Lopinavir/ritonavir monotherapy after 24 weeks of second-line antiretroviral therapy in Africa: a randomized controlled trial (SARA)**. *Antiviral therapy* 2012; 17(7):1363-1373.
36. Sunpath H, Wu B, Gordon M, Hampton J, Johnson B, Moosa MY, et al. **High rate of K65R for antiretroviral therapy-naive patients with subtype C HIV infection failing a tenofovir-containing first-line regimen**. *AIDS* 2012; 26(13):1679-1684.
37. Ciaffi L, Koulla-Shiro S, Sawadogo A, Moing VL, Eymard-Duvernay S, Izard S, et al. **Efficacy and safety of three second-line antiretroviral regimens in HIV-infected patients in Africa**. *AIDS* 2015; 29:1473-1481.
38. Bartlett JA, Ribaudo HJ, Wallis CL, Aga E, Katzenstein DA, Stevens WS, et al. **Lopinavir/ritonavir monotherapy after virologic failure of first-line antiretroviral therapy in resource-limited settings**. *AIDS* 2012; 26(11):1345-1354.
39. Paton NI, Kityo C, Hoppe A, Reid A, Kambugu A, Lugemwa A, et al. **Assessment of second-line antiretroviral regimens for HIV therapy in Africa**. *N Engl J Med* 2014; 371(3):234-247.
40. **Dart Trial Team. Virological response to a triple nucleoside/nucleotide analogue regimen over 48 weeks in HIV-1-infected adults in Africa**. *AIDS* 2006; 20(10):1391-1399.
41. Wensing AM, Calvez V, Gunthard HF, Johnson VA, Paredes R, Pillay D, et al. **2014 Update of the drug resistance mutations in HIV-1**. *Topics in antiviral medicine* 2014; 22(3):642-650.
42. Mauskopf JA, Sullivan SD, Annemans L, Caro J, Mullins CD, Nuijten M, et al. **Principles of good practice for budget impact analysis: report of the ISPOR Task Force on good research practices--budget impact analysis**. *Value in health : the journal of the International Society for Pharmacoeconomics and Outcomes Research* 2007; 10(5):336-347.
43. Caro JJ, Briggs AH, Siebert U, Kuntz KM, Force I-SMGRPT. **Modeling good research practices--overview: a report of the ISPOR-SMDM Modeling Good Research Practices Task Force--1**. *Value in health : the journal of the International Society for Pharmacoeconomics and Outcomes Research* 2012; 15(6):796-803.
44. Weidle PJ, Malamba S, Mwebaze R, Sozi C, Rukundo G, Downing R, et al. **Assessment of a pilot antiretroviral drug therapy programme in Uganda: patients' response, survival, and drug resistance**. *Lancet* 2002; 360(9326):34-40.
45. Cleary SM, McIntyre D, Boulle AM. **The cost-effectiveness of antiretroviral treatment in Khayelitsha, South Africa--a primary data analysis**. *Cost effectiveness and resource allocation : C/E* 2006; 4:20.
46. Mellors JW, Munoz A, Giorgi JV, Margolick JB, Tassoni CJ, Gupta P, et al. **Plasma viral load and CD4+ lymphocytes as prognostic markers of HIV-1 infection**. *Annals of internal medicine* 1997; 126(12):946-954.
47. Luz PM, Morris BL, Grinsztejn B, Freedberg KA, Veloso VG, Walensky RP, et al. **Cost-effectiveness of genotype testing for primary resistance in Brazil**. *Journal of acquired immune deficiency syndromes* 2015; 68(2):152-161.

48. Schackman BR, Goldie SJ, Freedberg KA, Losina E, Brazier J, Weinstein MC. **Comparison of health state utilities using community and patient preference weights derived from a survey of patients with HIV/AIDS**. *Medical decision making : an international journal of the Society for Medical Decision Making* 2002; 22(1):27-38.
49. Holtgrave DR, Pinkerton SD. **Updates of cost of illness and quality of life estimates for use in economic evaluations of HIV prevention programs**. *Journal of acquired immune deficiency syndromes and human retrovirology : official publication of the International Retrovirology Association* 1997; 16(1):54-62.
50. Robberstad B, Olsen JA. **The health related quality of life of people living with HIV/AIDS in sub-Saharan Africa - a literature review and focus group study**. *Cost effectiveness and resource allocation : C/E* 2010; 8:5.