Ph.D. Dissertation Proposal

Department of Epidemiology
Johns Hopkins University
Bloomberg School of Public Health

Predictors and Prediction Modeling of Mortality, Drop Out and Immuno-virologic outcomes among HIV-1 infected Adults receiving highly active antiretroviral therapy in Nigeria

By

Chuka Anude, MD MS MPH

Principal Investigator: Dr. William Blattner (IHV)
Ph.D. Candidate Investigator / Study Coordinator: Dr. Chuka Anude (IHV)
Co-investigators: Dr. Man Charurat (IHV), Dr John Farley (IHV)
Co-investigators: Drs. Patrick Dakum, Mary-Ann Etiebet, Usman Gebi, Oluyemisi Akinwande (IHVN)
Asokoro District Hospital Co-investigator: Dr Onyegbutulam
University of Abuja Teaching Hospital Co-investigator: Dr Ajayi
University of Benin Teaching Hospital Co-investigator: Dr Eze

2008©
# TABLE OF CONTENTS

LIST OF ABBREVIATIONS .................................................................................. 4

1. **Introduction** ............................................................................................. 6

2. **Study Rationale and significance** ............................................................. 8
   2.1. Overall study goal .................................................................................. 8
   2.2. Study Aims and Hypothesis .................................................................. 10

3. **Background** ............................................................................................ 15
   3.1. PEPFAR ............................................................................................... 15
   3.2. IHV Nigeria program ............................................................................. 17

4. **Literature Review** ................................................................................... 20
   4.1. Global HIV Epidemiology ................................................................. 20
   4.2. Epidemiology of HIV in Nigeria ......................................................... 20
   4.3. HIV Virology and Immunology ......................................................... 22
   4.4. Anti-retroviral therapy ....................................................................... 23
       4.4.1. Overview ....................................................................................... 23
       4.4.2. Classes and modes of action of anti-retroviral agents ................. 24
       4.4.3. Treatment schedules (WHO and Nigeria) ................................... 26
       4.4.4. Monitoring ART ......................................................................... 26
   4.5. Adherence to Antiretroviral Therapy ................................................... 27
   4.6. HIV treatment failure ......................................................................... 28
       4.6.1. Definitions and progression ......................................................... 28
       4.6.2. Measures and Predictors of Immuno-virologic treatment failure ... 28

5. **Research Methodology** .......................................................................... 29
   5.1. Summary of Study Hypothesis ............................................................ 29
   5.2. Study Design ........................................................................................ 30
   5.3. Study Population ................................................................................ 31
   5.4. Study Setting ....................................................................................... 32
   5.5. Eligibility criteria ................................................................................ 33
   5.6. Sample size calculations .................................................................... 33
   5.7. Flowchart of study participants ......................................................... 36
   5.8. Sources and methods of selection ....................................................... 37
   5.9. Definition and Sources of outcome variables ..................................... 38
   5.10. Definition and source of predictor variables ..................................... 39
LIST OF ABBREVIATIONS

3TC Lamivudine
ABC Abacavir
ADH Asokoro District Hospital, Asokoro, Abuja
AIDS Acquired Immunodeficiency Syndrome
ARV Antiretroviral
ART Antiretroviral therapy
AUROC Area Under Receiver Operating Curve
UATH University of Abuja Teaching Hospital, Gwagwalada
AZT Azidothymidine / Zidovudine
BMI Body mass index
CD4 Cluster of differentiation 4 T-helper cells
D4T Stavudine
EFV Efavirenz
FTC Emtricitabine
GLOBAL FUND Global Fund to fight AIDS, TB and Malaria
HAART Highly active antiretroviral therapy
HIV Human immune-deficiency virus
IHV Institute of Human Virology, Baltimore, Maryland
IHVN Institute of Human Virology Nigeria
LPV/r Lopinavir boosted with ritonavir
MAP Multi-country AIDS Program of the World Bank
M&E Monitoring and Evaluation
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>MOHN</td>
<td>Ministry of Health, Nigeria</td>
</tr>
<tr>
<td>MTCT</td>
<td>Mother-to-child transmission of HIV</td>
</tr>
<tr>
<td>NHREC</td>
<td>Nigerian Health Research Ethics Committee</td>
</tr>
<tr>
<td>NNRTI</td>
<td>Non-nucleoside reverse transcriptase inhibitor</td>
</tr>
<tr>
<td>NRTI</td>
<td>Nucleoside reverse transcriptase inhibitor</td>
</tr>
<tr>
<td>NtRTI</td>
<td>Nucleotide reverse transcriptase inhibitor</td>
</tr>
<tr>
<td>NVP</td>
<td>Nevirapine</td>
</tr>
<tr>
<td>OI</td>
<td>Opportunistic Infections</td>
</tr>
<tr>
<td>PEPFAR</td>
<td>President Emergency Fund for AIDS Relief</td>
</tr>
<tr>
<td>PI</td>
<td>Protease inhibitor</td>
</tr>
<tr>
<td>PMM</td>
<td>Patient monitoring and management forms</td>
</tr>
<tr>
<td>TB</td>
<td>Tuberculosis</td>
</tr>
<tr>
<td>TDF</td>
<td>Tenofovir</td>
</tr>
<tr>
<td>UBTH</td>
<td>University of Benin Teaching Hospital, Benin</td>
</tr>
<tr>
<td>UNAIDS</td>
<td>Joint United Nations Agency for AIDS</td>
</tr>
<tr>
<td>VL</td>
<td>HIV viral load</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
</tbody>
</table>
Predictors and Prediction Modeling of Mortality, Drop-outs and Immuno-virologic outcomes among HIV-1 infected Adults receiving highly active antiretroviral therapy in Nigeria

1. INTRODUCTION:

Almost three decades after its recognition\(^1,2\) and discovery\(^3-5\), Human Immunodeficiency Virus (HIV) infection remains a serious global public health and development challenge. According to the World Health Organization (WHO) and the United Nations Joint Program on AIDS (UNAIDS)\(^6\), about 25 million persons have died since 1981, and 33 million are currently infected with HIV. In 2007 alone, there were 2 million deaths and 2.7 million new infections contributed from every country in the world. Africa has 11% of the world’s population and 1.5% of the health workforce but contributes 65% of adult and 90% of pediatric HIV infections\(^7\). Fortunately, since 2002-3 there has been an explosion in the funding for HIV globally with the President Emergency Plan for AIDS relief (PEPFAR)\(^8\), the Global Fund to fight AIDS, TB and Malaria\(^9\), the World Bank’s Multi-country AIDS program (MAP)\(^10\) and other bilateral, multilateral and private philanthropic organizations contributing millions for HIV prevention, care, treatment and support programs. By 2007 ending, almost one million (950,000) people in low and middle income countries were on antiretroviral therapy bringing the total number of people on treatment to three million – a seven fold increase in about four years.\(^7\)

With the increase in PEPFAR funding for HIV treatment and the drop in prices of drugs and commodities\(^11,12\), HIV treatment scale up with highly active antiretroviral therapy (HAART) is now a reality\(^7,13\). However, concerns have shifted from access and availability of HIV treatment programs to issues of late presentations, quality of care, HIV drug resistance and HIV treatment failure\(^14-20\). In particular, treatment failure is a huge problem as it leads to transmission of HIV...
resistant virus, increase in treatment complexity and cost, worsening morbidity and mortality and ultimately, failure of the HIV treatment program\textsuperscript{13,20,21}. Diagnosis of the earliest forms of HIV treatment failure (virologic failure) is not commonly done as the WHO or PEPFAR does not recommend routine viral load testing in public HIV treatment programs in resource-constrained settings, mainly due to cost and logistics constraints\textsuperscript{13}. Thus by the time most patients are diagnosed with immunological failure (using CD4 counts) or clinical failure (by clinical history/examination or presence of new opportunistic infections), multiple drug resistance mutations have developed compromising and complicating future drug treatment options\textsuperscript{22,23}. Even though treatment outcomes in public run African HIV treatment programs are generally favorable\textsuperscript{18,24-27}, virologic responses are less than what obtains in prospective randomized trials\textsuperscript{28,29}. Thus there is a huge need to determine the clinical and non-clinical predictors of virologic and immunologic failure in public treatment programs offering widespread ART in Africa in order to address specific modifiable predictors and categorize patients at high risk for treatment failure for further preventive interventions and treatment preparation through the development of a HIV treatment failure prediction model. In addition, early mortality and drop out has also been identified as a threat to successful treatment outcomes\textsuperscript{30,31}. In some centers, about 20\%-30\% of all patients started on HAART die before their first year of treatment\textsuperscript{32}. Thus prediction models that help to predict probability of patients’ outcome using baseline predictors will help in the development of intervention protocols to reduce mortality, drop-out and improve general treatment outcomes\textsuperscript{33}. There are currently no published studies from Nigeria and other PEPFAR funded ART programs in public facilities in Africa looking at predictors of mortality, drop-out and immuno-virologic failure and no prediction models have been proposed to help predict these outcomes using baseline clinical and non-clinical characteristics of patients at program enrollment.
2. STUDY RATIONALE AND SIGNIFICANCE

2.1. Overall Study Goal

The overall goal of this study is to determine significant clinical and non-clinical predictors and to develop a prediction model for mortality, drop-out and immuno-virologic outcomes in unique government owned PEPFAR funded program settings offering comprehensive HIV prevention and treatment services, free drugs and commodities, and liberal use of Tenofovir as a component of the NRTI backbone. The prediction model will be developed and interpreted within the challenging structural and health system context of the treatment sites and used to guide policy and programs to address modifiable baseline predictors related to negative outcomes, improve treatment preparation and education, identify high risk patients who need priority attention and improve overall treatment outcomes. Further, the possibility of the transportability of the prediction model to other sites and its inclusion into the annual HIVQual program will help streamline program quality with clinical outcomes and improve overall treatment outcomes simultaneously in all IHVN sites.

A cohort of patients will be assessed at one year to determine mortality, drop-out and immuno-virologic failure among patients still active on the program and the predictors of those outcomes. Since an undetectable viral load at 12 months is regarded by some investigators as highly predictive of sustained viral response and long term clinical benefit\(^{34,35}\), the patients with virologic suppression at 12 months will be the reference group for the four mutually exclusive outcomes of mortality, drop-out, active with virologic failure and active with virologic suppression. A prediction model and score will be developed using significant clinical and non-clinical predictors which will help guide a policy and programmatic framework to address
modifiable predictors, improve treatment preparation and education, identify high risk patients and improve treatment outcomes.

The significance and novelty of this study include

- The IHVN ACTION project is one of the first and largest programs in any developing country using Tenofovir (TDF) liberally as a first line ARV agent in adults. About 30% of all patients on the project are on a TDF + 3TC backbone which have been recently recommended by the WHO and has shown efficacy and safety better than AZT or d4T. No other studies on treatment outcomes in Africa have been done in programs using TDF even though it has been shown to be more effective than AZT or d4T as TDF is considered costly both in terms of the drug itself and in terms of renal toxicity monitoring.

- the use of clearly defined indicators as predictors to develop a prediction model of HIV treatment failure outcomes in a developing country setting

- research in the context of PEPFAR, the largest HIV treatment program in the developing world and in the unique context of a network model of care, no patient rationing, free drugs, free laboratory tests and commodities, funding for clinical and non-clinical providers and a back-up M&E system used in field settings. This is unlike most other published research in treatment outcomes which are in settings that have co-payments or are run by NGOs like MSF or government through the help of the Global Fund.

- Research in the context of comprehensive HIV program settings providing a one-stop sites for HIV counseling and testing, prevention of mother to child HIV transmission, HIV treatment with readily available free 1st and 2nd line agents for adults and children, home-based care, care for orphans and vulnerable children.
The possibility of transportability of the prediction model and its use in the annual multi-site HIVQual program to match assessment of program quality with patient clinical outcomes.

There are currently no published studies on predictors of treatment outcomes in public government owned, PEPFAR funded African ART programs utilizing freely supplied Tenofovir as first line agents in a significant number of patients. No prediction models of mortality, drop-out and immuno-virologic failure using baseline clinical and non-clinical indicators have ever been proposed in published literature from Africa.

2.2. Study aims and hypothesis

AIM 1:

(1a). **To determine clinical predictors important in developing a prediction model for mortality, drop-out and immuno-virologic outcomes at 12 months among HIV-1 infected treatment naïve adults commenced on HAART.**

Predictors of interest here include baseline (pre-HAART) CD4 count, baseline hemoglobin level, prior non-HAART therapy and TB diagnosis.

(1b). **To determine non-clinical predictors important in developing a prediction model for mortality, drop-out and immuno-virologic outcomes at 12 months among HIV-1 infected treatment naïve adults commenced on HAART.**

Predictors of interest here include age, gender, HIV disclosure (or presence of treatment partner) and distance from treatment sites.
**Hypothesis:**

The hypothesis here is that among persons enrolled into ART programs, patients with low baseline (pre-HAART) CD4 count, low baseline hemoglobin level, history of or current TB diagnosis, younger age, male gender, non-HIV disclosure and far distance from treatment sites will independently have a statistically significantly increased risk of mortality, drop-out or immuno-virologic failure by the first 12 months after commencement of HAART

**AIM 2:**

(2a). To **develop a prediction model** for mortality, drop-out and immuno-virologic failure at 12 months after HAART commencement in HIV-1 infected treatment naïve adults in Nigeria using significant baseline clinical and non-clinical predictors.

(2b). To **develop a policy framework** for addressing significant baseline predictors and the prediction model of mortality, drop out and immuno-virologic failure among HIV-1 infected adults commenced on HAART.

**AIM 3:**

**To determine the program-related (contextual and structural) and quality of life related indicators that can influence HIV treatment outcomes.**

The indicators include human resource capacity (physician-patient ratios), facility infrastructure, quality and accessibility of services, adherence, educational and support services and perceptions.
of stigma and quality of health will be assessed using structured interview questionnaires and facility information.

**Hypothesis:**

*The hypothesis here is that compared with patients who have good program-related (contextual and structural) and quality of life related indicators, patients with poor indicators will independently have adverse HIV treatment outcomes*

**AIM 4:**

To determine the population-level effectiveness and association of immuno-virologic outcomes to NRTI backbone comparing Tenofovir/3TC backbone to an AZT/3TC or a d4T/3TC

The predictor variable here is NRTI backbone and the outcome here is immuno-virologic outcomes at 12 months. Data on **adherence and socio-economic status** will be collected using a standardized questionnaire as adherence and socio-economic status will be additional confounders in the association of immuno-virologic outcome and NRTI backbone. Adherence will be assessed using objective (pharmacy re-fill records) and subjective (self-report and visual analogue adapted from the MASRI interview from ACTG) means and socio-economic status will be assessed using educational level, employment type.

**Hypotheses:**

*The hypothesis here is that among patients enrolled into HAART and active and alive at 12 months, patients using AZT/3TC or d4T/3TC regimen, or who have poor adherence (<95% adherence) or low socio-economic status will have statistically significantly*
increased risk of immuno-virologic failure as determined by the results of viral load and CD4 count testing during the routine 12 month visit and hence demonstrate a poorer population-level effectiveness of the regimen.
Figure 1: Flow Chart Summarizing the Methodology and Aims 1-4

Aim 1

Determination of significant clinical and non-clinical predictors for development of a prediction model

Aim 2

Development of a prediction model for mortality, drop-out and immuno-virologic outcomes

Aim 3

Determination and Development of the health systems context to interpret the predictors and prediction model

Aim 4

Determination of the independent population-level effectiveness of the NRTI backbone on immuno-virologic outcomes adjusted for adherence and socio-economic status
3. BACKGROUND

3.1. President Emergency Plan for AIDS Relief (PEPFAR)

The President Emergency Plan for AIDS Relief (PEPFAR) is the boldest, most ambitious and largest financial commitment to a single disease by any one country in history\textsuperscript{36}. In January 2003, President Bush announced a 5 year $15 billion commitment for HIV prevention, treatment and care for 14 focus countries (one more country, Vietnam was later added to make it 15) representing around 50\% of the world-wide infection and an 114 additional countries through bilateral support mechanisms\textsuperscript{37}. Twelve of the 15 PEPFAR focus countries are sub-Saharan African countries severely affected by HIV. The US congress passed the United States Leadership Against HIV/AIDS, Tuberculosis and Malaria Act which established PEPFAR with specific authorizations including development of a global strategy for programming based on sound science and available best practices, budgetary allocations for categories of programmatic activities, creation of the oversight position of the US Global AIDS Coordinator, coordination among US government agencies and international stakeholders, and identification of priority countries for action.

The goal of PEPFAR when it was authorized in 2003 was to prevent 7 million new HIV infections, \textit{treat 2 million persons with HIV} and provide care for 10 million affected persons including orphans and vulnerable children mainly in the 15 focus countries. Since the current PEPFAR authorization ended in 2008, President Bush presented the congress with a new plan of committing $50 billion over the next five years with the goal of prevent 12 million new infections, treating an additional 2.5 million persons infected with HIV and providing care for 12 million HIV affected persons including orphans and vulnerable children. This will support 18
focus countries (3 new countries added – Malawi, Lesotho, Swaziland), another 114 countries in bilateraI HIV programs and the Global Fund.38

PEPFAR is a vertical, bilateral initiative coordinated by the Office of the Global AIDS Coordinator (OGAC) based in Washington DC which was mandated by the congressional Act that establis hed PEPFAR. International and national collaborators work with the core team and members of the in-country USAID and CDC GAP office to set national priorities and determine the focus of the programs. The National Ministries of Health are integrally involved in all the activities and must consent for the activities before they are signed off and implemented. International non-governmental organizations, US-based universities and local organizations called “Implementing Partners” (IPs) directly implement PEPFAR program with the support of the Ministry of Health but outside of the government bureaucracy. For the purposes of budgeting and performance targets, PEPFAR is categorized into 4: prevention, treatment, care and support for orphans and vulnerable children.

PEPFAR defines treatment as anti-retroviral therapy (ART) as distinct from all other related care services. Thus treatment involves only activities that directly or indirectly support ART provision including drug procurement, essential laboratory infrastructure and monitoring, equipment and training of personnel and support for supply chain management systems. Treatment for prevention of mother-to-child HIV transmission is classified under PEPFAR’s prevention category and treatment for co-infections like tuberculosis and malaria, palliative care as well as non-clinical care is classified under the care category. The main rationale of PEPFAR treatment strategy and its administrative and implementing system is to move huge funds, human resources, expertise and commodities to implement projects quickly for rapid scale-up of HIV treatment activities through an emergency plan. This is accompanied by extensive monitoring.
and evaluation plans with numerous indicators. The infusion of large amounts of funds for procurement of drugs and commodities, refurbishment of clinics and laboratories, strengthening of health systems, provision of equipment, hiring of local and international HIV experts serve not only to close the huge funding gap stalling AIDS treatment programs but provide a complimentary strengthening of the health system to support rapid scale-up of care delivery.  

About 55% of PEPFAR’s funds are mandated for HIV treatment alone.

In July 2008, based on request for renewal from President Bush, PEPFAR was re-authorized by the US congress at the amount of US$48 billion for 2008-2013 to cover for 18 focus countries, 104 additional countries and the Global Fund.

3.2. Institute of Human Virology, Nigeria (IHVN)

The Institute of Human Virology (IHV), University of Maryland was founded by Drs Robert Gallo, William Blattner and Robert Redfield in 1996 and has been a world leader in HIV virology, immunology, epidemiology and clinical research in the area of diagnostics, therapeutics and vaccinology. Initially formed as a partnership between the State of Maryland, the City of Baltimore and the University of Maryland and University of Maryland Medical System, IHV has evolved into a global institution revered for pioneering advances in all aspects of HIV prevention and treatment. In addition, IHV has pioneered HIV program implementation and has been funded since 2004 by PEPFAR to provide comprehensive, expanded, integrated, family-oriented, patient-centered HIV care and treatment.

IHV Nigeria is the Nigerian country program of IHV Maryland and is arguably the largest, most productive and most influential of all the PEPFAR funded HIV treatment programs in Nigeria and perhaps in all of PEPFAR. Since 2005, IHVN has been funded through the AIDS Care and Treatment in Nigeria (ACTION) project to provide comprehensive, expanded,
integrated, family-oriented, patient-centered HIV care, treatment and support. The ACTION project partners with the Nigerian Federal Ministry of Health, the National Action Committee on AIDS (NACA), state and local governments and medical institutions and hospitals to provide HIV related services. By mid 2008, there were about 34 comprehensive treatment sites based in secondary and tertiary hospitals, including university hospitals supported by IHV. In addition to having over 70,000 patients on ART, the program sites have the largest number of patients in any single program on Tenofovir, a nucleotide reverse transcriptase inhibitor with about 21,000 patients (30%) on the drug. No other studies on treatment outcomes in Africa have been done in programs using Tenofovir even though it has been shown to be more effective than AZT or d4T as it is not routinely used because of concerns about the cost of the drug and the renal toxicity monitoring. IHV follows the Nigerian national treatment guidelines which are modeled after the WHO treatment guidelines for resource limited settings. Thus immunologic, clinical and personal factors are used to determine eligibility for HIV treatment. In addition, viral load testing is not routinely offered to patients on ART because of cost and logistic concerns. However, patients receive thorough clinical and treatment preparation assessment, hematological and biochemical laboratory tests and CD4 count tests at baseline and every 6 months. A multidisciplinary team of health care providers ensure adherence to treatment protocol and follow up at scheduled visits. A home based care team comprised of paid and unpaid volunteers engage in patient follow-up and support activities.

Attempts at improving the quality of care has necessitated the adaptation of the HIVQual program developed by the New York Department of Health AIDS Institute and funded by the Department of Health and Human Services of the US government into PEPFAR programs through the support of the CDC Global AIDS programs. HIVQual assists in capacity-building to
help HIV treatment facilities build and sustain quality improvement programs through monitoring of selected indicators. HIVQual has been adapted into the ACTIONQual program of the ACTION project of IHVN. However, HIVQual monitors program level indicators and not individual clinical indicators and outcomes. Selected patient and health systems related indicators will be used for this study.

Patients receiving routine HIV care and treatment at three representative IHVN ACTION project sites (University of Abuja Teaching Hospital, Gwagwalada, University of Benin Teaching Hospital, Benin and Asokoro District Hospital, Abuja) will be participants in this proposed study.

Figure 2: Location of IHVN sites in Nigeria
4. LITERATURE REVIEW

4.1. Global HIV Epidemiology

Almost three decades after its initial recognition\textsuperscript{1,2} and discovery\textsuperscript{3-5}, Human Immunodeficiency Virus (HIV) remains a serious global public health and development problem. According to the World Health Organization (WHO) and the United Nations Joint Program on AIDS (UNAIDS)\textsuperscript{6}, about 25 million persons have died since 1981, and 33 million are currently infected with HIV. In 2007 alone, there were 2 million deaths and 2.7 million new infections coming from every country of the world. Africa has 11\% of the world’s population and 1.5\% of the health workforce but contributes 65\% of adult and 90\% of pediatric HIV infections\textsuperscript{7}. The HIV epidemic varies globally across geographical areas and can be categorized as low, centralized or generalized\textsuperscript{40}. The African epidemic is generalized unlike most other regions of the world where it is concentrated in high risk groups like injecting drug users, men who have sex with men and commercial sex workers\textsuperscript{40,41} and also characterized by a predominant heterosexual mode of transmission. In addition, women and youth aged 15-24 are disproportionately affected according to the UNAIDS report\textsuperscript{6} leading to large numbers of AIDS orphans, child-headed households and subsequent medical, social, economic and development challenges in the worst affected countries\textsuperscript{42-45}.

4.2. Epidemiology of HIV in Nigeria

Nigeria with about 148 million people is African’s most populous country and the 7\textsuperscript{th} most populous country in the world\textsuperscript{46}. With a serious generalized HIV epidemic with prevalence among 15-49 year olds reaching 3.1\% (range 2.3, 3.8\%) in 2007\textsuperscript{6}, about 2.6 million people are infected with HIV in Nigeria, the second highest of any country in the world. With 170,000 annual
AIDS deaths in 2007, AIDS was mainly responsible for the decline in life expectancy at birth between 1991 (54 years for females and 53 years for males) and 2007 (46 for females and 48 for males)\textsuperscript{47}.

The Nigerian HIV epidemic is mainly driven by unprotected heterosexual intercourse and mother-to-child transmission and is characterized by feminization (55% women) and significant regional and state variations\textsuperscript{48, 49}. Using surveillance to map the HIV geographical distribution in Nigeria indicates the south eastern and middle belt regions of Nigeria are the most affected\textsuperscript{50}. In the survey of HIV knowledge, attitude and behavior\textsuperscript{49}, results indicated that only 20% of adults are knowledgeable about correct HIV prevention methods, 78% of males and 29% of females aged 15-24 have had sex with a casual partner in the past 12 months and only 46% of males and 24% of females used condoms during sex with a non-regular partner. In addition, 20% of women in this survey have had sex before the age of 15 years. With its huge population, high rate of internal and external migration, low condom usage and an unregulated sex industry, many investigators are still puzzled as to why the HIV prevalence in Nigeria is not higher than the current 3.1%\textsuperscript{51}. This may be because of the reduced prevalence of homosexual transmission, intravenous drug use and high rates of male circumcision which is almost universal in Nigeria\textsuperscript{52, 53}.

The Nigerian national response to HIV is multi-sectoral and involves both national, state, local and private organization and government together with bilateral and multilateral agencies. This response is under girded by a National Strategic Framework, the Health Sector Strategic Plan (HSP) on HIV/AIDS 2005, the National Anti-retroviral Therapy (ART) scale up plan 2006 and the Health Sector Operational Plan of the Federal Ministry of Health and coordinated by the National Action Committee on AIDS (NACA).

There is a dearth of published studies from Nigeria on HIV treatment outcomes even though Nigeria has the one of largest number of persons on HIV treatment in any donor funded free ART program and

Doctoral Dissertation_Chuka Anude
has the second largest number of persons eligible for ART in the world. This study will help bridge the knowledge gap on treatment outcomes and also help provide information about the value of prognostic prediction models in predicting HIV treatment outcomes in African settings

4.3. **HIV Virology and Immunology**

HIV is an enveloped RNA retrovirus that infects T-cells with primary infection causing high plasma viremia up to a viral set point determined by immunologic response, infectivity, viral mutation rates, replicative capacity, and number of available target cells. Once HIV DNA is integrated into host DNA, a state of latency in several tissues develops complicating attempts at eradication. The natural history of HIV infection is divided into three: acute infection, chronic asymptomatic infection and terminal HIV infection. Acute infection is characterized by high viremia, CD4 decline, raised transaminases, flu-like clinical symptoms (acute viral syndrome) and typical spontaneous resolution with the development of a cytotoxic T-cell response. The chronic phase is characterized by continued viral replication with progressive CD4 decline with later appearances of opportunistic infections. Clinical deterioration is determined by the viral set point, HLA class, switching of circulating viral strains to the syncitia-inducing form and associated mononuclear receptor types (CCr5 or CXCR4). Lastly, the terminal phase of HIV infection is characterized by pronounced depletion of CD4 cells, susceptibility to severe opportunistic infections resulting in AIDS and inevitable death if untreated. Numerous natural history studies show three patterns of HIV disease progression from infection to AIDS: rapid progressors (median of 3-4 years), typical progressors (median of 8-10 years), and long term non-progressors (> 12 years). The CD4 T-cell count and the plasma HIV RNA viral load are two surrogate laboratory markers for monitoring disease...
progression. While the CD4 count is a marker for immune competence, the viral load is a marker for disease progression\textsuperscript{13}.

4.4. Anti-retroviral Therapy

4.4.1. Overview

The goal of antiretroviral therapy in HIV treatment is to maximize HIV viral suppression, preserve and restore immune function, reduce HIV-related morbidity and mortality and improve the quality of life\textsuperscript{13, 73}. Combination therapy using three or more antiretroviral drugs from at least two different classes in therapeutic doses is termed “Highly active antiretroviral therapy” (HAART). HAART has been associated with sustained virologic suppression\textsuperscript{74, 75}, improvement in CD4 counts\textsuperscript{76, 77}, increased survival and quality of life\textsuperscript{78}, improvement in HIV testing uptake in some African settings\textsuperscript{79} and prevention of new infections\textsuperscript{80-83}. Numerous studies of the impact of HAART in Africa has shown high survival rates: \textbf{81\%} at 1-3 years in Senegal\textsuperscript{26, 27}, \textbf{86\%} at 24 months in South Africa\textsuperscript{25}, \textbf{92\%} in 12 months in Cameroun\textsuperscript{84}, and \textbf{84\%} at 12 months in Ivory Coast\textsuperscript{85} in spite of late presentations. Programs with lower survivals rates are mainly affected by high attrition\textsuperscript{14}.

It is important to note that all these studies were done in settings where Tenofovir was not in use, patients were required to co-pay for drugs or laboratory tests, or treatment was provided in NGO settings (like Doctors Without Borders/Médecins Sans Frontières (MSF) clinics) or government clinics without substantial external funding support, all of which can impact on treatment outcomes. Currently, there are no published well powered studies on predictors of treatment (clinical, immuno-virologic, programmatic) outcomes in public government owned PEPFAR funded African ART programs utilizing freely supplied Tenofovir as first line agents in a significant number of patients. There are also
no publications on prediction models developed using significant baseline clinical predictors in African ART programs.

4.4.2. Classes and modes of action

The main classes of antiretroviral agents include nucleoside reverse transcriptase inhibitors (NRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI), protease inhibitors (PI) and Fusion (entry) inhibitors. The NRTIs work by competitively antagonizing HIV replication by incorporating their synthetic analogues into viral DNA and inhibiting reverse transcriptase while the NNRTI work by non-competitive inhibition of HIV reverse transcriptase. NNRTIs are not active against HIV-2 and easily succumb to rapidly emerging resistance through single point mutations. Tenofovir disoproxil fumarate (TDF) is the only nucleotide reverse transcriptase inhibitor (NtRTI) and competitively incorporates into viral DNA inhibiting viral replication. PIs are excellent inhibitors of HIV by inhibition of viral protease. The only licensed entry inhibitor, Enfurvitide (T-20) and other new agents awaiting approval (CCr5 antagonists, Integrase inhibitors, etc) are not available in Africa. Different combinations of drugs are available to reduce pill burden and simplify dosing.

The table below illustrates the antiretroviral agents and their different classes.

Table 1: NRTI (NtRTI) agents, dosage, dietary rules and selected toxicities

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dosage</th>
<th>Dietary rules</th>
<th>Selected adverse effects, toxicities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zidovudine (AZT)</td>
<td>300 mg twice daily</td>
<td>None</td>
<td>Anaemia, neutropoenia</td>
</tr>
<tr>
<td>Stavudine (d4T)</td>
<td>&gt;60 kg, 40 mg &lt;60 kg, 30 mg twice daily</td>
<td>None</td>
<td>Peripheral neuropathy, pancreatitis</td>
</tr>
<tr>
<td>(Use is discouraged due to long term toxicity)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lamivudine (3TC)</td>
<td>150 mg twice daily</td>
<td>None</td>
<td>Peripheral neuropathy, lactic acidosis (rare)</td>
</tr>
<tr>
<td>Drug</td>
<td>Dosage</td>
<td>Dietary rules</td>
<td>Selected adverse effects, toxicities</td>
</tr>
<tr>
<td>--------------------</td>
<td>---------------------------------------------</td>
<td>------------------------------------</td>
<td>-------------------------------------</td>
</tr>
<tr>
<td>Didanosine (ddI)</td>
<td>&gt;60 kg, 400 mg once daily &lt;60 kg, 250 mg once daily</td>
<td>Take on empty stomach</td>
<td>Peripheral neuropathy, lactic acidosis, (rare)</td>
</tr>
<tr>
<td>Abacavir (ABC)</td>
<td>300 mg twice daily</td>
<td>None</td>
<td>Hypersensitivity reaction</td>
</tr>
<tr>
<td>Tenofovir (TDF)*†</td>
<td>300 mg once daily (See Table 1.2 below.)</td>
<td>None</td>
<td>Renal toxicity</td>
</tr>
<tr>
<td>Emtricitabine (FTC)</td>
<td>200 mg once daily</td>
<td>None</td>
<td>Skin discoloration</td>
</tr>
</tbody>
</table>

Table 2: NNRTI agents, dosage, dietary rules and selected toxicities

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dosage</th>
<th>Dietary rules</th>
<th>Selected adverse effects, toxicities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nevirapine (NVP)</td>
<td>200 mg once daily for 14 days, then 200 mg twice daily</td>
<td>None</td>
<td>Rash, hepatotoxicity</td>
</tr>
<tr>
<td>Efavirenz (EFV)</td>
<td>600 mg once daily, preferably at night</td>
<td>Do not take with high-fat meals</td>
<td>Teratogenic, CNS effects, rash</td>
</tr>
</tbody>
</table>

Table 3: PI agents, dosage, dietary rules and selected toxicities

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dosage</th>
<th>Dietary rules</th>
<th>Selected adverse effects, toxicities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indinavir (IDV)</td>
<td>800 mg thrice daily or 800 mg indinavir when boosted with 100 mg ritonavir twice daily</td>
<td>Drink plenty of water—at least 6 glasses every 24 hrs</td>
<td>Renal stones, metabolic changes</td>
</tr>
<tr>
<td>Indinavir + ritonavir (IDV+RTV)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nelfinavir (NFV)</td>
<td>1250 mg twice daily</td>
<td>Take with food</td>
<td>Metabolic change, diarrhoea</td>
</tr>
<tr>
<td>Lopinavir + ritonavir (LPV/r)</td>
<td>133.3 mg/33.3 mg [3 capsules] twice daily</td>
<td>None</td>
<td>Metabolic changes</td>
</tr>
<tr>
<td>Saquinavir (SQV)</td>
<td>1200 mg thrice daily or 1000 mg saquinavir when boosted with 100 mg ritonavir twice daily</td>
<td>None</td>
<td>Metabolic changes</td>
</tr>
<tr>
<td>Saquinavir + ritonavir (SQV+RTV)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atazanavir (ATV)</td>
<td>400 mg once daily</td>
<td>Take with food</td>
<td>Hyperbilirubinaemia</td>
</tr>
</tbody>
</table>

4.4.3. Treatment guidelines and schedules (WHO and Nigeria)

The Nigerian HIV treatment guideline is an adaptation of the WHO treatment guidelines for scaling up antiretroviral therapy in resource-limited settings which aims to simplify protocols, integrate HIV treatment into primary health care and employ a public health / health systems approach to HIV treatment. The preferred first line treatment consists of:

```
TDF or AZT or d4T or ABC
AND
3TC or FTC
AND
EFV or NVP
```

This recommended first line regimen has been evaluated in clinical trials and found to have sustained virologic potency\(^{29, 91-97}\) and safety\(^{93, 98}\). The proposed second line regimen includes introducing Lopinavir/ritonavir and changing one NRTI while keeping 3TC or FTC to maintain the M184V mutation which increases AZT, d4T and TDF activity if too many thymidine associated mutations are not present\(^{23, 99, 100}\).

4.4.4. Monitoring ART

Carefully and strategically planned monitoring is an essential component of effective ARV treatment, permitting early detection of adverse events, ongoing reinforcement of patient adherence, and periodic assessment of treatment efficacy. Monitoring of ART involves clinical and laboratory parameters and involves first establishing a baseline assessment which includes detailed medical history and physical examination, nutritional assessment and counseling, adherence counseling, family and treatment support counseling and laboratory tests. At a minimum laboratory tests will include HIV antibody test, completed blood count, liver function test, CD4 count test, pregnancy test for women and a chest x-ray to rule out tuberculosis. At each scheduled follow-up visit,
thorough medical history and physical examinations are done as well as laboratory tests to monitor CD4 count and drug adverse events.

4.5. Adherence to Antiretroviral Therapy

Adherence which is defined as the degree of consistency between the providers prescribed medication and the patient’s behavior is complex and difficult to measure in clinical and epidemiological studies\textsuperscript{101-105} but has been associated with sustained viral suppression\textsuperscript{106}, reduction in onset of resistance\textsuperscript{107-109} and favorable clinical outcomes\textsuperscript{110,111}. Initial skepticism and reluctance to scale up ART in Africa was mainly founded on fears of prohibitive costs and medication non-adherence\textsuperscript{112,113}. However, numerous studies have shown that medication adherence in Africans is good and sometimes better than that obtained in developed countries\textsuperscript{114,115}. A meta-analysis of 51 studies done by Mills, et al showed that medication adherence was better in developing countries (pooled estimates of 77%; 95% CI:68-85%) than in developed countries (pooled estimates of 55%; 95% CI:49-62%)\textsuperscript{111}, how since adherence decreases over time\textsuperscript{116}, this difference might be due to the earlier onset of treatment in developed countries. Generally, a near perfect adherence rate of $\geq 95\%$ is accepted to be ideal for sustained viral suppression\textsuperscript{117}.

Measures of adherence used in ART programs include objective (blood drug level monitoring, electronic monitoring of medication bottles, pharmacy refill) and subjective (self-report, patient diary, pill count and clinic attendance) measures\textsuperscript{101,103,106}. Predictors of medication adherence may include patient-related, regimen-related, provider-related and health systems-related factors\textsuperscript{118-124}. In addition, other factors like low socio-economic status\textsuperscript{125}, stigma\textsuperscript{126} and HIV non-disclosure\textsuperscript{127}, can predict poor adherence which will lead to treatment failure, drug resistance and poor clinical and program
outcomes. In the absence of ideal adherence measurement tools and in the context of lack of routine viral load measurements and resistance testing, widespread treatment failure with drug resistance is a grave possibility, thus efforts must be made to improve adherence measurements tools, and adherence counseling and treatment preparation activities.

4.6. HIV treatment failure

4.6.1. Definitions and progression

HIV treatment failure occurs when patients develop negative virologic, immunologic and clinical outcomes while still on therapy. Treatment failure normally progresses from virologic, to immunologic and then clinical failure\(^{13,19}\). Even though limits of RNA viral load detection vary from 50-400, for this study virologic failure is defined as failure to suppress RNA viral load levels to < 400 copies / ml at 12 months after HAART initiation (failure to suppress). Some investigators have shown that viral load estimations at 12 months predict sustained virologic suppression and long term clinical outcomes\(^{34,35}\).

Immunologic failure is defined in this study as failure of CD4 lymphocyte count to improve by at least 50 cells /mm3 or worsening to or below baseline pre-HAART CD4 level at 12 months after HAART initiation. There will not be an assessment of clinical failure in this study.

4.6.2. Measures and Predictors of Immuno-virologic treatment failure

Sustained virologic suppression on first-line therapy is the goal of treatment, since potency of subsequent regimen is affected by accumulated resistance mutations.

Currently, clinical and immunological failure (with the use of CD4 counts) diagnosis can be routinely made in Nigeria since viral load testing is not yet approved for routine
patient monitoring while on ART. Numerous studies in some settings in Africa have demonstrated good rates of survival in ART patients\textsuperscript{27, 85, 128}, high CD4 count restoration\textsuperscript{24, 125, 129}, sustained viral suppression\textsuperscript{24, 125, 130, 131} and good clinical outcomes. However, failure rates on first line therapy is still high in some settings and numerous studies demonstrate that this may be due to late presentation\textsuperscript{14, 132, 133}, advanced disease\textsuperscript{30, 134}, low baseline CD4 count level\textsuperscript{27}, malnutrition\textsuperscript{135}, low body mass index\textsuperscript{128}, presence of opportunistic infection especially tuberculosis\textsuperscript{9, 136, 137}, co-infection with hepatitis B\textsuperscript{138} and C\textsuperscript{139, 140}, long distance from treatment sites\textsuperscript{141}, HIV non-disclosure\textsuperscript{142, 143}, stigma\textsuperscript{126, 144}, low baseline hemoglobin count\textsuperscript{128}, low socio-economic status\textsuperscript{125}, prior non-HAART experience\textsuperscript{145}, WHO stages 3 and 4, alcohol and drug abuse\textsuperscript{146, 147}, clinical depression\textsuperscript{118}, perception of treatment efficacy\textsuperscript{119} and self efficacy\textsuperscript{148}.

No published studies from Africa have looked at predictors of mortality, drop-outs and immune-virologic outcomes by the first year of HAART enrollment with the intention of developing prediction models. As HIV treatment scales up rapidly across Africa and as resources become increasingly scarce, gaps in knowledge and new lessons need to be learned about HIV treatment outcomes in order to streamline future program activities, identify at-risk patients at baseline, address modifiable patient and program characteristics and improve survival and immune-virologic outcomes by the first year and beyond.

5. **RESEARCH METHODOLOGY**

5.1. **Summary of Study Hypothesis**

The main hypothesis of this study is that clinical predictors, particularly baseline (pre-HAART) CD4, prior non-HAART therapy, tuberculosis, baseline hemoglobin and NRTI
regimen backbone and non-clinical predictors, particularly age, gender, HIV disclosure, distance from treatment site, socioeconomic status and adherence can affect HIV treatment outcomes (mortality, drop-out, immuno-virologic failure or immuno-virologic success) by 12 months after HAART commencement. Mortality here is defined as death anytime from date of HAART commencement to 12 months post HAART commencement. Drop-out is defined as default from treatment program (clinic visits and pharmacy refill) for greater than 2 months after the next clinic appointment or discontinuation of treatment or self-transfer to another site where follow-up is impractical. Virologic failure is defined as a viral load estimation > 400 copies / ml at the 12 month visit. Immunologic failure is defined in this study as failure of CD4 lymphocyte count to improve by at least 50 cells /mm3 or worsening to or below baseline pre-HAART CD4 level at 12 months after HAART initiation.

5.2. Study Design

This will be a multi-center observational clinic-based cohort study using prospective data abstracted from medical records and a cross-sectional study involving patient interviews and laboratory work-up at the 12 month clinic visit after HAART commencement. This study will involve patients whose baseline exposure predictor variables were measured and collected before the occurrence of the outcomes of interest. A cohort of all new treatment naïve patients commenced on HAART from 3 representative sites during a 3 month period in the second quarter of 2008 will be assessed at 12 months post-HAART visit for mortality, drop-out or immuno-virologic failure for those alive and active at 12 months. Data abstraction will be done with a standardized tool and information will be retrieved by medical record chart abstraction, and review of laboratory, clinical,
pharmacy and follow-up records. The interview at the 12 month visit will be done with a standardized tool and questionnaire assessing adherence, socio-economic status and other indictors. Laboratory testing will involve viral load testing and routine 12 month tests (CD4 count, hematology, creatinine, liver function tests). Since medical record information will be routinely and prospectively collected by clinical providers, investigator and recall bias will be reduced.

5.3. Study Population

The target study population will comprise of 1164 HIV-1 infected adults on HAART attending routine care in 3 IHVN PEPFAR funded sites located in government owned public secondary and tertiary health facilities in Nigeria. These treatment naïve patients all started on HAART in the second quarter of 2008 (April-June) and are being routinely followed up at the recommended three monthly intervals or sooner as the clinical providers or patients deem fit.

Table 4: List of sites and patient population enrolled into HAART

<table>
<thead>
<tr>
<th>Month Enrolled</th>
<th>Asokoro Hospital</th>
<th>University of Benin Teaching Hospital</th>
<th>University of Abuja Teaching Hospital</th>
</tr>
</thead>
<tbody>
<tr>
<td>April 2008</td>
<td>53</td>
<td>189</td>
<td>116</td>
</tr>
<tr>
<td>May 2008</td>
<td>40</td>
<td>235</td>
<td>143</td>
</tr>
<tr>
<td>June 2008</td>
<td>59</td>
<td>234</td>
<td>95</td>
</tr>
<tr>
<td>TOTAL</td>
<td>152</td>
<td>658</td>
<td>354</td>
</tr>
</tbody>
</table>

Before HAART commencement, all individuals are first enrolled into care based on a confirmed HIV positive test result. Treatment preparation consisting of educational sessions and personal and group counseling are subsequently conducted to prepare patients for life-long care and identify obstacles to effective HIV care and treatment. Patients are encouraged to disclose their HIV status to a family member, bring along a
treatment partner and agree to home visits from home-based care volunteers. Screening is done for opportunistic infections including tuberculosis and baseline laboratory work-up including CD4 count test, full blood count, liver function tests, serum creatinine, hepatitis B surface antigen are done free of charge. Cotrimoxazole is provided free of charge to eligible patients whose CD4 counts are < 200 according to the Nigerian national protocol.

Patients are commenced on HAART based on the following criteria:

| Documented HIV positive serology by parallel testing with 2 different test kits PLUS Immunological criteria of CD4 ≤ 200 irrespective of WHO staging OR Clinical criteria of WHO Staging 3 or 4 irrespective of CD4 count level AND Absence of contraindications to treatment and possibility of adherence, clinical monitoring and follow-up |

5.4. Study Setting

Three representative public government-owned treatment sites located in tertiary hospitals (University of Abuja Teaching Hospital, Gwagwalada and University of Benin Teaching Hospital, Benin) and a secondary hospital (Asoko District Hospital, Abuja) will be used. By mid 2008, these three hospitals have a total of 19,000 patients on ART. All sites are cohort sites of the IHVN program run by government staff but with support of IHVN staff. These sites were chosen because they are representative of typical Nigerian medical facilities providing ART, and have the same contextual, programmatic, structural and health systems issues facing ART programs in Nigeria, thus increasing the generalizability of study findings. CAREWare, the free US government (Department of Health and Human Services) electronic database used for HIV care in the US has been adapted for use in Nigeria by the ACTION project of IHVN. Clinical providers have been
trained to complete standardized patient management and monitoring (PMM) forms which include forms covering medical and social history, demographic information, drug adherence information, pharmacy refill information and information about laboratory tests, drug side effects, switches in medication and other HIV related information. These forms are sent periodically to dedicated monitoring and evaluation (M&E) staff who log in the contents of the PMM forms into the CAREWare database. Summary reports are then issued from the M&E department to clinical providers at each patient’s visit and regular communication maintained to improve quality of data management.

5.5. Eligibility criteria

The proposed eligibility criteria for participant selection include

i. HIV-1 Infected adults (men and non-pregnant women) on HAART in the selected sites

ii. Commencement of HAART between April-June 2008 (should be coming for 12 month visit between April-June 2009)

iii. Aged 18 years or above

iv. Willingness to consent for study and the routine 12 month blood draw done for all patients. Willing to consent for extra test on the blood (viral load)

To confirm eligibility for participation, information in CAREWare database will be cross-checked with the medical records in the clinic charts.

5.6. Sample Size Calculations

Several studies done in public HIV treatment programs and settings similar to Nigeria report viral load suppression rates ranging from 65-85% in the first 12 months of
HAART using a viral load cut off point of < 400 copies/ml\textsuperscript{24, 27, 133, 149}. Using a mid-point value of 75% viral suppression at 12 months, this means that the virologic failure rate is approximately 25%. This is used to calculate the sample size for the number of patients needed for viral load testing.

For a one sample comparison of proportion to hypothesized value, the following table summarizes the sample sizes needed for different assumptions:

<table>
<thead>
<tr>
<th>Alpha ((\alpha))</th>
<th>Beta ((\beta))</th>
<th>Power</th>
<th>Alternatives</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>(p_1=0.15) ((-0.1))</td>
</tr>
<tr>
<td>0.05</td>
<td>0.3</td>
<td>0.7</td>
<td>108</td>
</tr>
<tr>
<td>0.05</td>
<td>0.2</td>
<td>0.8</td>
<td>133</td>
</tr>
<tr>
<td>0.05</td>
<td>0.1</td>
<td>0.9</td>
<td>171</td>
</tr>
</tbody>
</table>

Thus at the 0.05(5\%) level of significance for a two-sided test of 80\% power with a minimum detectable alternative of \(\pm 5\%\) from hypothesized value, a minimum of 610 viral load samples will be needed.

With an assumed laboratory error rate of 5\%, the total viral load samples to be done will be \(610 \times 1.05 = 641\). Using information from historical data and results from studies in similar settings in Africa, an assumption of a mortality rate of 15\%\textsuperscript{26, 85} and drop-out rate of 15\%\textsuperscript{14} is made. Thus, approximately 30\% of the patients will be lost before 12 months. Consequently, the total minimum number for the study will be \(641 \times 1.3 = 834\).
Assuming a study refusal rate of 10%, total minimum needed to be will be \(834 \times 1.1 = 918\) from all three sites. The three sites proposed for the study have a total of 1164 patients enrolled into HAART from the second quarter of 2008 who will be evaluated for their outcome at their 12 month visit in the second quarter of 2009. This is more than the number required to power the study.

5.7. Flow Chart of Study Participants

The flow chart below illustrates the recruitment and study plan:
Figure 3: Flow chart of study plan

1164 patients enrolled on HAART from 3 representative sites – UATH, UBTH, ADH

Mortality by 12 month after HAART enrollment (Outcome 1)

Drop-out by 12 month after HAART enrollment (Outcome 2)

Alive and attend 12 month post HAART visit

Random sample of 650 patients from the 3 sites enrolled into the study

Immuno-virologic failure (Outcome 3)

Immuno-virologic success (Outcome 4)
5.8. **Sources and Methods of Data Collection**

Data for this study will be abstracted from the CAREWare database and the medical chart records of patients who are enrolled into the PEPFAR HIV program in the three participating sites. The CAREWare database contains information about patient demographics including residential addresses, medical history, physical examination including WHO staging, medications, laboratory tests (CD4 count, complete blood count, liver function test, creatinine, etc), adherence information, pharmacy refill records, HIV disclosure information, diagnosis of opportunistic infections including tuberculosis, presence of other co-infections, details about clinic visits. A summary of the patient’s information will be obtained and cross-checked with the medical records and abstracted using a standardized abstraction tool.

An analysis of the 1164 patients will be done and patients who are active and attend the 12 month visit will be recruited for an interview and laboratory work-up. Defaulting patients will be followed up to ascertain their condition. Since most of the patients (>90%) have active cell phone numbers, attempts will be made to reach them in a confidential manner and ascertain their condition. If a patient is confirmed dead, efforts will be made to verify this and obtain a verbal autopsy if possible. Patients who default beyond 2 month after their 12 month clinic visit appointment will be classified among drop-out. It is inevitable that some of the persons classified as drop-out may have actually died and sensitivity analysis will be used to determine the impact of possible misclassification. People who discontinue treatment or self-transfer to another treatment site where follow-up is impractical will also be classified as drop-outs.
Additional information will be collected during the 12 month interview including information on the socio-economic status, adherence, height (for calculation of body mass index) before laboratory tests are done. The laboratory tests will cover viral load test, routine 12 month tests (CD4 count, complete blood count, liver function test, creatinine).

After all the information are abstracted and verified, they are recorded in excel and transferred to STATA for data management.

5.9. Definition and Sources of Outcome Variables

There will be four mutually exclusive outcome variables for all 1164 patients being studied. These outcome variables and their definitions include

a. Mortality by 12 months – death of patient between the date of HAART initiation and the 12 month visit. This will be verified by home visits and phone calls for defaulting patients. Attempts will be made to obtain a verbal autopsy from treatment patients or other close relatives without breaching patient confidentiality. This is already being done as part of routine care.

b. Drop-out- This is defined as discontinuation of treatment, self-transfer to another site where follow-up is impractical or default from clinic visit appointment/pharmacy refill appointment for > 2 months from the last clinic appointment. This information will be verified two months after the last clinic appointments.

c. Virologic success is defined by viral load ≤ 400 copies / ml and immunologic success is defined as change of > 50 CD4 cells from baseline to 12 months while on HAART.

d. Virologic failure is defined by viral load > 400 copies / ml and immunologic failure is defined in this study as failure of CD4 lymphocyte count to improve by at least 50
cells /mm$^3$ or worsening to or below baseline pre-HAART CD4 level at 12 months after HAART initiation.

5.10. Definition and Source of Predictor Variables

Clinical and non-clinical variables and laboratory results will be abstracted from medical records and from the CAREWare database. Only socio-economic status / score and adherence information will be obtained at 12 months in those still active. Adherence will be categorized as dichotomous outcomes ($\geq 95\% = \text{yes}$ and $<95\% = \text{no}$). Adherence of $\geq 95\%$ means less than one missed dose in 1 week in a twice daily dosing schedule or less than one missed dose in 2 weeks in a daily dosing schedule. An adherence questionnaire will be used to probe for obstacles and factors affecting adherence.

A unique socioeconomic score using indicators of highest educational achievement, employment type, household size and household income which have been shown to impact socio-economic status will be used$^{150,151}$. This score is classified in quintiles as follows:
For single patients

Table 6: Socioeconomic indicators that define the socioeconomic score

<table>
<thead>
<tr>
<th>Highest educational achievement</th>
<th>Score</th>
<th>Employment status</th>
<th>Score</th>
<th>Household size (members)</th>
<th>Score</th>
<th>Monthly Household Income (naira)</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
<td>None</td>
<td>0</td>
<td>≥ 12</td>
<td>0</td>
<td>&lt; 15,000</td>
<td>0</td>
</tr>
<tr>
<td>Primary</td>
<td>1</td>
<td>Unskilled workers (e.g. drivers, security guards, cleaners) and related workers</td>
<td>1</td>
<td>9-11</td>
<td>1</td>
<td>15,000 – 30,000</td>
<td>1</td>
</tr>
<tr>
<td>Secondary</td>
<td>2</td>
<td>Lower level office workers, petty traders, bus drivers and related workers</td>
<td>2</td>
<td>6-8</td>
<td>2</td>
<td>31,000 – 60,000</td>
<td>2</td>
</tr>
<tr>
<td>Tertiary</td>
<td>3</td>
<td>Middle level bureaucrats, technicians, skilled artisans, traders and related workers</td>
<td>3</td>
<td>3-5</td>
<td>3</td>
<td>61,000 – 90,000</td>
<td>3</td>
</tr>
<tr>
<td>Advanced (MSc., PhD)</td>
<td>4</td>
<td>Professionals, top civil servants, politicians, businessmen in formal sectors</td>
<td>4</td>
<td>≤ 2</td>
<td>4</td>
<td>&gt; 90,000</td>
<td>4</td>
</tr>
</tbody>
</table>
Table 7: Aggregate socioeconomic score quintiles for singles

<table>
<thead>
<tr>
<th>Aggregate score</th>
<th>Socio-economic class</th>
</tr>
</thead>
<tbody>
<tr>
<td>13-16</td>
<td>Class I</td>
</tr>
<tr>
<td>10-12</td>
<td>Class II</td>
</tr>
<tr>
<td>7-9</td>
<td>Class III</td>
</tr>
<tr>
<td>4-6</td>
<td>Class IV</td>
</tr>
<tr>
<td>0-3</td>
<td>Class V</td>
</tr>
</tbody>
</table>

ii. For married patients

For married patients, the singles socioeconomic score will be administered in addition to spouse highest educational achievement and employment status

Table 8: Additional socioeconomic score for married persons

<table>
<thead>
<tr>
<th>Spouse Highest educational achievement</th>
<th>Score</th>
<th>Spouse Employment status</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>Primary</td>
<td>1</td>
<td>Unskilled workers (e.g. drivers, security guards, cleaners) and related workers</td>
<td>1</td>
</tr>
<tr>
<td>Secondary</td>
<td>2</td>
<td>Lower level office workers, petty traders, bus drivers and related workers</td>
<td>2</td>
</tr>
<tr>
<td>Tertiary</td>
<td>3</td>
<td>Middle level bureaucrats, technicians, skilled artisans, traders and related workers</td>
<td>3</td>
</tr>
<tr>
<td>Advanced (MSc., PhD)</td>
<td>4</td>
<td>Professionals, top civil servants, politicians, businessmen in formal sectors</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 9: Aggregate socioeconomic score quintiles for married persons

<table>
<thead>
<tr>
<th>Aggregate score</th>
<th>Socio-economic class</th>
</tr>
</thead>
<tbody>
<tr>
<td>21-24</td>
<td>Class I</td>
</tr>
<tr>
<td>16-20</td>
<td>Class II</td>
</tr>
<tr>
<td>11-15</td>
<td>Class III</td>
</tr>
<tr>
<td>6-10</td>
<td>Class IV</td>
</tr>
<tr>
<td>0-5</td>
<td>Class V</td>
</tr>
</tbody>
</table>

Doctoral Dissertation_Chuka Anude
6. **QUALITY ASSURANCE AND QUALITY CONTROL**

IHVN has a quality assurance and quality control program for clinical care and laboratory services in order to maintain a consistently high level of quality in all 34 IHVN sites. As a result, the quality of the laboratory testing at the three different sites is expected to be similarly high.

7. **DATA MANAGEMENT**

Data from 1164 patients comprising of 152 from ADH, 658 from UBTH and 354 from UATH will be abstracted from the CAREWare database. Before the beginning of the study, information on each patient will be collated and analyzed for possible missing information which will be filled up in those who are alive and attend the 12 month visit.

The level of completeness of the baseline data as reviewed during a feasibility study trip was good with less than 7% level of missingness in important baseline variables. Data from CAREWare will be compared to medical records and all will be entered into a standardized abstraction tool by trained research assistants and then to an excel database for importation into STATA. Data will be double-entered and cross-checked to ensure quality and linkage to the unique identification number. The database will have checks for incorrect or out of range data entry. A dedicated computer with password protection will be used by locally hired data clerk for data entry and daily regular back-ups will be carried out. All abstraction forms, CAREWare summaries, laboratory results, back up CDs and laptop will be stored in securely locked cabinets and preserved for future audits.

Prior to data analysis, data merging, cleaning and editing will be done in STATA. (STATA version 10, StataCorp Inc., College Station, Texas, USA)
8. STATISTICAL ANALYSIS AND MODELING

8.1. Overview

STATA version 10 will be used for all statistical analysis. Exploratory data analysis will help describe the characteristics of the study population, check for errors and anomalies, allow distributions of variables to be checked for skewness and kurtosis, produce summary statistics and help with initial understanding of the nature and strength of the relationship among variables. Correlations between variables will be assessed. This exploration will help in initially conceptualizing the final statistical model. Normal Q-Q plots, box plots and frequency tables will be used to describe the summaries of the exploratory data analysis. Characteristics of patients from the three participating sites will be compared for differences in predictor and outcome variables using Wilcoxon rank sum tests for continuous variables and Pearson chi square test for dichotomous or categorical variables.

The statistical analysis to be used for each specific aim is outlined below:

8.2. Statistical Analysis for AIM 1

(1a). To determine clinical predictors important in developing a prediction model for mortality, drop-out and immuno-virologic outcomes at 12 months among HIV-1 infected treatment naïve adults commenced on HAART.

Predictors of interest here include baseline (pre-HAART) CD4 count, baseline hemoglobin level, prior non-HAART therapy and TB diagnosis.
To determine non-clinical predictors important in developing a prediction model for mortality, drop-out and immuno-virologic outcomes at 12 months among HIV-1 infected treatment naïve adults commenced on HAART. Predictors of interest here include age, gender, HIV disclosure (or presence of treatment partner) and residential distance from treatment sites.

The four mutually exclusive outcome variables here will be mortality, drop-out, immuno-virologic failure and immuno-virologic success at 12 months. Immuno-virologic outcomes will be determined by CD4 count and viral load tests at the 12 month visit for patients still alive and active on the program as described before. The clinical predictor variables will be baseline CD4 count (categorized into 4 groups: <50, 50-99, 100-199, and ≥ 200 cells/ul), diagnosis of tuberculosis (categorized as a dichotomous outcome (yes=1 or no=0) and low baseline hemoglobin (categorized as a dichotomous outcome: <10g/dl=yes=1 or ≥ 10g/dl =no=0) and prior non-HAART therapy(categorized as a dichotomous outcome (yes=1 or no=0) . The non-clinical predictor variables of interest here are age (continuous and categorical), gender (dichotomous), HIV disclosure and availability of treatment partner (dichotomous outcome, yes = 1, no = 0), residential distance from treatment site (dichotomous outcome, yes=1 if ≤ 100 kilometers, no=0 if >100 kilometers), socioeconomic class (categorized in quintiles from Class 1 to 5 using indicators of highest educational achievement, employment status, household size and household income to calculate socio-economic scores), and adherence (dichotomous outcomes, yes = 1 if adherence ≥ 95%, no = 0 if adherence is < 95%) . Socio-economic score and adherence are not assessed at baseline and can only be assessed for patients still
alive and who attend the 12 month clinic visit. However, educational achievement and employment status which are collected at baseline will still be used to determine relationships with mortality and drop-outs. The baseline demographic characteristics and the predictors outlined above will be compared among patients using the four mutually exclusive outcomes.

Proportions will be calculated and compared using chi square tests within the different levels of the covariates. Continuous variables will be categorized or dichotomized at reasonable levels. The unadjusted and adjusted effects of the baseline predictors will be determined for each of the four outcomes of interest using multinomial logistic regression models and final model will be fit using model specification tests. The 12 month immuno-virologic success outcome will be the reference category to be compared to the mortality, drop-out and 12 month immuno-virologic failure categories for each predictor variable of interest. All results will be assessed at the 0.05 level of significance for a 2-sided test. And final predictor model will be selected by backward selection. All statistically significant predictors will be used to develop the prediction model.

To minimize confounding, predictors and baseline demographic characteristics will not be included in the multivariable model if either they yielded a univariate p-value of $\geq 0.2^{152,153}$ or if removing them changes the coefficient for the predictor of interest by less than 10%$^{154}$. Covariates known to be associated with the outcomes will be forced into the model for face validity regardless of their level of significance. All results will be assessed at a 0.05 level of statistical significance (p-value < 0.05) for a two-sided test. All final models containing all clinical and non-clinical predictors will be fit to assess the final independent predictors affecting mortality, drop-out and immuno-
virologic failure. Final predictor model will be selected through a backward selection procedure as it is less likely that negatively confounded sets of variables will be omitted since the whole completed set will be initially included in the model.

8.3. Statistical Analysis for AIM 2

(2a). To develop a prediction model for mortality, drop-out and immuno-virologic failure at 12 months after HAART commencement in HIV-1 infected treatment naïve adults in Nigeria using significant baseline clinical and non-clinical predictors.

(2b). To develop a policy framework for addressing significant baseline predictors and the prediction model of mortality, drop out and immuno-virologic failure among HIV-1 infected adults commenced on HAART.

A prediction model will be developed using baseline clinical and non-clinical predictors as determined in Aim 1 since temporality is demonstrated by the collection of the baseline parameters before the onset of outcomes. The main goal here is to increase predictive accuracy and minimize prediction error unlike in aims 1 and 4 where the main goal is to identify important independent predictors of the outcomes of interest. The prediction model will be constructed using the generalized cross-validation method (learning set/ test set approach) after dividing the patient population into two (40% for the learning set and 60% for the test or validation set). Model construction and selection will employ significant predictors of interest. Predictors will be modeled in combinations to get the highest meaningful C statistic. Model calibration will be assessed by the Hosmer Lemeshow chi square statistic. Model discrimination will be assessed by the C statistic of
at least 0.8. Final model will be based on parsimony, high area under receiver operating curve (AUROC), and common sense judgment using predictors that are easily obtainable in out-patient clinics in resource-challenged settings. A simplified prediction score will be subsequently developed to help rapidly identify and prioritize vulnerable patients with high probabilities of mortality, drop-out and immuno-virologic failure. Thus limited resources and individualized treatment preparation, educational and support activities can be channeled to patients most likely to benefit from it.

The possibility of transportability of this prediction model to all 34 ACTION sites as part of the HIVQual annual program evaluation exercise will be done to improve linkage of program quality outcomes with clinical outcome indicators.

A policy framework to address and translate the findings of the prediction model will include the health policy principles of patient-centeredness, cost-effectiveness and efficiency, task-shifting, public-private partnerships, referral system strengthening, collaborations with NGOs, CBOs, and FBOs, monitoring and accountability, responsiveness and inclusiveness, sustainability and equity.

8.4. **Statistical Analysis for AIM 3**

*To determine the program-related (contextual and structural) and quality of life related indicators that can influence HIV treatment outcomes.*

*The indicators include human resource capacity, facility infrastructure, quality and accessibility of services, adherence, educational and support services and perceptions of*
For active patients who attend the 12 month clinic visit, a cross-sectional interview with a standardized questionnaire will be administered together with the normal blood draw after consent is obtained. The questionnaire will contain adherence questions, socio-economic status questions and questions related to contextual, structural and quality of life issues. Since it is important to analyze HIV treatment outcomes in the context of the challenging context of HIV treatment programs in the site. General site specific questions include physician-patient ratios, services provided on site and availability of adequate ventilation on site. Patient specific questions include average waiting time, availability of adherence counseling, educational programs, treatment preparation programs, prevention with positives programs, support groups, and questions on perceptions of stigma and quality of care.

The patient specific variables will be analyzed according to the different sites and the different immuno-virologic outcomes. Since all the questions are categorical or dichotomous, Pearson’s chi square test will be used to test for statistically significant differences in sites and immuno-virologic outcomes. Wilcoxon sum tests will be used for continuous outcomes in the site specific variable information.

The baseline predictors identified in Aim 1 and the prediction model developed in Aim 2 will subsequently be analyzed and discussed in the context of the site specific and patient specific structural issues identified all of which can impact HIV treatment outcomes.
Policies and programs will be suggested to address significant contextual, structural and quality of life issues that are found to be associated with negative treatment outcomes.

8.5. Statistical Procedure for AIM 4

To determine the population-level effectiveness and association of immuno-virologic outcomes to NRTI backbone comparing Tenofovir/3TC backbone to an AZT/3TC or a d4T/3TC backbone. The predictor variable here is NRTI backbone and the outcome here is immuno-virologic outcomes at 12 months. Data on adherence and socio-economic status will be collected using a standardized questionnaire as adherence and socio-economic status will be additional confounders in the association of immuno-virologic outcome and NRTI backbone. Adherence will be assessed using objective (pharmacy re-fill records) and subjective (self-report and visual analogue adapted from the MASRI interview from ACTG) means and socio-economic status will be assessed using educational level, employment type.

The hypothesis here is that among patients enrolled into HAART and active and alive at 12 months, patients using AZT/3TC or d4T/3TC regimen, or who have poor adherence (<95% adherence) or low socio-economic status will have statistically significantly increased risk of immuno-virologic failure as determined by the results of viral load and CD4 count testing during the routine 12 month visit and hence demonstrate a poorer population-level effectiveness of the regimen.

Using the baseline NRTI backbone as the predictor variables (TDF/3TC vs. ATZ/3TC vs. d4T/3TC), the four outcomes of mortality, drop-out, immuno-virologic failure and
immuno-virologic success will be compared and proportions tested in all 3 sites using chi-square tests. The predictors of adherence and socio-economic status will be compared and proportions determined in the levels of immuno-virologic outcome variables. Univariate logistic regression models will be used to identify the dependent effects of adherence and socio-economic status on immuno-virologic outcomes. Independent effects of the 3 different drug regimens will be determined using logistic regression analysis and odds ratios of mortality, drop-out and immuno-virologic failure calculated for each drug regimen category. Immuno-virologic success group will be the reference group. Adjustment will be made for disease severity at baseline, adherence and socio-economic status. Significance will be at the 5% level for a 2-sided test. Adherence and socio-economic status will be assessed using a standardized questionnaire. Adherence will be assessed using objective (pharmacy re-fill records) and subjective (self-report and visual analogue scale adapted from the Medication Adherence Self-Report Inventory (MASRI)) tool from ACTG and socio-economic status will be assessed using educational level, house-hold size and employment type.

9. POLICY ANALYSIS IMPLICATION

As part of Aim 4 in this proposed study, a policy framework will be developed to integrate the findings of this study into real public health interventions. A translational research approach involving the use of significant study results and development of relevant context-specific tools and scores that can be applied easily in busy clinic settings to screen patients and help clinical providers improve patient management and outcomes. Efforts will be made to outline the process of engagement of all stakeholders with the
results in order to influence better policies for management of HIV-1 infected adults on HAART and improving treatment outcomes in a comprehensive sustainable locally relevant context specific manner.

10. HUMAN SUBJECT PROTECTION

Ethical approval for the proposed study will be obtained from the ethics committees in the three participating hospitals, the Nigerian National Health Research and Ethics Council (NHREC), and the Institutional Review Board (IRB) of the University of Maryland (UMD). After approval of the study protocol by the UMD, approval will be obtained from the John Hopkins University Bloomberg School of Public Health using the federal wide assurance (FWA) from the UMD.

Informed consent will be obtained from all study participants who present at the 12 month visit for blood draws and interviews. Persons who are dead or dropped out will not be available for the 12 month visit. However, their information in the CAREWare database and medical records charts will be abstracted for use in the analysis.

There will be minimal risks in participating in the study as it will be done as part of routine clinical care in the HIV treatment clinics. A very remote additional risk will be breach of confidentiality and additional steps will be taken to forestall this remote possibility.

It is anticipated that there will be an approximate male to female ratio of 0.45:0.55 based on indicator data reported monthly to PEPFAR and the Centers for Disease Control and Prevention (CDC). This proposed study is limited to adults only and Nigeria is comprised of almost exclusively black Africans with no minority populations.
The confidentiality and privacy of participants will be actively protected. To protect participant confidentiality, all interviews and laboratory results will be kept confidential. All information will be stored in locked cabinets and all staff trained in methods to protect confidentiality. All participants will be assigned a unique identification number. The name and phone number of the study director will be provided to participants. A back-up copy of all information will be kept in a locked file cabinet.

Every effort will be made to emphasize the voluntariness of this study. Decisions to stop or discontinue in the study will be respected and will not affect the regular care of the patient in any way.

11. LIMITATIONS AND STRENGTHS

The main strengths of this study is the real world context of HIV treatment programs in resource-challenged settings with all the infrastructural, contextual and health systems challenges. In addition, using public health treatment facilities available to everyone, use of secondary and tertiary treatment sites, a large sample size, ease of generalizability, use of prospectively entered clinical information, minimization of investigator bias all add up to strengthen the methods used in this study.

Limitations of this proposed study include possibility of measurement error, possibility of missing data with no indication on the mechanism of missingness (assumption of missing completely at random will be made), inability to validate mortality outcomes through a vital statistics database and transportability of the prediction model. In addition, the absence of a control group of HIV-uninfected adults, absence of viral load testing at baseline, the participation of only adults and possible selection bias at HAART initiation.
and recall bias from the 12 month questionnaire administration are also potential limitations. These limitations are however normal experiences in real world public health ART program in many developing countries.

12. **PUBLIC HEALTH SIGNIFICANCE**

There are currently no published studies of predictors of HIV-related mortality, drop-out and immuno-virologic failure together with development of prediction models in any PEPFAR funded public ART program in Africa. This is partly because PEPFAR does not support routine viral load testing and research in general until recently. This study will help provide additional information and tools to bridge the knowledge gap and improve quality of patient care and treatment outcomes which is the current challenge in many ART programs. In addition, information gleaned from this study will help identify and screen patients with a high likelihood of mortality, drop-out and immuno-virologic failure so that they can be prioritized for tailored individualized treatment preparation and other interventions to improve treatment outcomes. This is relevant for all real world ART programs rapidly scaling up in resource challenged settings with fractured health systems.

13. **APPENDICES**

(See attachments)
REFERENCES


98. Sax PE, Gallant JE, Klotman PE. Renal safety of tenofovir disoproxil fumarate. 


158. Chesney MA, Ickovics JR, Chambers DB, et al. Self-reported adherence to antiretroviral medications among participants in HIV clinical trials: the AACTG
