SECOND-LINE ANTIRETROVIRAL THERAPY IN NORTHERN TANZANIA

Habib Ramadhani Omari

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Approved by:

William C. Miller

Steven R. Meshnick

Brian W. Pence

John A. Bartlett

Nathan M. Thielman

ABSTRACT

Habib Ramadhani Omari: Second-Line Antiretroviral Therapy in Northern Tanzania
(Under the direction of William C. Miller)

Following rapid expansion of anti-retroviral therapy (ART) in resource limited settings, some patients are failing and require switching to second line ART. The diagnosis of treatment failure in these settings depends on relatively poorly performing WHO immunological failure criteria. As a consequence, physicians are reluctant to switch patients to second-line and hence, times to switch to second line varies substantially in different programs. Despite the efforts to address the importance of ART adherence, some patients receiving second-line ART are still non-adherent. Limited treatment options underscore the need to explore adherence as well as switching times among patients receiving second-line.

A review of 637 adolescents and adults meeting WHO immunological failure criteria was conducted. Immediate and delayed switching to second-line ART were defined when switching happens at < 3 and ≥ 3 months respectively following failure diagnosis. Those receiving second-line were administered questionnaires that assessed adherence. Optimal and suboptimal cumulative adherence were defined as percentage adherence of ≥ 90% and < 90% respectively. Cox proportional hazard marginal structural models were used to assess the effect of switching to second-line ART and the risk of opportunistic infections and binomial regression models were used to assess the prevalence of suboptimal adherence percentage by pre-switch adherence status. Among 322 participants who had suboptimal adherence to first-line ART, 117 (36.3%) had suboptimal adherence to second-line ART compared to 17/114 (14.9%) who had optimal adherence to first-line. Of 637 participants 74% (n=471) were either delayed or did not switch to second-line. Participants who had suboptimal adherence to first-line

ART were more likely to have suboptimal adherence to second-line ART (APR 2.4, 95% CI 1.5 – 3.9).Switching to second-line ART reduced the risk of opportunistic infections (adjusted hazards ratio [AHR] 0.4, 95% CI 0.2 – 0.6). Compared to patients who switched to second-line ART immediately after failure diagnosis is made, those who delayed switching exhibited a trend toward more opportunistic infections (AHR 1.7, 95% CI 0.6 – 4.4).

Interventions to improve adherence to patients with suboptimal adherence prior to switch as well as increasing physician's awareness about when to switch to second-line ART is critical to improve patient outcomes.

To my one and only daughter Maimuna Habib.

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3TC	Lamivudine
ABC	Abacavir
AHR	Adjusted hazard ratio
AIDS	Acquired immune deficiency syndrome
AITRP	Aids international training research programme
APR	Adjusted prevalence ratio
ART	Anti retroviral therapy
ATZ/r	Atazanavir/ritovir
AZT	Zidovudine
CI	Confidence interval
CM	Cryptococcal meningitis
CTC	Care and treatment center
D4T	Stavudine
EGPAF	Elizabeth glacier pediatric aids foundation
EVF	Efavirenz
HAART	Highly active antiretroviral therapy
HIV	Human immunodeficiency virus
HZ	Herpes zoster
ID	Identification number
IQR	Inter quartile range
IRB	Institutional review board
KCMC	Kilimanjaro Christian Medical Center
KS	Kaposis sacorma
LMIC	Lower and middle income countries

LPV/R	Lopinavir/ritonavir
MEMS	Medication event monitoring systems
MRH	Mawenzi regional hospital
MSM	Marginal structural models
N/A	Not applicable
NACP	National AIDS Control Programme
NC	North Carolina
NIH	National Institute of Health
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NRTI	Nucleoside reverse transcriptase inhibitor
NVP	Nevirapine
OHRP	Office of human research protection
PCP	Pneumocystis carinii pneumonia
PI	Protease inhibitor
PR	Prevalence ratio
RLS	Resource limited settings
RNA	Ribonucleic acid
SAS	Statistical Analysis Software
TB	Tuberculosis
TDF	Tenofovir
USA	United States of America
VAS	Visual analogue scale
VCT	Voluntary counselling and testing
WHO	World Health Organization

CHAPTER 1: SPECIFIC AIMS

As the access to anti-retroviral therapy (ART) expands in resource limited settings, substantial proportions of patients are failing and hence require switch to second-line therapy.[1-4] Due to the laboratory infrastructure and costs associated with routine viral load monitoring, switching to second-line in resource limited settings is entirely dependent on clinical grounds and immunological failure criteria. In this context, virological relapse may not be recognized and the identification of treatment failure may be delayed until participants have a decline in CD4+ cells or clinical manifestations of progressive disease. Despite high potency of second-line ART in viral suppression and restoration of immunity, its success requires optimal adherence to medication.[5-8]

Suboptimal adherence continues to be the main concern among patients receiving second line ART. Given that second line ART is the last treatment option in many parts of resource limited settings, optimal adherence is critically important. Patients switched to second line ART for reasons other than non-adherence to first line appear to be about twice as likely to achieve viral suppression, suggesting that adherence during first line therapy may be an important indicator of adherence during second line therapy.[2] Patients who were non-adherent during firs line therapy may also be non-adherent during second line therapy. If true, targeted intervention could be implemented for these patients prior to switching to second line therapy in order to improve patients' outcomes.

Timing of switching into second line therapy after first line failure is another target for possible intervention to improve patient's outcomes, particularly in programs using immunological failure criteria. Given the relatively poor specificity and positive predictive values of immunological failure criteria in predicting virological failure, many patients with good

treatment success on first line therapy are misclassified as treatment failures.[9,10] Due to the potential of misclassification, physicians delay in deciding when to switch; however, delayed switching is associated with high mortality.[11] Delayed switching may also pose the risk of opportunistic infections among those experiencing unrecognized treatment failures. The magnitude of delay switching on the risk of opportunistic infections in this subset of population meeting immunological failure criteria is unknown. Due to the absence of future treatment options, exploring adherence and impact of switching times on the risk of opportunistic infections is vital in informing physician's decision making to improve patient's outcome.

In this study, we assessed the effect of pre-switch adherence on post-switch adherence among second-line users. We also evaluated the effect of delay in switching and causal impact of switching among patients eligible to second-line by immunological failure criteria on the risk of opportunistic infections. To do this we used prospectively collected clinical data from Kilimanjaro Christian Medical Centre (KCMC), Mawenzi Regional Hospital (MRH), Kilema, Kibosho and Machame infectious disease clinics.

AIM 1: To assess the association of adherence to first-line ART with adherence to second-line ART.

Overview: The primary outcome is the percentage of self-reported adherence to second-line ART. Optimal and suboptimal adherence was defined as percentage adherence of (≥ 90%) and (< 90%) respectively. Binomial regression models were used to assess the association of adherence to first-line ART with adherence to second-line ART. I hypothesized that patients who had suboptimal adherence (< 90%) prior to switch to second-line will have a higher proportion of suboptimal adherence after switching than those who had optimal (≥ 90%) adherence prior to switch.

AIM 2A: Among patients eligible for second-line by immunological failure criteria, what is the effect of immediate versus delayed (≥ 3 months) switching to second-line on the incidence of opportunistic infections?

Overview: The primary outcome is time from immunological failure to the first occurrence of opportunistic infections tuberculosis (TB), pneumonia, Kaposi's sarcoma (KS), cryptococcal meningitis (CM), herpes zoster).[12] Cox proportional hazards marginal structural models (MSM) were used to assess the differences of time to the first occurrence of opportunistic infections by switching status. I hypothesized that among patients eligible for second-line by immunological failure criteria, delayed switching to second-line would result into a higher incidence of opportunistic infections compared to immediate switching.

AIM 2B: Among patients eligible for second-line by immunological failure criteria what is the effect of switching to second-line on the incidence of opportunistic infections?

Overview: The primary outcome is time from immunological failure to the first occurrence of opportunistic infections (tuberculosis, pneumonia, Kaposi's sarcoma, cryptococcal meningitis, herpes zoster).[12] Cox proportional hazards marginal structural models (MSM) were used to assess the differences of time to the first occurrence of opportunistic infections by switching status. I hypothesized that among patients eligible for second-line by immunological failure criteria, switching to second-line would result into a lower incidence of opportunistic infections compared to those who continued with the failed regimen.

CHAPTER 2: BACKGROUND AND SIGNIFICANCE

High potency of second line ART revealed early good treatment outcomes among HIV-infected adults failing first line ART.[2,11] Most patients switched to second line ART have undetectable viral load and improved immunity at 6 and 12 months of follow up.[6,13] Despite good treatment success in many patients, some patients are failing relatively quickly, suggesting medication non-adherence may be a major concern.[13-15] Realizing second line therapy is the last treatment option in many parts of resource limited settings, maximizing its potential benefits by addressing adherence and switching times in these settings is vitally important.

Adherence during first line ART may be an important predictor of adherence during second line ART**.** Sub-optimal adherence in first line ART has been associated with stigma, low social economic status, and long travel distances to the care sites. Provision of free ART and decentralization of ART programs from referral hospitals to health care centers was implemented to improve adherence in many resource limited settings. Despite these efforts, some second-line users are still non-adherent.[5,6,16] Patients switched to second line because of medication non-adherence were less likely to achieve viral suppression. Since success of second line ART depends on high levels of adherence, such an observation may suggest adherence on first line may be an important indicator of adherence during second line.[2,14] Since whether individuals who were non-adherent prior to their switch continue to be non-adherent after switching to second-line ART is unclear, evaluating the association of adherence to first line ART with adherence to second line ART is critical. The findings may help identify this group of patients and calls for targeted intervention prior to their switching into second line therapy in order to improve their outcomes.

Times to switch into second line therapy is another hurdle in the success of second line ART. Lack of routine virological monitoring systems in many parts of resource limited settings causes switching times to vary substantially.[5,16-18] Physicians are reluctant to switch patients to second line because the decision on when to switch is based on relatively poorly performing immunological failure criteria. The longer the time patients remain on failed regimen, the longer they may take to have immunity recovery and be virologically suppressed after switching to second line therapy.[19] As a consequence of delayed switching, patients with unrecognized treatment failure may develop opportunistic infections. Delayed switching is associated with increased risk of mortality.[11] Although causes of deaths were not described, some patients might have had opportunistic infections prior to death. The importance of switching to second line immediately after the diagnosis of treatment failure is made was also evidenced in program with biannual routine viral load monitoring. Patients switched after the first detectable viral load (HIV RNA ≥ 1000c/ml) are more likely to achieve viral suppression than those switched after a second detectable viral load.[2] Substantial variation on switching times calls for further evaluation to explore the impact it may have on the risk of opportunistic infections.

Generally, most of the studies on second line ART in resource limited settings were done in South Africa where patients are routinely monitored using HIV RNA testing. In addition, these studies were done when a few patients were initiated second line ART and therefore their sample sizes are relatively small. Furthermore, the causal effect of switching on the risk of opportunistic infections among patients meeting immunological failure criteria has never been evaluated. We therefore evaluated the association of adherence to first line ART and adherence to second-line. In addition, we explored the effect of delayed switching and the causal effect of switching on the risk of opportunistic infections among patients meeting immunological failure criteria in northern Tanzania. This study analyzed data collected from KCMC, Mawenzi, Kilema, Kibosho and Machame Hospitals, which are located within Moshi Municipality. According to hierarchy of Tanzania health care delivery systems, these hospitals take the first three positions

as referral and regional and district hospitals. All hospitals have access to CD4 cell count testing, and therefore are ideal places for this study.

ART uptake and adherence

In 2011, approximately 1.6 million Tanzanians were living with HIV and an estimated 660,000 persons are in need of ART.[20] Forty one percent of those in need were receiving ART, equivalent to an eight percent increase compared to 2009. In 2004 before ART were made freely available, about 1.4 million persons were estimated to live with HIV, however, fewer than 5000 persons were receiving ART. An increase ART coverage rate was facilitated by international donor supports. Early in 2005, at the time of transitioning to free ART approximately 84% of adults were reported to have 100% adherence (not missing any dose in the last 3 days) in northern part of Tanzania.[21] Due to the variability of adherence measurements, other studies reported adherence of up to 96% in later years.[22,23] Information about adherence to ART after decentralization of ART programs is limited in this region; however, adherence is expected to have improved.

Adherence measurements

Various methods of medication adherence assessments have been developed. The methods include measuring plasma concentration levels of medications, Medication Event Monitoring System (MEMS), pill count, pharmacy records, self-report questionnaires and visual analogue scale (VAS).[21,24-27] Other than plasma drug concentration, none of the remaining methods is superior. For example, MEMS caps measure the number of times medication bottle is open to indicate the patient has taken his/her pill. Neither a missing pill in pill count nor an opened medication bottle necessarily indicates that the patient has taken his/her medication.[28-30] In addition, self-report questionnaires and visual analogue scales are subject to recall bias. Logistical issues including technical expertise and costs associated with some adherence assessment methods impairs their extensive use in many parts of resource limited settings.[31]

Despite the deficiencies of adherence assessments methods, a majority of the patients reporting high level of adherence have undetectable viral load.[21,32,33]

We chose to use self-reported adherence in this study. Patients who failed first line ART by immunological failure criteria were interviewed by phone or in person. Several studies have documented an association between self-reports adherence and viral load, which suggests that self-reports, may be a valid indicator of adherence.[21, 32]

Barriers to ART adherence and efforts to alleviate

ART is a life-long treatment. Its success depends on sustained medication use. The main concern of the interrupted medication use is the development and the spread of resistant strains of viruses leading to treatment failure.[34,35] Many challenges arose when ART became available for treatment of HIV-infected patients. Among the challenges associated with treatment adherence are poverty, stigma associated with HIV infection, long distance travelled to clinics, long waiting hours at the clinic, attitude of the health care workers, substance abuse, forgetfulness, being away from home, side effects etc.

Poverty

Financial constraints have been constantly reported to be associated with treatment interruptions.[21,35] Due to the expenses of purchasing ART medications, few patients were able to continually purchase them. In one study, evaluating risk factors of medication non-adherence at the time when ART was not extensively made free of charge, we asked patients if they had moments spending the money they needed for health care for something else such as paying house rent etc. A substantial proportion of participants agreed to have sacrificed health care for other necessities, suggesting financial constraint as the risk factor of medication non-adherence. Cost associated with long distance travelled to the clinics for regular appointments has also been documented as the reason for medication non-adherence.[36,37]

Recognizing the consequences of medication non-adherence, through donor support, interventions to make ART accessible to people with different economic status were implemented. First, formulations of generic medication by pharmaceutical companies have lowered the cost of ART.[38] Secondly, in many parts of resource limited settings, ART was made free of charge.[23,39] Moreover, ART care and treatment programs have been decentralized from consultant and regional hospitals to district and health care centers in order to minimize the travelling costs.[40-43] Decentralization of ART programs also reduced long waiting hours as patients have options to attend various infectious disease clinics.

Stigma associated with HIV

Fear of discrimination by the family members of community impaired HIV-infected patients to disclose their HIV status and negatively impacts ART medication use.[21,44] Earlier on it was hoped that increased access to ART would turn HIV/AIDS into a more manageable condition and consequently reduce stigma. While this is the case in some parts of world, stigma still persist in many resource limited settings. Efforts to reduce stigma have also included improvement of clinical services, bolstering of health care providers morale, and community health education about HIV.

Adherence as the salvage intervention

The use of immunological failure criteria in identifying treatment failure, lack of resistance testing and limited availability of second line ART makes targeted adherence as the main salvage intervention of improving patient's outcome. Medication non-adherence among patients receiving first line ART proved to be associated with treatment failure as a consequence a number of patients failing first line ART switch to second line.[45,46] In resource limited settings, diagnosis of treatment failure is often late due to the lack of routine viral load testing. Most patients failing first line ART have accumulated resistance mutations.[1,8,21,47,48] The accumulated resistance mutations following medication non-adherence and delayed switching is

considered to have negative impacts on the outcome of patients receiving second line.[19] In addition to the resistance mutation patients might have accumulated following first line treatment failure, medication non-adherence may persist and negatively impact patient outcome among second line users.[5,6,49]

Many ART programs have addressed and implemented adherence support mechanisms. Despite these efforts, for unknown reasons some patients receiving second line ART are still non-adherent.[46] A few treatment sites have third line ART options in resource limited settings and patient failing second line will still be kept on failed regime.[15] To maximize potential benefits of high potency second line ART, further evaluation of predictors of medication non-adherence is critical. Whether patients who were not optimally adherent during first line continue to be non-adherent during second line is unknown. If this relationship exists, limited treatment options calls for identification of this subgroup of patients who might benefit from targeted adherence support mechanisms that involves, in addition to routine counseling, HIV education sessions for patients and family members, and possibly supervisory home-based care that proved to improve adherence elsewhere.[50] Although home-based care seemed challenging due to shortage of staff and long distance to patient's premises, its implementation may not be in demand in these moments when treatment and care programs have been decentralized.

Magnitude of opportunistic infections

Patients failing first line ART may be as equally at risk of acquiring opportunistic infections as those not initiated on ART. Opportunistic infections such as TB, pneumonia, KS, herpes zoster and CM are significant contributors of morbidity and mortality among HIV-infected patients. However, the magnitude of these infections in this group of population is not known.

Tuberculosis

Pulmonary TB is one of the principal causes of death of the infectious diseases among HIV-infected adults in resource limited settings and the disproportionate burden of the disease is seen in this region. Annual notification rate of TB in sub-Saharan Africa has been on the rise since 1980's.[51] It is not surprising the increase in the number of TB cases is related to HIV infections. In Tanzania, the annual cumulative incidence of TB was 107 cases per 100,000 populations in 1990 (figure 2.3). The cumulative incidence increased by approximately 69% in 2004 suggesting that TB is still a huge concern in the country. Despite poor sensitivity of microscopic examination of the sputum in the diagnosis of TB, a combination of chest x-ray and sputum culture improved the diagnosis. Reported prevalence of TB among patients attending infectious disease clinics ranged from (7.2 – 21.6) percent.[52,53] Compared to HIV-infected adults with CD4+ cell > 200 cell/ mm^3, those with CD4+ cell less than 200 cell/mm^3 were more likely to have TB.[53] Since severe immunosuppression is a risk factor of developing TB, it is likely that individuals failing first line ART may also be at risk of acquiring TB.

Cryptococcus meningitis (CM)

CM is one of the most lethal opportunistic infection among HIV-infected patients. Compared to developed countries, sub-Saharan Africa bears a high burden with estimated annual incidence of 720,000 cases.[54] The reported prevalence of CM among admitted HIV-infected adults and among HIV-infected adults presented with headache and altered mental status in Tanzania were 4.4 and 26.8 percent respectively.[55] Severe immunosuppression defined by a CD4+ cell less than 100 cells / mm^3 is predictive of CM. Since some of the patients meeting immunological failure criteria might have persistently low CD4+ cell below 100 cells /mm^3, it is likely that these patients are at risk of acquiring CM. In addition, due to the delay in the diagnosis, up to about 70% of the patients diagnosed with CM in resource limited settings die, suggesting the importance of preventing acquisition of deadly disease.[56]

Pneumocystis pneumonia (PCP)

PCP is another respiratory illness commonly observed in HIV-infected patients. In many African countries, the prevalence of PCP is estimated to be as high as 27 %.[56] Lack of sensitive diagnostic equipment in diagnosing PCP probably underestimate the magnitude of PCP in this region. Prevalence of PCP in Tanzania is similar to those reported in other resource limited settings. Among HIV-infected patients presented with cough in two tertiary hospitals, the prevalence of PCP was 7.5 and 10.4 percent respectively.[52,53] In the developed world, the median CD4+ cell count among patients on HAART diagnosed with PCP is 29 cell/ mm^3 compared to 13 cell/ mm^3 among those not on HAART, suggesting that very low CD4+ cell count may be associated with the development of PCP.[57]

Kaposi's sarcoma (KS)

Of the malignancies affecting HIV-infected patients, KS is the most commonly observed. In a study evaluating the impact of HAART on the incidence of KS, incidence densities among ART naïve patients were 1876, 596 and 624 per 100,000 person-years in Kenya, Uganda and Southern Africa respectively. The corresponding incidence densities among patients on HAART were 201, 270 and 174.[58] Although these findings suggest significant reduction on the incidence of KS, the magnitude of KS among patients failing first line ART is not known. The prevalence of KS among 700 biopsies of oral lesion in Tanzania was 11.1 % of which 95% were associated with HIV.[59]

Herpes zoster

One of the common dermatological manifestations of HIV-infected patients in resource limited settings is development of herpes zoster. Almost all young adults diagnosed with herpes zoster are HIV co-infected. In the pre-ART era, the prevalence of herpes zoster among infectious disease clinic attendees in Tanzania was about 26%.[60] The incidence of herpes zoster after the introduction of free ART in this region is unknown. Herpes zoster is associated

with severe morbidity and hence exploring its magnitude among patients failing first line ART is critical.[61]

Delayed switching and possible risk of opportunistic infections

Identification and management of the patients failing first line ART remained to be a challenge in resource limited settings. Although viral load testing is a gold standard for identification of treatment failure, it is not available, and if available is not for routine use in many parts of resource limited settings.[18,62-64] As a consequence, ART programs in these settings use WHO algorithm to identify treatment failure. The accuracy of WHO criteria in identifying treatment failure is questionable. The reported ranges of sensitivities and positive predictive values are (16 – 58) and (21– 54) percent respectively.[10,65-67] Low sensitivities suggest that a high proportion of patients that should have been switched to second line remained on failed regimen while low positive predictive value suggest a high proportion of patients that should not have been switched to second line, switched unnecessarily. Due to these unintended misclassifications and to maximize the potential benefits of more affordable fist line ART; health care providers are reluctant to switch patients on second line immediately after the diagnosis of treatment failure is made.[11] Low rates of switching into second line (0.5 – 3.3) per 100 person-years further justifies physician's reluctance.[18,68,69] Lack of viral suppression (HIV RNA > 400 c/ml) was seen for each additional month in delaying switching.[19] It is therefore likely that delayed switching may lead to the occurrence in patients with unrecognized treatment failure the opportunistic infections. Diagnosis of treatment failure by using WHO immunological failure criteria is associated with higher levels of HIV RNA and multi nucleoside/nucleotide resistance mutation.[46,70] High levels of HIV RNA is suggestive of continuing viral replication among patients on failed first line regimen. Continued viral replication further poses a risk of opportunistic infection in these patients. Moreover, since standard second line ART includes in addition to boosted protease inhibitors the nucleoside/nucleotide reverse transcriptase inhibitors, a class of

medication that was also used during first line, the effectiveness of second line ART may also be compromised.

Compared to patients meeting WHO immunological failure criteria and switched to second line ART, patients who met WHO immunological failure criteria and remained on first line ART were more likely to die.[63] Although these findings are critical to inform physician decisions on whether to switch patients meeting immunological failure criteria, the study has methodological issues. Patients who switched were more likely to be severely immune compromised than those who did not switch as indicated by the median CD4+ cell count at the time of switch of 82 c/mm^3 and 174 c/mm^3 respectively. Since switching was influenced by CD4+ cell, confounding by indication is a potential problem. In this study, we will assess the causal effect of switching on the risk of opportunistic infections using marginal structural models that take care of confounding by indication.[71]

Due to the relatively poor performance of immunological failure criteria in identifying treatment failure, physicians may find delayed switching of patients to second line to be beneficial for the sake of preserving current treatment options, avoiding exposing patients to more toxic second line, and minimizing the cost of managing toxic effects. However, patients with true treatment failure may develop opportunistic infections.

With these remarks, we explored the impact of delayed switching and the causal effect of switching to second line on the risk of opportunistic infection among patients meeting immunological failure criteria. We believe our findings will add to the existing knowledge the effects of switching times and risk of opportunistic infection as well as informing physician's decision making.

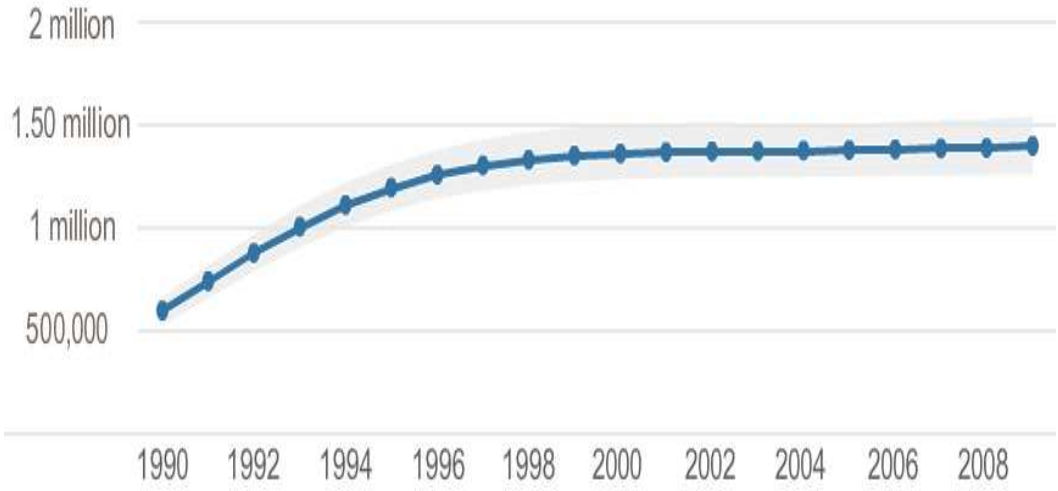


Figure 2.1: Trend in the number of people living with HIV since 1990

Figure 2.2: ART coverage rate: Percent of those receiving ART to those in need between 2009 and 2010

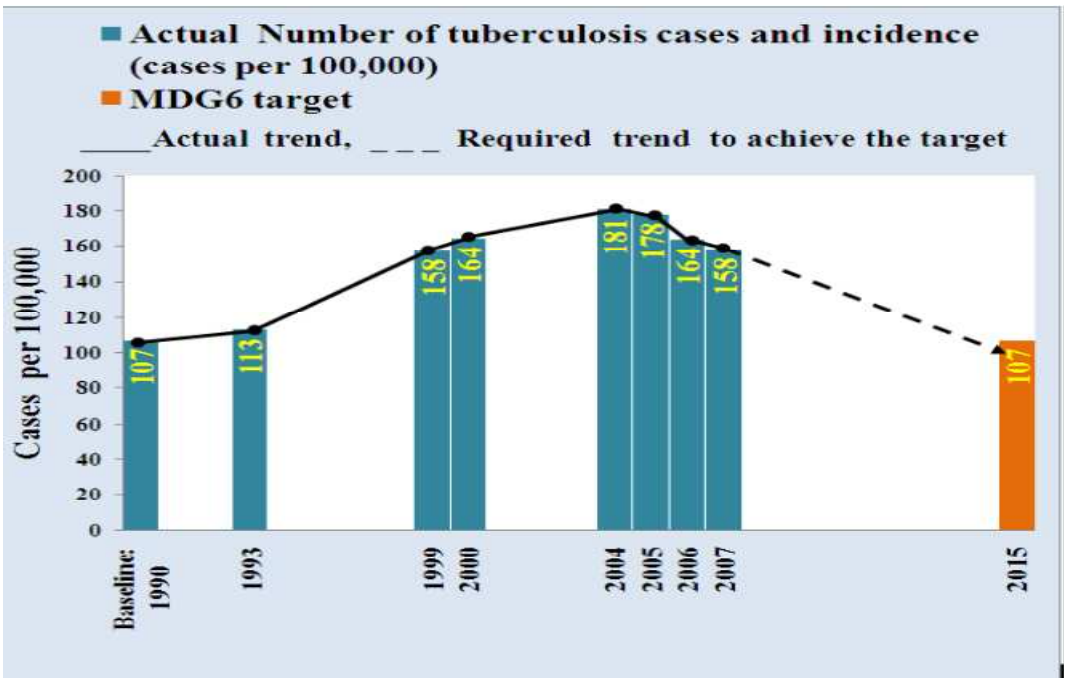


Figure 2.3: Trends in TB incidence in Tanzania

Source: Ministry of Health and Social Welfare

CHAPTER 3: RESEARCH DESIGN AND METHODS

Overview

For our first aim, which explores the association between adherence to first-line ART and adherence to second-line ART, we conducted a cross-sectional study among HIV-infected patients switched to second-line ART. In our second aim that explores switching and delayed switching to second-line ART on the risk of opportunistic infections, we abstracted data from patient's medical records.

Aim 1: Adherence to antiretroviral therapy

To assess the association of adherence to first-line ART with the adherence to second-line ART.

Study population

The study population consisted of HIV-infected adolescent and adult patients attending CTC's at the Kilimanjaro Christian Medical Center (KCMC), Mawenzi Regional Hospital (MRH), and Kibosho, Kilema and Machame Hospitals in Northern Tanzania between January 2004 and August 2013. According to the hierarchy of Tanzania health system, KCMC is a tertiary referral hospital, MRH is a regional hospital, and Kibosho, Kilema and Machame serve as district hospitals. These CTC's offer treatment according to the Tanzanian Ministry of Health treatment guidelines for the provision of ART. Patients received fixed-dose combination of stavudine, lamivudine and nevirapine (D4T/3TC/NVP) as first-line ART. Zidovudine (AZT) and efavirenz (EVF) were used in place of D4T and NVP respectively, depending upon toxicities and concurrent medications. Each patient was seen on a monthly basis and their prescriptions were refilled at each visit. At the time of this study, routine viral load monitoring was not available in these CTC's; therefore, patients were switched to second-line ART based on clinical and

immunological criteria according to WHO Guidelines.[72] We used immunological failure criteria to identify study participant.

The drugs used for second-line ART included tenofovir, abacavir and lopinavir/ritonavir; atazanavir/ritonavir (ATZ/r) was substituted for LPV/r as needed.

The second-line nucleoside reverse transcriptase inhibitor (NRTI) choice for adolescents and adults depended on the first-line ART. For patients on AZT or D4T in first-line ART, the default second-line option was TDF combined with 3TC or FTC and LPV/r. For those who had received TDF in first-line, the second-line option was an AZT-based regimen. For those who were on TDF during first-line because of intolerance to AZT or D4T, an alternative second-line option was abacavir (ABC) combined with 3TC or FTC and LPV/r. Patients who were less than 13 years old were excluded.

The prevalence of HIV infection in 2008 among individuals attending voluntary and testing counseling centers (VCT) in this region was 4.3% and 5.8% for men and women respectively. The lower prevalence of HIV in men may be attributed to the low number of men testing compared to women due to stigma reasons that seem to affect more men than women. The overall prevalence of HIV infection in the country is estimated to be (5.1) %. Given that the overall prevalence of HIV infection in Kilimanjaro region is not very different from that of the country and all patients receive free ART throughout the country, then this was an ideal study population for our study.

Outcome

The primary endpoint was the cumulative percentage adherence to second-line ART. We defined optimal and suboptimal adherence as percentage of self-reported cumulative adherence of ≥ 90% and < 90% respectively. A cut off of 90% was chosen for two reasons: first, we assessed cumulative adherence as opposed to 3 -day recall, which would normally result in higher adherence percentages; and secondly, a previous study showed that variation in plasma

viral load is not increased when adherence is between 90% and 100%; however, adherence below 90% had a significant effect in terms of plasma viral load.[73]

Exposure

The exposure of interest was the cumulative percentage adherence to first-line ART. Optimal and suboptimal adherences were defined as the percentage of self-reported cumulative adherence of ≥ 90% and < 90% respectively.

Other covariates

Other variables evaluated were age, gender, duration on first-line ART, treatment sites, CD4 cell count at the time of treatment initiation, CD4 cell count at time of switch, and the patient's weight. Patient date of birth was used to compute age in years which was categorized as (age < 30, 30 – 55 and > 55). Duration on first-line line was calculated in months and categorized as (duration < 36, 36 – 60 and > 60). Patient's weights were measured in kilograms (kg) and categorized as (weight < 45, 45 – 70 and > 70). Sites were categorized as KCMC, Mawenzi and others. CD4 cell counts were measured in c/mm^3 and were dichotomized as (< 200 and ≥ 200).Gender was assessed as male or female.

Data analysis

Statistical analyses were conducted using Statistical Analysis Software (SAS) version 9.3. Institute Inc., Cary, NC, USA. The distribution of continuous variables was explored to decide whether or not to categorize. Frequencies of categorical variables were calculated as the proportions of patients sampled. Crude and adjusted binomial regression models were used to assess the association of adherence to first-line ART with adherence to second-line ART. All associations were presented as adjusted prevalence ratios (APRs) with 95% confidence intervals (CI).Estimates whose confidence intervals excluded 1, were considered statistically significant. The final multivariate model included established demographic and clinical factors

associated with adherence. Sensitivity analyses were performed with adherence redefined at 85% and 95% cutoffs.

Aim 2: Switching and risk of opportunistic infections

2a. *Among patients eligible for second-line by immunological failure criteria, what is the effect of immediate versus delayed (≥ 3 months) switching to second-line on the incidence of opportunistic infections.*

Study population

The study population consisted of 396 HIV-infected adolescent and adult patients receiving second-line ART from CTC's at the Kilimanjaro Christian Medical Center (KCMC), Mawenzi Regional Hospital (MRH), and Kibosho, Kilema and Machame Hospitals in Northern Tanzania between January 2004 and August 2013.

The drugs used for second-line ART included tenofovir, abacavir and lopinavir/ritonavir; atazanavir/ritonavir (ATZ/r) was substituted for LPV/r as needed. The second-line nucleoside reverse transcriptase inhibitor (NRTI) choice for adolescents and adults depended on the first-line ART. For patients on AZT or D4T in first-line ART, the default second-line option was TDF combined with 3TC or FTC and LPV/r. For those who had received TDF in first-line, the second-line option was an AZT-based regimen. For those who were on TDF during first-line because of intolerance to AZT or D4T, an alternative second-line option was abacavir (ABC) combined with 3TC or FTC and LPV/r. Patients who were less than 13 years old were excluded.

Outcome

The primary end point was the time from immunological failure to the first occurrence of opportunistic infections [tuberculosis (TB), pneumonia, Kaposi's sarcoma (KS), cryptococcal meningitis (CM) and herpes zoster (HZ)]. Time of immunological failure began at the visit when the failure diagnosis was made. All TB, KS, CM and some pneumonia infections were confirmed by the laboratory, histopathology or x-ray, while herpes zoster infections were diagnosed

clinically. We defined loss to follow-up as the absence of a documented clinic visit six months from date of the previous clinic visit.

Exposure

The exposure of interest was delayed switching to second-line ART. We defined immediate and delayed switching if switching happens at < 3 months and ≥ 3 months respectively the diagnosis of immunological failure was made. A cut-off of 3 months was chosen because following a failure diagnosis; patients receive 1-2 months of intensive adherence counselling prior to making the switch.

Other covariates

Other variables evaluated were age, gender, duration on first-line ART, treatment sites, adherence to first and second-line ART. Patient date of birth was used to compute age in years which was categorized as (age < 30, 30 – 55 and > 55). Duration on first-line was calculated in months and categorized as (duration < 36, 36 – 60 and > 60).Adherence to ART was measured in percentage and dichotomized as suboptimal (< 90%) and optimal (≥ 90%). Sites were categorized as tertiary, regional and district hospitals. Gender was assessed as male or female.

Data analysis

Cox proportional hazards marginal structural models were used to assess the differences of time to the first occurrence of opportunistic infections by switching status. Sensitivity analysis was performed by defining time from failure diagnosis to death or first occurrence of opportunistic infections as the outcome.

2b. *Among patients eligible for second-line by immunological failure criteria what is the causal effect of switching to second-line on the incidence of opportunistic infections?*

Study population

The study population consisted of 637 HIV-infected adolescent and adult patients who met WHO immunological failure criteria from CTC's at the Kilimanjaro Christian Medical Center

(KCMC), Mawenzi Regional Hospital (MRH), and Kibosho, Kilema and Machame Hospitals in Northern Tanzania between January 2004 and August 2013.

Outcome

The primary end point was the time from immunological failure to the first occurrence of opportunistic infections [tuberculosis (TB), pneumonia, Kaposi's sarcoma (KS), cryptococcal meningitis (CM) and herpes zoster (HZ)].

Exposure

The exposure of interest was time until switching to second-line ART. We defined switched to second-line as initiation of a boosted PI-based regimen; otherwise, the patient was considered not switched.

Other covariates

Other variables evaluated were age, gender, duration on first-line ART, treatment sites, adherence to first and second-line ART. Patient date of birth was used to compute age in years which was categorized as (age < 30, 30 – 55 and > 55). Duration on first-line was calculated in months and categorized as (duration < 36, 36 – 60 and > 60).Adherence to ART was measured in percentage and dichotomized as suboptimal (< 90%) and optimal (≥ 90%). Sites were categorized as tertiary, regional and district hospitals. Gender was assessed as male or female.

Data analysis

Cox proportional hazards marginal structural models were used to assess the differences of time to the first occurrence of opportunistic infections by switching status. Sensitivity analysis was performed by using propensity scores weighting to assess the effect of switching to second-line ART on the risk of opportunistic infections. In this case the main exposure switching to second-line ART was considered as time fixed mimicking the design of randomized controlled trial where every eligible patient would be assigned either to be switched or not at baseline.

CHAPTER 4: ASSOCIATION OF FIRST-LINE ANTIRETROVIRAL THERAPY ADHERENCE WITH ADHERENCE TO SECOND-LINE ANTIRETROVIRAL THERAPY AMONG HIV-INFECTED PATIENTS IN TANZANIA.

Introduction

Global efforts towards universal access to antiretroviral treatment (ART) have led to an increase in the number of patients receiving ART in low- and middle-income countries (LMIC).[73] ART coverage rose from about 3 million persons in 2007 to 9.7 million in 2012.[19,74] Although clinical, immunological and virological outcomes of the HIV-infected patients receiving first-line ART are promising,[69,75-79], many patients are failing first-line and requiring a switch to second-line ART.[2] About 6% of patients receiving first-line therapy in sub-Saharan Africa need to switch to second-line regimens in any given year.[74] For patients failing first-line ART, the World Health Organization (WHO) recommends switching from non-nucleoside reverse transcriptase inhibitor (NNRTI)-based regimens to protease inhibitor (PI)-based regimens. Most patients switched to second-line PI-based regimens experience good early treatment outcomes [2,11], with undetectable viral load and increased CD4 counts after 6 and 12 months of follow-up.[6,14] Despite treatment success in many patients on second-line ART, some patients fail relatively quickly; an estimated 33% – 40% of patients receiving second-line ART are failing,[8,80] potentially due to medication non-adherence.[13,16]

Medication non-adherence in first-line ART has been associated with stigma, food insecurity, low socioeconomic status, and long travel distances to the care sites.[21,26,36] Provision of free ART and decentralization of ART programs from referral hospitals to health care centers has been implemented in an attempt to improve adherence in many LMIC. Despite these efforts, some second-line users are still non-adherent.[5,6,16] Patients switched to second-line because of medication non-adherence were less likely to achieve viral suppression.[14]

Due to the apparent high genetic barrier to resistance mutations in patients receiving boosted PIs,[80,81] most patients failing PI-based second-line regimens do not have PI resistance mutations, suggesting that non-adherence may be the main reason for treatment failure. [14,80,82] Moreover, compared to patients who switched to second-line due to accumulated resistant viruses, those who switched with wild type viruses were less likely to achieve viral suppression.[14] This observation may also suggest that medication non-adherence is responsible for treatment failure among patients switched into second-line ART. Since success of second-line ART depends on high levels of adherence, these observations imply that adherence on first-line may be an important indicator of adherence to second-line ART.[2,13] If true, targeted interventions could be implemented for these patients prior to switching to second-line therapy, and may improve patient outcomes.

Whether individuals who were non-adherent prior to their switch continue to be non-adherent after switching to second-line ART is unclear. Thus, evaluating the association of adherence to first-line ART with adherence to second-line ART is critical. Furthermore, second-line ART is associated with higher costs, and second-line ART is the final salvage regimen in many LMIC, underscoring the need for evaluation.[80] We used cross-sectional survey data and linked it with prospectively collected clinical data from five care and treatment centers (CTC) located in northern Tanzania to assess the effect of adherence to first-line ART on the adherence to second-line ART.

Methods

Study design and population

We used a cross-sectional study design to evaluate the association of adherence to first-line ART with adherence to second-line ART. The study population consisted of all HIV-infected adolescent and adult patients on second-line ART attending CTC's at the Kilimanjaro Christian Medical Center (KCMC), Mawenzi Regional Hospital (MRH), and Kibosho, Kilema and

Machame Hospitals in Northern Tanzania between January 2004 and August 2013. According to the hierarchy of Tanzania health system, KCMC is a tertiary referral hospital, MRH is a regional hospital, and Kibosho, Kilema and Machame serve as district hospitals. These CTC's offer treatment according to the Tanzanian Ministry of Health treatment guidelines for the provision of ART. Patients received fixed-dose combination of stavudine, lamivudine and nevirapine (D4T/3TC/NVP) as first-line ART. Zidovudine (AZT) and efavirenz (EVF) were used in place of D4T and NVP respectively, depending upon toxicities and concurrent medications. Each patient was seen on a monthly basis and their prescriptions were refilled at each visit. At the time of this study, routine viral load monitoring was not available in these CTC's; therefore, patients were switched to second-line ART based on clinical and immunological criteria according to WHO Guidelines.[72] We used immunological failure criteria to identify study participants.

The drugs used for second-line ART included tenofovir, abacavir and lopinavir/ritonavir; atazanavir/ritonavir (ATZ/r) was substituted for LPV/r as needed. The second-line nucleoside reverse transcriptase inhibitor (NRTI) choice for adolescents and adults depended on the first-line ART. For patients on AZT or D4T in first-line ART, the default second-line option was TDF combined with 3TC or FTC and LPV/r. For those who had received TDF in first-line, the second-line option was an AZT-based regimen. For those who were on TDF during first-line because of intolerance to AZT or D4T, an alternative second-line option was abacavir (ABC) combined with 3TC or FTC and LPV/r. Patients who were less than 13 years old were excluded.

Data collection

After obtaining informed consent, standardized questionnaires translated into Kiswahili were administered to participating patients by trained research nurses. Those who were not captured at their CTC's were interviewed by telephone. The questionnaire addressed demographic characteristics and the patient's adherence before and after switching to second-

line ART. Using a visual analogue scale, participating patients were asked to rate their adherence percentages before and after switching to second-line therapy.

As part of routine HIV clinical care, all patient data including demographics, medication use, opportunistic infections, adherence indicators (adherent = fewer than 2 missed days per month/non-adherent = 2 or more missed days per month), and laboratory values were collected on standardized forms, and entered into a database designed and funded by Tanzanian National AIDS Control Program (NACP) in collaboration with Elizabeth Glacier Pediatric AIDS Foundation (EGPAF). This database was searched for clinical data, and when information was missing, it was abstracted from their respective medical files. Treatment monitoring included clinical and immunological criteria; CD4 cell counts were checked at 4-6 month intervals using flow cytometry.

Definition of variables

The primary endpoint was the cumulative percentage adherence to second-line ART. We defined optimal and suboptimal adherence as percentage of self-reported cumulative adherence of ≥ 90% and < 90% respectively. A cut off of 90% was chosen for two reasons: first, we assessed cumulative adherence as opposed to 3 -day recall, which would normally result in higher adherence percentages; and secondly, a previous study showed that variation in plasma viral load is not increased when adherence is between 90% and 100%; however, adherence below 90% had a significant effect in terms of plasma viral load.[83]

The exposure of interest was the cumulative percentage adherence to first-line ART. Optimal and suboptimal adherences were defined as the percentage of self-reported cumulative adherence of ≥ 90% and < 90% respectively. Other variables evaluated were age, gender, duration on first-line ART, treatment sites, CD4 cell count at the time of treatment initiation, CD4 cell count at time of switch, and the patient's weight.

Institutional review board (IRB) approval was obtained from the University of North Carolina and Kilimanjaro Christian Medical Center (KCMC).

Statistical analyses

Statistical analyses were conducted using Statistical Analysis Software (SAS) version 9.3. Institute Inc., Cary, NC, USA. The distribution of continuous variables was explored to decide whether or not to categorize. Frequencies of categorical variables were calculated as the proportions of patients sampled. Crude and adjusted binomial regression models were used to assess the association of adherence to first-line ART with adherence to second-line ART. All associations were presented as adjusted prevalence ratios (APRs) with 95% confidence intervals (CI).Estimates whose confidence intervals excluded 1, were considered statistically significant. The final multivariate model included established demographic and clinical factors associated with adherence. Sensitivity analyses were performed with adherence redefined at 85% and 95% cutoffs.

Results

From May through August 2013, 11,289 medical files were reviewed, and 656 (5.8%) identified patients who met WHO immunological failure criteria (Figure 4.1). Of these, 456 (69.5%) switched to second-line ART. Of those switched to second-line 20 (4.4%) were children less than 13 years and were excluded. Among the 436 adolescent and adult patients on second-line ART, 279 (64%) were female, and 298 (68.4%) were between 30 and 55 years (Table 4.1). Suboptimal adherence on first-line ART was reported by 322 (73.9%) patients. Most (351; 80.5%) had CD4 cell counts less than 200 c/mm^3 at ART initiation, and 378 (86.7%) had CD4 cell counts less than 200 c/mm^3 at the time of switch. Slightly higher than half of patients (246; 54.4%) spent less than 36 months on first-line ART, and 270 (62%) came from tertiary referral hospital (KCMC) CTC. The majority (278; 63.8%) weighed between 45kg and 70kg.

Suboptimal adherence.

One hundred and thirty-four persons (30.7%) reported cumulative suboptimal adherence to second-line ART. Patients who had suboptimal adherence to first-line ART were much more

likely to have suboptimal adherence to second-line ART than those who had optimal adherence to first-line (PR 2.4, 95% CI 1.5 – 3.9; Table 4.2). In bivariable analyses, compared to patients who weighed less than 45kg, those weighing above 70kg were less likely to have suboptimal adherence to second-line ART (PR 0.5, 95% CI 0.3 –1.0).

After adjusting for age, gender, site, duration on first-line ART, weight, baseline CD4 cell count and the CD4 cell count at the time of switch, the effect of adherence to first-line ART on adherence to second-line ART persisted (APR 2.4, 95% CI 1.5 – 3.9; Table 4.2). The effect of adherence to first-line ART on adherence to second-line ART was substantially stronger than other available factors. Several factors showed positive, but relatively imprecise, associations with suboptimal adherence to second-line ART. For example, patients switched to second-line ART at CD4 cell count less than 200 c/mm^3 were slightly more likely to report suboptimal adherence during second-line than those switched at CD4 cell count more than 200 c/mm^3 (APR 1.2, 95% CI 0. 7 – 1.9). Compared to patients switched into second-line at less than 3 years on first-line, those switched into second-line ART after 5 years were slightly more likely to report suboptimal adherence (APR 1.2, 95% CI 0.8 – 1.8). Patients who weighed more than 70kg continued to be less likely to demonstrate suboptimal adherence to second-line, (APR 0.6, 95% CI 0.3 – 1.1).

Sensitivity analyses

When optimal and suboptimal adherence were defined as percentage of self-reported cumulative adherence of ≥ 95% and < 95% respectively, patients who had suboptimal adherence to first-line ART were more likely to have suboptimal adherence to second-line ART than those who had optimal adherence to first-line (APR 3.0, 95% CI 1.7 – 5.2; Table 4.3). Defining adherence at 85% cutoff, the effect of suboptimal adherence to first-line on suboptimal adherence to second-line persisted (APR 6.0, 95% CI 3.0 – 12.2).

Discussion.

Following increased access to ART in LMIC, a substantial proportion of patients are failing first-line ART and need a switch to second-line ART. Non-adherence to second-line ART negatively affects its potential benefits.[4-6,72] In this study, we have shown that adherence to first-line ART is an important predictor of adherence to second-line ART among HIV-infected adolescents and adults attending five CTC's in Northern Tanzania. Compared to patients reporting optimal adherence to first-line ART, patients with suboptimal adherence to first-line ART were 2.4 times more likely to report suboptimal adherence to second-line ART.

An association between adherence to first-line and second-line ART has been reported in South Africa,[14] in which the odds of >90% adherence to second-line ART was 2.5 times as high among patients whose adherence to first-line ART was above the median (67%) compared to those with first-line adherence below the median. These findings support our hypothesis that patients who are non-adherent to first-line ART are more likely to be non-adherent to second-line.

Others have made similar observations using plasma viral load as the primary end point. For example, fifty-five percent of patients who had sub-therapeutic drug concentrations on first-line ART failed to achieve viral suppression on second-line ART.[72] Compared to patients who switched into second-line for reasons other than non-adherence, those who switched for non-adherence reasons were less likely to achieve viral suppression.[2,83] Patients who were non-adherent on first-line likely continued to be non-adherent after switching into second-line ART, which explains treatment failure after switching.

In this study, about 15% of patients who had optimal adherence to first-line ART had suboptimal adherence to second-line ART. A decline of medication adherence and adherence practices over time has been reported previously.[84,85] Although 36% of our patients who had suboptimal adherence before switching continued to have suboptimal adherence after the switch, the proportion of optimal adherence increased after switching. This may reflect the role

of current counseling efforts on the importance of regular and consistent use of medication. Using medication possession ratio as adherence assessment method, others have shown an increase in median adherence from less than 67% prior to switching to second-line to 92% twelve months post-switch.[14]

Patients with low CD4 cell counts at the time of switch reported suboptimal adherence after switching. Low CD4 cell count values in these patients may be attributed to non-adherence before switching, and possibly these patients continued to be non-adherent after their switches. Our findings can be substantiated by previous studies demonstrating that low CD4 cell count at the time of switch was associated with virological failure and high mortality on second-line ART.[16,49]

Our study does have a number of limitations. We used self-reported adherence to assess adherence to first and second line ART; however, self-reported adherence is subject to both correlation to recall bias and overestimation of adherence percentages.[80,86] Although most patients were on first-line ART for less than three years, some spent more than five years. Since adherence assessment occurred after switching into second-line ART, correlation recall bias is a potential concern, and misclassification could be differential (patients may have overestimated both their first-line and second-line adherence to a similar extent). If most patients overestimated their adherence percentages, patients who had suboptimal adherence to second-line would have reported optimal adherence; however, among patients who had suboptimal adherence to second-line, 87.3 % had suboptimal adherence to first-line ART. Given high sensitivity of first-line adherence in detecting second-line adherence, it is likely that overestimation was minimal. In addition, despite the anticipated bias and overestimations, self-reported assessment has been used extensively and it has being shown to be associated with virological outcome. [87]

We used a cross-sectional design to assess cumulative self-reported adherence on both first-line and second-line ART, and hence failed to account for variation of adherence over time.

As noted previously, adherence and adherence practices may decline over time. For example, adherence may be high when patients are seriously ill. Regaining health may tempt people to engage into practices that may lower their adherence. Smoking and alcohol consumption are among notable practices resumed following recovery, which in turn lowered patients adherence.[84] While 3-30 days recall can produce high levels of adherence,[21] reported median life-time adherence was between 60% and 62%,[88,89] suggesting change of adherence over time. The cross-sectional design of our study did not allow an assessment of the effect of adherence to first-line on adherence to second-line longitudinally.

The standard of care guidelines changed over the study period of 9 years. For example, different antiretroviral drugs became available over time, which could have increased their tolerability, potentially improving adherence. Such a change might result in to higher adherence percentages, yet, about 31% of patients had second-line adherence below 90%.

Conclusion

This study reports adherence to first-line ART as an important predictor of adherence to second-line ART. Although most patients with suboptimal adherence prior to switch had improved adherence after switch, a substantial proportion of patients reported suboptimal adherence after switching. Targeted adherence interventions are needed in patients with low levels of adherence prior to switching to second-line ART to improve patient outcomes.

Table 4.1: Demographic and clinical characteristics of HIV-infected adolescents receiving second -line ART at five infectious disease clinics in Kilimanjaro Region, Moshi, Tanzania, 2004 – 2013.

Characteristics		All patients (n = 436)	Optimal Adherence to second-line ART (n = 302)	Suboptimal Adherence to second-line ART (n = 134)
Adherence to first-line ART	Optimal	114 (26.1)	97 (32.1)	17 (12.7)
	Suboptimal	322 (73.9)	205 (67.9)	117(87.3)
Age	< 30 years	113 (25.9)	77 (25.5)	36 (26.9)
	30 – 55 years	298 (68.4)	207 (68.5)	91 (67.9)
	> 55 years	25 (5.7)	18 (6.0)	7 (5.2)
Gender	Male	157 (36.0)	106 (35.1)	51 (38.1)
	Female	279 (64.0)	196 (64.9)	83 (61.9)
Duration on first-line ART	< 36 months	246 (56.4)	172 (57.0)	74 (55.2)
	36 – 60 months	136 (31.2)	97 (32.1)	39 (29.1)
	> 60 months	54 (12.4)	33 (10.9)	21 (15.7)
CD4 cell count at ART initiation	< 200 c/mm^3	351 (80.5)	240 (79.5)	111 (82.8)
	≥ 200 c/mm³	85 (19.5)	62 (20.5)	23 (17.2)
CD4 cell count at time of switch	< 200 c/mm^3	378 (86.7)	258 (85.4)	120 (89.5)
	≥ 200 c/mm³	58 (13.3)	44 (14.6)	14 (10.5)
Sites	KCMC	270 (61.9)	190 (62.9)	80 (59.7)
	Mawenzi	100 (22.9)	66 (21.9)	34 (25.4)
	Others	66 (15.1)	46 (15.2)	20 (14.9)
Weights	< 45 kg	94 (21.5)	65 (21.5)	29 (21.6)
	45 – 70 kg	278 (63.8)	184 (60.9)	94 (70.2)
	> 70 kg	64 (14.7)	53 (17.6)	11 (8.2)

Abbreviations: ART, Antiretroviral Therapy

Table 4.2: Crude and Adjusted risk factors of suboptimal adherence to second-line ART among HIV-infected adolescents and adults at five infectious disease clinics in Kilimanjaro Region, Moshi, Tanzania, 2004 – 2013.

		Bivariable Analysis	Multivariable Analysis
Variable		Prevalence ratio (95 % CI)	Adjusted prevalence ratio (95 % CI)
Adherence to first-line ART	Optimal	1	1
	Suboptimal	2.4 (1.5 – 3.9)	2.4 (1.5 – 3.9)
Age	< 30 years	1	1
	30 – 55 years	0.9 (0.7 – 1.3)	1.1 (0.7 – 1.5)
	> 55 years	0.9 (0.4 – 1.7)	1.0 (0.5 – 2.0)
Gender	Male	1	1
	Female	0.9 (0.7 – 1.2)	0.9 (0.7 – 1.2)
Duration on first-line ART	< 36 months	1	1
	36 – 60 months	1.0 (0.7 – 1.3)	1.0 (0.7 – 1.3)
	> 60 months	1.3 (0.9 – 1.9)	1.2 (0.8 – 1.8)
CD4 cell count at ART initiation	< 200 c/mm^3	1	1
	≥ 200 c/mm^3	1.2 (0.8 – 1.7)	0.9 (0.6 – 1.3)
CD4 cell count at time of switch	< 200 c/mm^3	1	1
	≥ 200 c/mm^3	1.3 (0.8 – 2.1)	1.2 (0.7 – 1.9)
Sites	KCMC	1	1
	Mawenzi	1.1 (0.8 – 1.6)	1.0 (0.7 – 1.5)
	Others	1.0 (0.7 – 1.5)	1.0 (0.6 – 1.5)
Weights	< 45 kg	1	1
	45 – 70 kg	1.0 (0.8 – 1.5)	1.0 (0.6 – 1.5)
	> 70 kg	0.5 (0.3 – 1.0)	0.6 (0.3 – 1.1)

Abbreviations: CI, Confidence Interval

Table 4.3: Sensitivity Analyses on the effect of adherence to first-line on adherence to second-line ART among HIV-infected adolescents and adults at five infectious disease clinics in Kilimanjaro Region, Moshi, Tanzania, 2004 – 2013.

	Proportion of suboptimal adherence to second-line ART (%)			
Cutoffs	Suboptimal Adherence to first-line ART	Optimal Adherence to first-line ART	Crude Prevalence ratio (95% CI)	Adjusted Prevalence ratio (95% CI)
85%	77/267 (28.8)	8/169 (4.7)	5.3 (2.8 – 9.9)	6.0 (3.08 – 12.2)
95%	241/386 (62.4)	8/50 (16.0)	4.0 (2.1 – 7.5)	3.0 (1.7 – 5.2)

Models adjusted for age, gender, CD4 cell count, sites, duration on first-line ART and weight.

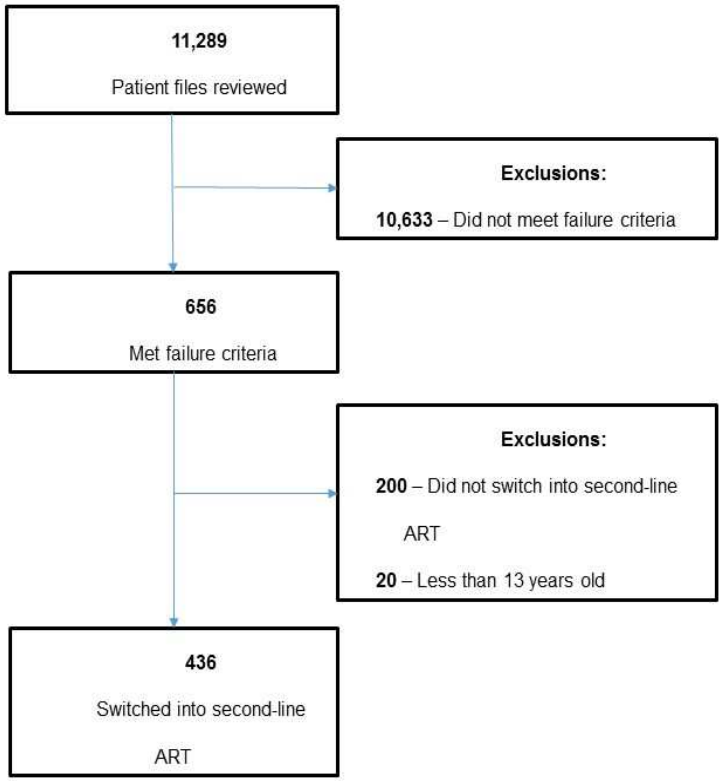


Figure 4.1: Study flow diagram. Selection of HIV-infected adolescents and adults receiving second-line ART at five infectious disease clinics in Kilimanjaro Region, Moshi, Tanzania 2004 – 2013

CHAPTER 5: THE EFFECT OF SWITCHING TO SECOND-LINE ANTIRETROVIRAL THERAPY ON THE RISK OF OPPORTUNISTIC INFECTIONS AMONG HIV-INFECTED PATIENTS IN NORTHERN TANZANIA.

Introduction

Diagnosis and treatment of the patients failing first-line ART in low- and middle-income countries (LMIC) remains challenging.[17,90] Because of the lack of routine viral load monitoring, the definition of treatment failure to depends entirely on WHO clinical and immunological failure criteria.[16,18,62] Due to the potential misclassification of treatment failure resulting from using these failure criteria,[9,10,66,67] as well as, limited availability of treatment options, poor laboratory infrastructure, low health care provider confidence in making switches, and high costs of second-line ART, physicians are often reluctant[16,18,91] to switch. One consequence of unrecognized treatment failures and/or delayed switches may be an increased risk of opportunistic infections.

Delayed switching to second-line ART in programs without routine viral load monitoring is common. A higher proportion of patients remain on a failed first-line regimen in programs without routine viral load monitoring, as compared to those in programs with routine viral load monitoring.[67] Less than 45% of patients meeting WHO immunological and clinical failure criteria are switched to second-line ART.[63,92] The rate of switch is higher in Eastern Europe than in sub-Saharan Africa,[69] and the difference in the rates of switch is attributed to the lack of routine viral load monitoring in African programs. A delay in switching is also indicated by the variation in switching times from failure diagnosis.[17,69] The range of time from failure diagnosis to switch in programs without routine viral load monitoring is 2-15 months.[63] In addition, in resource-

limited settings, switching happens earlier in programs with routine viral load monitoring than in those without.[93]

Despite treatment success for many patients on second-line ART, mortality and virological failure rates are high.[6,14,19,69,80] Virological failure is defined as increasing levels of detectable HIV RNA and is associated with multiple nucleoside/nucleotide resistance mutations.[45,70] Higher levels of HIV RNA pose the risk of opportunistic infections. Other than medication non-adherence, high mortality and treatment failure may also be due to delayed switching among patients with unrecognized treatment failure. Compared to patients meeting clinical/immunological failure criteria and switched into second-line ART, those who met criteria and did not switch had poor survival.[63]

Reports about the frequency of opportunistic infections among patients failing first-line ART in LMIC are limited. Whether disproportionally higher opportunistic infections are observed in patients remaining on failed first-line regimen compared to those who switched to second-line ART is not understood. We sought to explore the influence of switching and delayed switching to second-line ART on the risk of opportunistic infections among patients meeting immunological failure criteria.

Methods

Study design and population.

We used a retrospective cohort study design to evaluate the effect of switching to second-line antiretroviral therapy on the incidence of opportunistic infections among HIV-infected patients in Northern Tanzania. Records of patients attending the Kilimanjaro Christian Medical Centre (KCMC), Mawenzi Regional Hospital (MRH), Kibosho, Kilema and Machame Hospitals between January 2004 and August 2013 were reviewed. Patients who were less than 13 years old were excluded. According to the hierarchy of the health system in Tanzania, KCMC is a tertiary referral hospital, MRH is a regional hospital, and Kibosho, Kilema and Machame are

district hospitals. These care and treatment centers CTC's offer treatment according to the Tanzanian Ministry of Health treatment guidelines for the provision of ART. According to these guidelines, patients receive a fixed-dose combination of stavudine, lamivudine and nevirapine (D4T/3TC/NVP) as first-line ART. Zidovudine (AZT) and efavirenz (EVF) are used in place of D4T and NVP respectively, depending upon toxicities and concurrent medications. Each patient is typically seen on a monthly basis and their prescriptions refilled. At the time of this study, routine viral load monitoring was not available in these CTC's; therefore, patients were switched to second-line ART based on clinical and immunological criteria according to the WHO guidelines[70] of a decline in CD4+ cell count to the pre-treatment value or below, ≥ 50% decline from the peak value CD4+ cell count value while on treatment or persistent low CD4+ cell count < 100 c/mm^3. The drugs used for second-line ART included tenofovir, abacavir and lopinavi/ritonavir; atazanavir/ritonavir (ATZ/r) was substituted for LPV/r as needed. The second-line nucleoside reverse transcriptase inhibitor (NRTI) choice for adolescents and adults depended on the first-line ART. For patients on AZT or D4T in first-line ART, the default second-line option was TDF combined with 3TC or FTC and LPV/r. For those who had received TDF in first-line, the second-line option was an AZT-based regimen. For those who were on TDF during first-line because of intolerance to AZT or D4T, an alternative second-line option was abacavir (ABC) combined with 3TC or FTC and LPV/r.

Data collection

As part of routine HIV clinical care, all patient data including demographics, medication use, opportunistic infections, adherence indicators (adherent = fewer than 2 missed days per month/non-adherent = 2 or more missed days per month), and laboratory values were collected on standardized forms, and entered into a database designed and funded by the Tanzanian National AIDS Control Program (NACP) in collaboration with Elizabeth Glaser Pediatric AIDS Foundation (EGPAF). This database was searched for clinical data, and when information was missing, it was abstracted from their respective medical files. Treatment monitoring included

clinical and immunological criteria; CD4+ cell counts were checked at 4-6 month intervals using flow cytometry.

Definition of variables

The primary end point was the time from immunological failure to the first occurrence of opportunistic infections [tuberculosis (TB), pneumonia, Kaposi's sarcoma (KS), cryptococcal meningitis (CM) and herpes zoster (HZ)]. Time of immunological failure began at the visit when the failure diagnosis was made. All TB, KS, CM and some pneumonia infections were confirmed by the laboratory, histopathology or x-ray, while herpes zoster infections were diagnosed clinically. We defined loss to follow-up as the absence of a documented clinic visit six months from date of the previous clinic visit.

The exposure of interest was time until switching to second-line ART. We defined switching to second-line as initiation of a boosted PI-based regimen; otherwise, the patient was considered not switched. Furthermore, among those switched to second-line, we defined immediate and delayed switching as switching occurring at < 3 months versus ≥ 3 months after the diagnosis of immunological failure was made, respectively. A cut-off of 3 months was chosen because following a failure diagnosis; patients receive 1-2 months of intensive adherence counselling prior to making the switch.

Institutional review board (IRB) approval was obtained from the University of North Carolina and KCMC.

Statistical analyses

Statistical analyses were conducted using Statistical Analysis Software (SAS) version 9.3, SAS Institute Inc., Cary, NC, USA. The distribution of continuous variables was explored to guide categorization. Frequencies and distributions of sociodemographic and clinical characteristics were computed.

Since switching to second-line ART is influenced by CD4+ cell counts, confounding by indication is a potential problem such that patients with low CD4+ cell are more likely to be

switched to second-line than those without low CD4+ cell. We assessed the effect of switching to second-line ART on the risk of opportunistic infections using Cox proportional hazards marginal structural models (MSM) which address confounding by indication. CD4+ cell count was considered as a time-varying confounder. To ensure switching to second-line ART happened after a time-varying confounder, CD4+ cell count values at failure diagnosis were assigned to the preceding month.

Other covariates included age, gender, adherence to first-line ART, adherence to second-line ART, treatment sites and duration spent on first-line ART. Patients began accumulating person time from the moment failure diagnosis was made, and were followed until first occurrence of an opportunistic infection, death, lost to follow-up or end of the study; whichever came first. All patients who died, were lost to follow-up, and did not experience opportunistic infections were censored in the primary analysis.

Marginal structural model analyses use inverse probability of exposure weights to control confounding by time-varying covariates that are also on the causal pathway from exposure to outcome. In this case, the persons were assigned weights inversely proportional to their probability of having the exposure, given their exposure and covariate histories. We used logistic regression models to compute the weights, and all weights were stabilized. Following computation of the stabilized weights, we used Cox proportional hazards marginal structural models to estimate the associations. All associations were presented as adjusted hazard ratios (AHR) with 95% confidence intervals (CI). Estimates whose confidence intervals excluded 1 were regarded as statistically significant.

Kaplan-Meier curves were used to assess the survival distributions among those who were switched and those who were not switched to second-line ART. We used log-rank tests to compare the hazard functions for those switched against those not switched to second-line ART. Poisson regression models were used to compute the incidence rate of opportunistic infections. Two sensitivity analyses were performed, first by defining time from failure diagnosis

to death or first occurrence of opportunistic infections as the outcome and secondly, by using propensity scores weighting to assess the effect of switching to second-line ART on the risk of opportunistic infections. In the second case the main exposure switching to second-line ART was considered as time fixed mimicking the design of randomized controlled trial where every eligible patient would be assigned either to be switched or not at baseline. A Logistic regression model was used to generate scores for which we considered the following variables into the model: Age (< 30, 30 – 55, > 55), gender, months patient spent on first-line ART (< 36, 36 – 60, > 60), CD4 cell count, (< 100, 100 – 200, > 200),sites (tertiary hospital, regional hospital, district hospital), percent adherence to first-line ART (< 90, ≥ 90) and percent adherence to second-line ART (< 90, ≥ 90). Furthermore, we assessed effect of switching on the risk of opportunistic infection by assuming the patient failed between the visit at which physicians have the most current CD4 cell count and the prior visit at which the CD4 cell count was measured. We also assessed if the effect of switching varied with the site.

Results

From May through August 2013, we identified 637 adolescent and adult patients who met WHO immunological failure criteria. Of these, 396 (62.2%) switched to second-line ART while 241 (38.3%) did not. Among those switched, 233 (58.8%) switched within the first 3 months from the time failure diagnosis was made.

Three hundred and ninety four (61.8%) were female and the median age was 39 years (Table 5.1). About half (327; 51.3%) came from the KCMC CTC. Most 476 (74.7%) had suboptimal adherence to first-line antiretroviral therapy and 360 (56.5%) spent less than 36 months on first-line ART. Over 60% (n=396, 62.2%) switched to second-line ART; among those switched, 260 (65.7%) had optimal adherence to second-line ART. At the time of switching to second-line ART, 174 (43.9%) had CD4+ cell count of less than 100/mm^3.

About one-fifth (n=115; 18.1%) experienced an opportunistic infection. The most common infections were pneumonia (n=46; 7.2%) and tuberculosis (n=35; 5.5%). About 5% experienced cryptococcal meningitis, Kaposi's sarcoma, or herpes zoster (n=34; 5.3%).

Survival distributions among those switched and those not switched to second-line ART.

During the follow-up, 637 patients contributed 1181 person-years with overall median of follow up of 1.4 years. Those who switched had a median follow up of 2.3 (IQR; 1.2 – 3.9) years, the corresponding median follow up among those who did not switch was 1.0 (IQR; 0.5 – 1.4) years. Among those who switched, 45 experienced an opportunistic infection (incidence rate=5.4 per 100 person-years [95% CI 4.0-7.5]), as compared to 70 among those who did not switch (incidence rate=15.9 per 100 person-years [95% CI 12.7-19.9]) The difference in probabilities of not developing opportunistic infections among those switched and those who did not switch is apparent from the start of follow-up (Figure 5.1). Kaplan-Meier curves showed a steep decline during the first 16 months among patients not switched to second-line. The six and twelve month probabilities of not having opportunistic infections among those switched to second-line ART were 0.97 and 0.93 respectively, as compared to. 0.87 and 0.64 among those who did not switch, log rank test,($p < 0.001$).

Compared to patients who met immunological failure criteria and did not switch to second-line ART, those who met and switched were less likely to acquire opportunistic infections (AHR 0.4, 95% CI 0.2 – 0.6; Table 5.2).

Compared to patients who switched to second-line ART immediately after their failure diagnosis was made, those with delayed switch were more likely to acquire opportunistic infections (AHR 1.7, 95% CI 0.6 – 4.4; Table 5.3).

When the primary end point was defined as death or the occurrence first opportunistic infections, compared to patients who met immunological failure criteria and did not switch to

second-line ART, those who met and switched were less likely to die or acquire opportunistic infections (AHR 0.4, 95% CI 0.2 – 0.6; Table 5.4).

The logistic regression model used to estimate the propensity score yielded a c-statistic of 0.8. The mean propensity to receive second-line ART was 0.7 (standard deviation, 0.2) compared with 0.4 (standard deviation 0.2) for those who did not receive second-line ART. The distribution of the propensity score for those switched to second-line ART was somewhat higher than those who did not switch to second-line, however, about 97.7% of the propensity scores overlapped between the two groups. Using propensity scores analyses, compared to patients who met immunological failure criteria and did not switch to second-line ART, those who met and switched were less likely to acquire opportunistic infections (AHR 0.2, 95% CI 0.1 – 0.2).

Using the visit at which physicians have the most current CD4 cell count and the prior visit at which the CD4 cell count was measured as the time of failure diagnosis, the effect of switching to second-line remains to be protective against opportunistic infection. The effect of switching to second-line ART did not significantly differ across the sites.

Discussion

The study attempted to assess the association between switching to second-line ART and the risk of opportunistic infections among HIV-infected patients meeting immunologic failure criteria from five CTCs in northern Tanzania. We demonstrated that switching to second-line ART reduced the incidence of opportunistic infections among patients meeting immunological failure criteria by an estimated 60%. Furthermore, time of switching impacted the risk of opportunistic infections with longer delays associated with a 70% increase in risk relative to immediate switching.

In the absence of routine viral load testing for determining treatment failure, delayed switching is common in many low- and middle-income countries.[17,19,69] In this study, only 62% of those meeting immunological failure criteria switched at all and the median time to switch from

the time of immunological failure diagnosis was 5.1 months. Limited availability of the second-line medications, low sensitivity of the WHO immunological failure criteria in predicting virological failure and low confidence of health care provider's in making switches may have contributed to the delays in switching. In addition to these factors delays in switching in this cohort could as also be explained by the low levels of adherence. About 75% of the patients had adherence levels below 90% prior to switch. Those with low levels of adherence may have been less likely to be switched. Similar delays in switching were documented in Haiti in which the median time to switching to second-line ART was 7 months and patients with adherence below 90% were less likely to be switched.[63] Substantial proportions of patients who met immunological failure criteria in a study from Mali [18] did not switch to second-line ART, and the median duration of follow-up from failure diagnosis was 5 months. These observations suggest that delays in switching to second-line ART are a serious management concern across low- and middle-income countries.

Among patients meeting immunological failure criteria, switching to second-line ART is associated with a 60% reduction in the risk of opportunistic infections compared to those who remained on a failed first-line regimen. The association between immunological failure and the incidence of herpes zoster has been reported previously,[94] in which the incidence of herpes zoster was 6.2 episodes per 100 person years. We observed a slightly lower incidence rate of 2.1 episodes per 100 person years. The high incidence of opportunistic infections is likely attributable to unrecognized treatment failure and relatively severe immunosuppression. Among those who switched, nearly half of the patients in our study had CD4+ cell count less than 100 c/mm^3 at the time of switch. Severe immunosuppression defined by CD4+ cell less than 100 c/mm^3 is a clear risk factor for opportunistic infections such as TB and pneumonia.[95,96]

Our sensitivity analyses results were robust with the results from the main analysis. Using propensity scores, switching to second-line ART is associated with an 80% reduction in the risk of opportunistic infections compared to those who remained on a failed first-line

regimen. Combining deaths and opportunistic infections as the end point, the effect of switching to second-line persisted; the risk of death or opportunistic infections was less among those switched compared to those continued on the failed first-line regimen. Among those who died, 31% had opportunistic infections before death. Others have shown previously that delayed switching is associated with an increased risk of mortality.[11,63] Although the causes of death were not ascertained in these studies, it is likely patients had life-threatening opportunistic infections prior to their deaths.

Many of the opportunistic infections in this study were clinically diagnosed. Misdiagnosis might have overestimated the incidence of opportunistic infections. While it is possible to misdiagnose pneumonia infections clinically, the dermatological manifestations of herpes zoster are obvious and unlikely to be misdiagnosed in HIV-infected patients. Moreover, clinical diagnosis of herpes zoster is common in many studies.[94,97,98] Although TB infections were confirmed by sputum examination, the presence of smear negative does not rule out TB especially so in HIV infected patients. The timing of sputum collection and processing also have impact in the diagnosis of TB. Variations in timing and processing from different laboratories might have underestimated the incidence of TB infections.

The timing of diagnosis of opportunistic infections could be measured with some errors in this study. Due to the factors related to patients, access to care and health care systems in LMIC, diagnoses of TB and KS and other AIDS defining cancers are likely to be made late.[99,100] All patients in this study were screened for TB at ART initiation, and were periodically evaluated for TB as they attended their CTC's. In addition, with monthly clinic visits and periodic evaluation errors in the timing of diagnosis of opportunistic infections are likely to be minimal.

Since CD4+ cell counts were measured at intervals of 4-6 months, the diagnosis of treatment failure was made late in most patients, especially those whose CD4+ cells were measured at 6 month intervals. In our analyses, the most current CD4+ cell measurements were assigned to the previous month to account for possible late treatment failure diagnosis.

We did not have HIV- RNA results for the patients and therefore CD4 cell count were used to make failure diagnosis. It is likely that some patients considered failed might have not failed and those with virological failure might have not been included into the study. Such misclassification of the failure diagnosis might have introduced selection bias. Moreover, practically, HIV-RNA is known to influence both opportunistic infections and physician's decision in making switches. However, since physicians did not have the knowledge of the HIV-RNA results, it is unlikely that their decision were directly influenced by the HIV-RNA. High plasma HIV-RNA leads to suppression of the patient's immunity which is being reflected by the low CD4 cell count that was controlled for in this study

Conclusion

The risk of opportunistic infections and death is reduced among patients switched to second-line ART after a diagnosis of immunological failure is made. Furthermore, the risk of opportunistic infection is higher when the delay to switch is 3 months or longer from the time of failure. Delay in switching could mainly be due uncertainties physicians have due to the relatively poor ability of the WHO immunological failure criteria to predict virological failure. If true, efforts should be put to invest on the viral load testing equipment in order to improve the timing of failure diagnosis and manage patients accordingly. This could be made possible by placing a central testing laboratory where patient's dry blood spots from different care and treatment centers could be evaluated. Dry blood spot is known to reduce logistic difficulties and the cost that is associated with the use of plasma.

Table 5.1: Demographic and clinical characteristics of HIV-infected adolescents and adults meeting WHO immunological failure criteria at five infectious disease clinics in Kilimanjaro Region, Moshi, Tanzania, 2004-2013.

Variable		Overall n = 637	Switched to second-line (n=396)	Did not switch to second-line (n=241)
Gender	Male	243 (38.2)	146 (36.9)	97 (40.2)
	Female	394 (61.8)	250 (63.1)	144 (59.8)
Age (IQR)	Median years	39 (32.0 – 46.0)	38 (30.0 – 45.0)	40 (34.0 – 48.0)
Duration on first-line ART	< 36 months	360 (56.5)	222 (56.1)	138 (57.3)
	36 – 60 months	187 (29.4)	146 (31.8)	61 (25.3)
	> 60 months	90 (14.1)	28 (12.1)	32 (17.4)
First-line adherence	Optimal	161 (25.3)	98 (24.8)	63 (26.1)
	Suboptimal	476 (74.7)	298 (75.2)	178 (73.9)
Second-line adherence*	Optimal	260 (40.8)	260 (65.7)	N/A
	Suboptimal	136 (21.3)	136 (34.4)	N/A
CD4 cells at time of switch*	< 100 c/mm^3	174 (27.3)	174 (43.9)	N/A
	100 – 200 c/mm^3	166 (26.0)	166 (41.9)	N/A
	> 200 c/mm^3	56 (8.8)	56 (14.1)	N/A
Sites	Tertiary hospital	327 (51.3)	244 (61.6)	83 (34.5)
	Regional Hospital	167 (26.2)	92 (23.2)	75 (31.1)
	District Hospital	143 (22.5)	60 (15.2)	83 (34.4)
Infections*	Tuberculosis	35 (5.5)	14 (3.5)	21 (8.7)
	Pneumonias	46 (7.2)	19 (4.8)	37 (15.4)
	Meningitis	1 (0.2)	0 (0.0)	1 (0.4)
	Kaposis sacorma	8 (1.3)	3 (0.8)	5 (2.1)
	Herpes zoster	25 (3.9)	9 (2.3)	16 (6.6)

*Percent will not add to 100, N/A = Not applicable

Table 5.2: Risk factors of opportunistic infections among HIV-infected adolescents and adults switched and those not switched into second-line antiretroviral therapy at five infectious disease clinics in Kilimanjaro Region, Tanzania, 2004-2013*

Variable		Number of infections	Person years	Rate/ 100 py	Adjusted HR 95% CI
Switched	No	77	483.3	15.9	1
	Yes	38	697.3	5.4	0.4 (0.2 – 0.6)
Gender	Male	43	489.1	8.8	1
	Female	72	691.5	10.4	1.2 (0.8 – 1.7)
Age	< 30 years	27	270.4	10.0	1
	30 – 55 years	80	827.0	9.7	0.9 (0.5 – 1.5)
	>55 years	8	83.2	9.6	0.6 (0.3 – 1.5)
Duration on first-line ART	< 36 months	76	647.4	11.7	1
	36 – 60 months	29	371.2	7.8	0.5 (0.3 – 0.8)
	>60 months	10	161.9	6.2	0.3 (0.1 – 0.6)
First-line adherence	Optimal	20	357.2	5.6	1
	Suboptimal	95	823.4	11.5	1.5 (0.9 – 2.4)
Second-line adherence	Optimal	43	686.3	6.2	1
	Suboptimal	72	494.3	14.6	1.3 (0.8 – 1.9)
Sites	Tertiary hospital	54	738.3	7.3	1
	Regional Hospital	30	251.8	11.9	1.1 (0.7 – 1.8)
	District Hospital	31	190.4	16.3	1.3 (0.8 – 2.1)

* Hazards ratios are based on analysis of Cox proportional marginal structural models on 636 patients
py = person years

Table 5.3: Risk factors of opportunistic infections among HIV-infected adolescents and adults switched into second-line antiretroviral therapy at five infectious disease clinics in Kilimanjaro Region, Tanzania 2004 – 2013.*

Variable		Number of infections	Person years	Rate/100 py	Adjusted HR (95% CI)
Delayed	No	11	413.4	2.7	1
	Yes	27	505.4	5.3	1.7 (0.6 – 4.4)
Gender	Male	15	368.9	4.0	1
	Female	23	550.0	4.2	1.4 (0.6 – 3.2)
Age	< 30 years	12	232.7	5.2	1
	30 – 55 years	22	632.8	3.5	0.3 (0.1 – 0.9)
	> 55 years	4	53.3	7.5	1.8 (0.1 – 4.2)
Duration on first-line ART	< 36 months	22	536.5	4.1	1
	36 – 60 months	11	290.3	3.8	1.0 (0.3 – 3.6)
	> 60 months	5	92.1	5.4	0.4 (0.1 – 1.3)
First-line adherence	Optimal	5	285.3	1.7	1
	Suboptimal	33	633.5	5.2	4.1 (1.4 – 12.6)
Second-line adherence	Optimal	26	675.6	3.8	1
	Suboptimal	12	243.3	4.9	1.7 (0.6 – 5.2)
Sites	Tertiary hospital	22	648.7	3.4	1
	Regional Hospital	11	172.6	6.4	2.1 (0.7 – 6.1)
	District Hospital	5	97.6	5.1	1.0 (0.3 – 3.7)

* Hazards ratios are based on analysis of Cox proportional marginal structural models on 396 patients
py = person years

Table 5.4: Analyses on the effect of delayed switching on the risk of opportunistic infections/deaths among HIV-infected adolescents and adults switched to second-line antiretroviral therapy at five infectious disease clinics in Kilimanjaro Region, Tanzania 2004 – 2013.*

Variable	Number of infections	Person years	Rate/100 py	Adjusted HR (95% CI)
Switched				
No	92	487.7	18.9	1
Yes	47	700.3	6.7	0.4 (0.2 – 0.6)

*Hazard ratio based on analysis of Cox proportional marginal structural models on 637 patients
Abbreviations: py; person-years, CI; Confidence Intervals

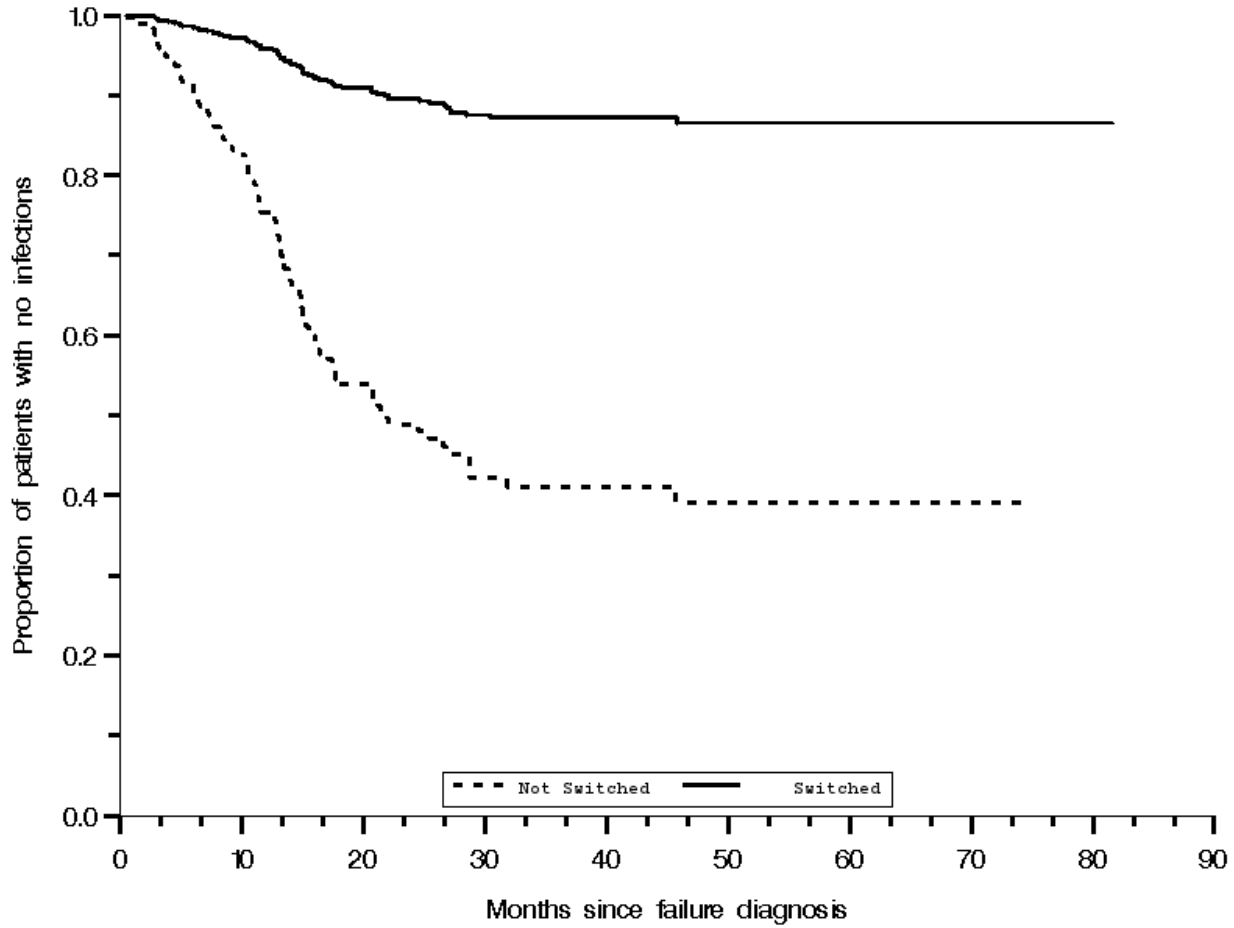


Figure 5.1: Kaplan-Meier curves for 637 HIV-infected adolescent and adult patients according to switching status

CHAPTER 6: DISCUSSION

The study attempted to explore adherence to antiretroviral therapy and the frequency of opportunistic infections among HIV-infected adolescent and adult patients meeting WHO immunological failure criteria in Northern Tanzania. The aims of this study were (1) To determine the association of adherence to first-line antiretroviral therapy with adherence to second-line antiretroviral therapy (2a) To determine the effect of delayed switching to second-line antiretroviral therapy on the risk of opportunistic infections among patients meeting WHO immunological failure criteria and (2b) To determine the effect of switching to second-line antiretroviral therapy on the risk of opportunistic infections among patients meeting WHO immunological failure criteria. To address the first aim we conducted a cross-sectional study in which participants were interviewed about their adherence practices before and after switching to second-line antiretroviral therapy. We conducted retrospective review of patient's medical charts to address the second aim.

Summary of the findings

Aim 1: Association of adherence to first-line ART with adherence to second-line ART

Most patients who had suboptimal adherence prior to switch continued to have suboptimal adherence even after switch. Patients who had suboptimal adherence to first-line ART were 2.4 times as likely to have suboptimal adherence to second-line as those who had optimal adherence to first-line.

Interpretation

Adherence to antiretroviral therapy is still a major concern. Most patients with poor adherence continued to be non-adherent even after receiving adherence counselling. The existing effect size of the association between adherence to first-line ART and adherence to second-line ART should be interpreted with care given the nature of the study design, biases and the method of adherence assessment. For example, adherence to first-line therapy was assessed when patients have already been switched to second-line therapy; given the long time the person has been on first-line, recall bias is likely.

Public health significance

This study is one the first to directly explore the association of adherence to first-line ART and that of adherence to second-line. Antiretroviral therapy reduced morbidity and mortality associated with HIV and its associated opportunistic infections and near perfect adherence is the key of the success. Given the limited availability of treatment options of the patients failing first-line and that second-line ART is the final salvage regimen in most resource poor settings, our findings underscore the need to re-address medication non-adherence in patients prior to switching to second-line therapy to improve their outcomes

Future directions

In this study, we identified adherence to first-line ART as an important predictor of adherence to second-line ART. Practices of improving adherence in these care and treatment centers involves patient provider counselling every time patients come for prescription refill. Although such practice is useful, it may not be sufficiently effective. In addition to routine individual counselling, we suggest involvement of peer supports and supervisory home based care that proved to improve adherence as well as retention of patients to care.[50] Although home-based care is challenging due to shortage of staff and long distance to patient's premises, its implementation may not be demanding in these moments when treatment and care programs have been decentralized. Since this was the first study to directly assess the association

between adherence to first-line ART and adherence to second-line ART, we suggest other investigators conduct studies to further explore this relationship.

Summary of the findings

Aim 2: Switching and risk of opportunistic infections

Among patients meeting WHO immunological failure criteria, switching to second-line reduced the risk of opportunistic infection by 60%. Among those switched to second-line, switching at 3 months or more after failure diagnosis is made is associated with increased risk of opportunistic infections.

Interpretation

Our study highlights that delayed switching to second line ART is common. About 74% of the patients who met WHO immunological failure criteria in our study did not receive second-line therapy in a timely manner, meaning they were either not switched or switching happen at 3 month or more after failure diagnosis was made. Timely switching reduced risk of opportunistic infections and deaths.

Public health significance

Low rates of switching to second-line appears common, despite increasing number of patients receiving first-line.[18,68,69] Furthermore, patients meeting clinical and immunological failure criteria also experience delayed switching to second line.[63,92] We hypothesized that delays in switching could pose an increased risk of opportunistic infections among patients with unrecognized treatment failure. Our observations supported our hypothesis: the risk of opportunistic infections was higher among patients who met WHO immunological failure criteria and did not switch to second line ART. Furthermore, the risk of death was also higher among those who did not switch. The research carries important public health significance, in order to improve outcome of the patients, switching has to be done immediately following failure diagnosis.

Future directions

In this study, we identified delayed switching to second-line ART increases the risk of opportunistic infections or deaths. We suggest more investment in viral load testing equipment is needed to improve the timing of failure diagnosis and manage patients accordingly. Viral load testing could be made possible using a central testing laboratory and shipped dry blood spots. Dry blood spot reduce logistic difficulties and the cost that is associated with the use of plasma. [101] In addition, physician education on the importance of adhering to treatment guidelines is critical in order to reduce variation in switching times.

Overall our study carries important implementation science messages that emphasize the need to incorporate adherence interventions to patients with mal-adherence problems. Involvement of peer support and home based care services are affordable and sustainable over long time at these times where ART services have been decentralized. Furthermore, the study increased physicians and health care provider's awareness on the timing of switching patients to second-line ART. It is hoped that improving the diagnosis of treatment failure would result in immediate switch thereby improving patient's outcomes.

APPENDIX 1: INFORMED CONSENT

Consent to participate in a Research Study Entitled “Causal effect of switching to second-line antiretroviral therapy and the risk of opportunistic infections in Northern Tanzania”.

IRB study # 13-1862

Consent Form Version Date: May 20, 2013.

Principal Investigator: Habib Ramadhani Omari

Funding source: Duke AIDS International Training Research Program (AITRP)

INTRODUCTION

You are being asked to take part in this research study because you are infected with HIV, the virus that causes AIDS, and you have been switched to second-line antiretroviral therapy. This study is sponsored by the Duke Aids International Training Research Program (AITRP) which is funded through the National Institute of Health (NIH) in the United States. This study is under the direction of Dr Habib Ramadhani and Dr Venance Maro at Kilimanjaro Christian Medical Centre (KCMC) and Dr. John Bartlett from Duke University Health Systems. Research studies are voluntary and include only people who choose to take part. Please read this consent form carefully and take your time making your decision. As your study staff member discusses this consent form with you, please ask him/her to explain any words or information that you do not clearly understand. The nature of the study, risks, inconveniences, discomforts, and other important information about the study are listed below. You are free to ask questions about this study at any time. If you agree to take part in this study, you will be asked to sign and date this consent form. You will get a copy to keep.

WHY IS THIS STUDY BEING DONE?

The purpose of this study is to evaluate the association of adherence to first-line antiretroviral therapy and adherence to second-line antiretroviral therapy.

WHAT DO I HAVE TO DO IF I AM IN THIS STUDY?

Screening

If you think you would like to join in this study, you will be asked to sign a consent form. You will be asked questions and examined to check your overall health. You will be asked questions about your adherence experience before and after you have been switched to second-line antiretroviral therapy.

If you do not enroll into the study

After signing the consent, if you decide not to take part in this study or if you don't meet eligibility requirements, we will still use some of your information. As part of the screening visit, data are being collected from you that might be useful for researchers. You are being asked to allow the researchers to keep some of the demographic (for example age, gender, race), clinical (for example disease condition, diagnosis) information that was collected during the screening visit in a database. You will be given a unique code number that will be used on all your information so that your identity will not be known.

Entry

If you have met all eligibility criteria to enter the study, you will be interviewed right away if you decide to take part in this study. You will be asked questions about your health and questions pertaining to medication adherence.

HOW MANY PEOPLE WILL TAKE PART IN THIS STUDY?

About 400 people will take part in this study. All study participants will be recruited from KCMC, Mawenzi, Kibosho, Kilema and Machame infectious disease clinics.

HOW LONG WILL I BE IN THIS STUDY?

If you agree to take part in this study you will only be available at the time of being interviewed. The interview is expected to last for about 30 minutes.

WHAT ARE THE RISKS OF THE STUDY?

Risk of loss of confidentiality

It is possible that participating in this study will make it difficult for you to keep your HIV status secret from people close to you. This may lead to unwelcome discussions about or reactions to your HIV status. Please talk with the study staff if you have any concerns in this regard. We will do everything we can to protect your confidentiality but this cannot be guaranteed.

ARE THERE BENEFITS TO TAKING PART IN THIS STUDY?

If you decide to take part in this study, there may or may not be direct medical benefit to you. Your health care provider will have results of adherence practices. He/she may plan to discuss with you how best you can be assisted to improve your adherence. We also hope the information learned from this study will benefit other people with HIV in the future.

WHAT OTHER CHOICES DO I HAVE BESIDES THIS STUDY?

Instead of being in this study you have the choice of treatment with the same or other anti HIV drugs available to you outside of the study.

WILL MY INFORMATION BE KEPT CONFIDENTIAL?

Study records that identify you will be kept confidential as required by law. Federal Privacy Regulations provide safeguards for privacy, security and authorized access. Except when required by law, you will not be identified in study records disclosed outside of Duke University or KCMC. For records disclosed outside Duke University or KCMC, you will be assigned a unique code number. The key to the code will be kept in a locked filed in the research staff offices. In addition, your records may be reviewed in order to meet Tanzanian and U.S. regulations. Reviewers include U.S. Office for Human Research Protections (OHRP), the National Ministry of Health in Tanzania, KCMC Ethic Committee, the Duke University Health Systems Institutional Review Board (IRB), the U.S. NIH, study staff, and study monitors. If any of these groups review your research record, they may also need to review your entire medical record. The study results will be retained in your research record forever. Any research

information in your medical record will also be kept indefinitely. If this information is disclosed to outside reviewers for audit purposes, it may be further disclosed by them and may not be covered by the federal privacy regulations. This information may be further disclosed by the sponsor of this study, the U.S. NIH. If disclosed by the sponsor, the information is no longer covered by the federal privacy regulations. While the information and data resulting from this study may be presented at scientific meetings or published in a scientific journal, your identity will not be revealed.

WHAT ARE THE COSTS TO ME?

There are no additional costs related to participating in this study.

WILL I RECEIVE ANY PAYMENTS?

There will be no financial compensation for participating in this study.

WHAT ABOUT RESEARCH RELATED INJURIES?

Immediate necessary care and support is available if an individual is injured because of participation in this research project, however, there is no provision for free medical care or for monetary compensation for such an injury. For questions about the study or research-related injury, contact Dr. Venance Maro at 0754 581444.

WHAT ARE MY RIGHTS AS STUDY PARTICIPANT?

Taking part in this study is completely voluntary. You may decide not to take part in this study or leave this study at any time. You will be treated the same no matter what you decide. Your decision to not participate or to withdraw from the study will not involve any penalty or loss of benefits to which you are entitled, and will not affect your access to health care at KCMC, Mawenzi, Kibosho, Kilema or Machame. If you do decide to withdraw, we ask that you contact Dr. Venance Maro in writing and let him know that you are withdrawing from the study. His mailing address is KCMC-Duke Collaboration, Box 3010, Sokoine Road, Moshi. We will tell you about new information from this or other studies that may affect your health, welfare or willingness to stay in this study. If you want the results of the study, let the study staffs know.

WHAT DO I DO IF I HAVE QUESTIONS OR PROBLEMS?

For questions about the study or a research-related injury, or if you have complaints, concerns or suggestions about the research, contact Dr. Venance Maro at 0754 581444. For questions about your rights as a research participant, or to discuss problems, concerns or suggestions related to the research, or to obtain information or offer input about the research, contact the Kilimanjaro Christian Medical Centre (KCMC) Ethics Committee at telephone number (255) 27 27-53909 or the Duke University Health Systems Institutional Review Board at +1-919-668-5111.

STATEMENT OF CONSENT

"The purpose of this study, procedures to be followed, risks and benefits have been explained to me. I have been allowed to ask the questions I have, and my questions have been answered to my satisfaction. I have been told whom to contact if I have additional questions. I have read this consent form and agree to be in this study with the understanding that I may withdraw at any time. I have been told that I will be given a signed and dated copy of this consent form to keep."

_____

Participant's Name (Print) Participant's Signature Date

"As the representative of the subject, I am acting on behalf of the subject and am aware of no factor that would be considered to create a conflict of interest (such as a potential independent personal benefit) for me in consenting to the subject's participation in this study."

_____

Participant's legally Authorized Representative's Signature Date

_____

Representative (print) (As appropriate)

_____

Study staff's Name Study staff's Signature Date

Witness Name Witness Signature Date

APPENDIX 2: ADHERENCE QUESTIONNAIRE

ASSOCIATION OF FIRST-LINE ANTIRETROVIRAL THERAPY ADHERENCE WITH ADHERENCE TO SECOND-LINE ANTIRETROVIRAL THERAPY AMONG HIV-INFECTED PATIENTS IN TANZANIA.

Study subject ID number: _____Date (*dd-mm-yyyy*): __ __ - __ __ - __ __ __ __

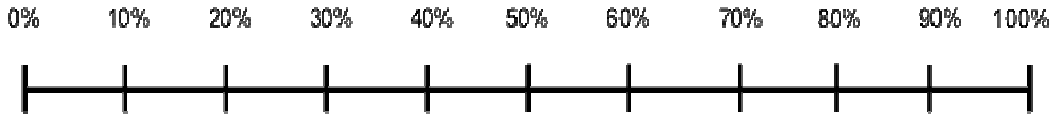
Study subject initials: | | | | Abstractor's (full name): _____

INSTRUCTIIONS

Check that the patient has been taking ARV's for 6 months or more before starting
Use the patient medical records when necessary
Ask the patient all the questions in this questinnaire
Following the instructions in italics in the right hand column
When you are filling boxes, right- justify your answer

1.1 What is your sex? *Choose only one.* *Circle the answer.*	Male.....1 Female.....2
1.2 What is your date of birth? *If only year is known, please code as 01-07-yyyy*	*(dd-mm-yyyy)* ___ ___ - ___ ___ - ___ ___ ___ ___
1.3 What district do you live in? *Choose only one* *Circle the answer*	Moshi rural.....1 Moshi urban.....2 Hai.....3 Rombo.....4 Mwanga.....5 Same.....6 Other.....7 *If answer is other, specify here*:_____
1.4 What region do you live in? *Choose only one* *Circle the answer*	Kilimanjaro.....1 Arusha.....2 Tanga .....3 Singida.....4 Dodoma.....5

	Manyara.....6 Other.....7 *If answer is other, specify here*:_____
1.5 Do you know CD4 cell count?	Yes……1 No……2
1.6 Is high CD4 cell count good or bad?	Good……..1 Bad……..2
1.7 When does the CD4 cell count rises?	When you take your medication constantly……..1 When you don't take your medication constantly……..2
1.8 What type of medications are you taking now?	1……. 2……. 3…….
1.9 How do you take your medications now	Once a day……1 Twice a day.……..2 Thrice a day……...3
1.10 How many doses of medication did you skip today?	☐☐☐
1.11 How many doses of medication did you skip yesterday?	☐☐☐
1.12 How many doses of medication did you skip a two days ago	☐☐☐
1.13 Ever since you switched to second-line, can you estimate the amount of medication you have been taking e.g 0% = you did not take any of the prescribed drugs 25%= you took only ¼ of the prescribed drugs 50%= you took only 1/5 of the prescribed	☐☐☐

drugs 75% = you took only ¾ of the prescribed drugs 100% = you tool all prescribed drugs	
	
Before you switched to second-line, can you estimate the amount of medication you have been taking e.g 0% = you did not take any of the prescribed drugs 25%= you took only ¼ of the prescribed drugs 50%= you took only 1/5 of the prescribed drugs 75% = you took only ¾ of the prescribed drugs 100% = you tool all prescribed drugs	
	

APPENDIX 3: QUESTIONNAIRE

CAUSAL EFFECT SWITCHING INTO SECOND LINE ART AND THE RISK OF OPPORTUNISTIC INFECTIONS IN NORTHERN TANZANIA
STUDY QUESTIONNAIRE

Study subject ID number: _____Date (*dd-mm-yyyy*): __ __ - __ __ - __ __ __ __

Study subject initials: | | | | Abstractor's (full name): _____

INSTRUCTIONS

Check that the patient has been taking ARV's for 6 months or more before starting
Use the patient medical records
Following the instructions in italics in the right hand column
When you are filling boxes, right- justify your answer

1.0 SOCIODEMOGRAPHICS

1.1 What is your sex? *Choose only one.* *Circle the answer.*	Male.....1 Female.....2
1.2 What is your date of birth? *If only year is known, please code as 01-07-yyyy*	*(dd-mm-yyyy)* ___ ___ - ___ ___ - ___ ___ ___ _
1.3 What district do you live in? *Choose only one* *Circle the answer*	Moshi rural.....1 Moshi urban .....2 Hai.....3 Rombo.....4 Mwanga…….....5 Same .....6 Other .....7 *If answer is other, specify here*:_____
1.4 What region do you live in? *Choose only one* *Circle the answer*	Kilimanjaro.....1 Arusha.....2 Tanga 3

	Singida…….....4 Dodoma…….....5 Manyara….....6 Other….....7 *If answer is other, specify here*:_____
1.5 Visit number	☐☐☐
1.6 Visit date	*dd-mm-yyyy)* ___ ___ - ___ ___ - ___ ___ ___ __
1.7 Patient weight	☐☐☐
1.8 Patient height	☐☐☐
1.9 Site	KCMC .....1 Mawenzi.....2 Kibosho.....3 Kilema….....4 Machame….....5
1.10 What is your marital status? *Choose only one* *Circle the answer*	Married (monogamous)….....1 Married (polygamous).....2 Engaged…….....3 Co-habitating .....4 Divorced/separated.....5 Widowed…….....6 Single.....7

2.0 ART

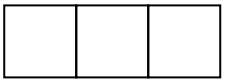
2.1 Date of ART initiation	*dd-mm-yyyy)* ___ ___ - ___ ___ - ___ ___ ___ ___
2.2 ART Regimen *Choose only one* *Circle the answer*	D4T + 3TC + NVP….....1 D4T + 3TC + EVF.....2 AZT + 3TC + NVP.....3 AZT + 3TC + EVF.....4 TDF + 3TC + LPVr/ATVr.....5 TDF + FTC + LPVr/ATVr.....6 ABC + DDI + LPVr/ATVr.....7
2.3 Type of ART? *Choose only one* *Circle the answer*	First line……1 Second line……..2
2.4 Is the patient switched? *Choose only one* *Circle the answer*	Yes……1 No……..2
2.5 Date switched	*dd-mm-yyyy)* ___ ___ - ___ ___ - ___ ___ ___ ___
2.6 Switch visit	☐☐☐

3.0 OPPORTUNISTIC INFECTIONS

3.0 Do you have opportunistic infections? *Choose only one* *Circle the answer*	None.....1 Tuberculosis.....2 Pneumonia.....3 Meningitis.....4 Kaposis sacoma.....5 Shingles.....6 Other…….....7
3.1 Date of diagnosis	*dd-mm-yyyy)* ___ ___ - ___ ___ - ___ ___ ___ ___
3.2 Diagnosis visit	☐☐☐

4.0 CD4 AND FAILURE CRITERIA

4.1 CD4 cell count (CD4)	☐☐☐
4.2 Is CD4 cell count less or equal to baseline? (If follow up is less than 6 months circle NA)	NA…….....1 No.....2 Yes.....3
4.3 Is CD4 cell count persistently less than 100 for the past 6 months? (If follow up is less than 6 months circle NA)	NA…….....1 No.....2 Yes.....3

4.4 Has CD4 cell dropped>50% of the maximum value? (If follow up is less than 6 months circle NA)	NA…….....1 No.....2 Yes.....3
4.5 Is the patient failed?	NA…….....1 No.....2
4.6 Date of failure (datefail)	*dd-mm-yyyy)* ___ ___ - ___ ___ - ___ ___ ___ ___
4.7 Failure visit	
In	
Out	

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