A STUDY OF CD4-STRATIFIED TIMING OF ANTIRETROVIRAL THERAPY AMONG PATIENTS RECEIVING INTEGRATED TUBERCULOSIS AND HIV TREATMENT IN A HIGHLY RESOURCE-LIMITED SETTING

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ABSTRACT

Monita R. Patel: A Study of CD4-Stratified Timing of Antiretroviral Therapy among Patients Receiving Integrated Tuberculosis and HIV Treatment in a Highly Resource-Limited Setting
(Under the direction of Annelies Van Rie)

In 2012, the World Health Organization recommended that HIV-infected patients starting tuberculosis (TB) treatment be initiated on antiretroviral therapy (ART) after 8 weeks if CD4 count ≥50 cells/mm3 and after 2 weeks if CD4 count <50 cells/mm3. Examination of this type of CD4-stratified ART timing strategy would be useful to inform development and implementation of this new recommendation. In the Integration of TB and AntiRetroviral Treatment study, nurses implemented a CD4-stratified timing strategy for ART initiation among HIV-infected patients starting TB treatment in Kinshasa, Democratic Republic of Congo. Participants were eligible for ART initiation at 1 month if CD4 count <100 cells/mm^3 or WHO clinical stage 4 for reason other than extrapulmonary TB, at 2 months if CD4 count 100-350 cells/mm^3, or at completion of TB treatment if subsequently CD4 count ≤350 cells/mm^3 or WHO clinical stage 4. We compared expected and observed timing of ART initiation and used logistic regression with backward stepwise elimination to determine predictors of delayed ART initiation, defined as deviation from strategy. Subsequently, we used the parametric g-formula to estimate the difference in 6-month mortality risk in the population with observed fidelity to CD4-stratified ART timing and in the population complete (100%) fidelity to CD4-stratifeid ART timing. Of 492 adult participants, 235 (47.8%) experienced delayed ART initiation. Contraindication to any ARV drug (adjOR 2.91, 95% CI 1.22-6.95), lower baseline CD4 count (adjOR 1.20, 95% CI

1.08-1.33 per 100 cells/mm^3), TB drug intolerance (adjOR 1.93, 95% CI 1.23-3.02), and non-disclosure of HIV-infection (adjOR 1.50, 95% CI 1.03-2.18) predicted delayed ART initiation. In the subset of 395 patients eligible at 1 or 2 months, mortality risk was 12.0% with observed fidelity and 7.8% with complete fidelity, corresponding to a risk difference of -4.2% (95% CI: -8.1, -0.3, %) and preventable fraction of mortality of 35.1% (95% CI: 2.9-67.9%).

Timing of ART initiation per CD4-stratified strategy in all patients may be a challenge to achieve in highly-resource settings; however, would be worthwhile to further reduce mortality among HIV-infected patients with TB. Pragmatic approaches to ensure timely ART initiation in those identified at-risk of delayed ART initiation are needed.

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AIDS	Acquired Immunodeficiency Syndrome
BMI	Body mass index
CD4	Cluster of differentiation 4
CI	Confidence interval
DRC	Democratic Republic of Congo
HR	Hazard ratio
HIV	Human immunodeficiency virus
IQR	Interquartile range
IRIS	Immune reconstitution inflammatory syndrome
ITART	Integration of Tuberculosis and Anti-Retroviral Treatment
LTFU	Lost-to-follow up
OR	Odds ratio
RCT	Randomized controlled trial
RD	Risk difference
RR	Risk ratio
SES	Socio-economic status
TB	Tuberculosis
UNC	University of North Carolina
WHO	World Health Organization

CHAPTER ONE: SPECIFIC AIMS

Tuberculosis (TB) is among the top fifteen causes of death in the world and is curable through a 6-9 month course of anti-tuberculosis treatment.[1] In 2012, there were approximately 8.6 million new cases of TB and 1.3 million deaths from TB; the majority of which were in limited-resource countries.[2] Human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) ranks as the sixth leading cause of death in the world and is manageable through life-long antiretroviral therapy (ART).[1] By end 2012, there were approximately 35 million people infected with HIV, 2.3 million of which were infected within the preceding year; and, 1.6 million deaths from AIDS.[3] Similar to TB, the majority of AIDS cases and deaths are in limited-resource countries. In fact, a large proportion of patients with TB disease are also HIV-infected; TB and HIV/AIDS are overlapping epidemics. In most countries, the HIV prevalence among patients with TB disease is typically higher than that in the general population; and in some sub-Saharan countries, as high as 50-80%.[4] A review conducted by Mukadi et al in 2001, found that without ART, case-fatality rates in HIV-infected TB patients across several sub-Saharan African countries range from 16-35%.[5] Thereafter, several observational studies have compared mortality between patients with and without ART. Lawn et al reviewed the findings from these studies in 2009 and found that ART reduced mortality in HIV-infected TB patients by 54% to 95%.[6]

Despite this evidence, the proportion of HIV-infected patients diagnosed with TB who initiated ART during TB treatment has only increased from 36% in 2005 to 57% worldwide in 2012.[2] One obstacle to ART initiation among HIV-infected TB patients is the need to refer patients from the TB clinic to the HIV clinic. TB treatment services are highly decentralized in most countries, while HIV treatment services are not. Implementation studies have demonstrated that the more integrated HIV services are into TB clinical settings, the higher the uptake among TB patients.[7, 8] A second obstacle to ART initiation is the concern among healthcare providers that co-treatment for TB and HIV may result in drug-drug interactions, immune reconstitution inflammatory syndrome (IRIS), and poor adherence due to high pill burden. These concerns may contribute to delay or failure to initiate ART during the course of TB treatment.

The World Health Organization (WHO) 2004 "Interim Policy on Collaborative TB/HIV Activities" recommended that ART be offered to all TB patients who are eligible per national guidelines, but provided no details on timing of ART treatment in patients receiving TB treatment. In 2006, the WHO recommended that ART be initiated between 2 weeks and 2 months after the start of TB treatment for patients with cluster of differentiation 4 (CD4) count <200 cells/mm^3, after 2 months for patients with CD4 count 200-350 cells/mm^3, or, after completion of TB treatment for patients with CD4 count >350 cells/mm^3. This recommendation weighs healthcare provider concerns with the level of patient immunosuppression, and provides more concrete criteria on when to initiate ART during TB treatment. No formal evaluation of this recommendation was done to date. Consequently, it is not clear whether this type of CD4-stratified ART initiation is efficacious or effective.

In 2009, WHO recommended that ART be initiated in all TB patients within 8 weeks of TB treatment, regardless of CD4 count. This recommendation was based on only moderate

evidence, and caters to settings in which CD4 cell count is not readily available. Several randomized controlled trials (RCTs) evaluated "early" vs. "delayed" ART initiation in TB patients. Results suggest a benefit to "early" ART initiation (within 2-4 weeks), but only among those with low CD4 cell count. The Starting Antiretroviral Therapy at Three Points in Tuberculosis (SAPiT) study in South Africa, concluded that starting ART during TB treatment, compared to waiting to start ART after completion of TB treatment, reduced the risk of death by 60%. Analysis of the two "early" ART arms in SAPiT demonstrated that initiating ART within 4 weeks v. 2-3 months of TB treatment, relatively reduced the risk of AIDS or death by 70% among those with CD4 cell count <50. The Immediate Versus Deferred Start of Anti-HIV Therapy in HIV-Infected Adults Being Treated for Tuberculosis (STRIDE) study, conducted across multiple countries, demonstrated that initiating ART within 2 weeks v. 2-3 months of TB treatment, absolutely reduced the risk of AIDS or death by 11%, only among those with CD4 cell count <50. The Cambodian Early versus Late Introduction of Antiretroviral Drugs (CAMELIA) study found that, in a population where 71% of participants have a CD4 cell count < 50 cells/mm^3, the risk of death was relatively reduced by 40% by initiating ART at 2 weeks v. 2 months of TB treatment. Taken together, these RCTs suggest that early ART initiation is efficacious among those with severe immunosuppression. For that reason, a CD4-based ART timing strategy, such as the one suggested in the 2006 WHO recommendation, may be appropriate. In fact, the recent 2012 WHO TB/HIV Policy for Collaborative TB/HIV Activities moves in this direction by recommending that patients with CD4<50 initiate ART within 2 weeks; and that patients with CD4 ≥50 initiate ART within 8 weeks.

The proposed analysis will formally evaluate a CD4-stratified ART timing using data from the Integration of Tuberculosis and Anti-Retroviral Treatment (ITART) study, conducted

by the University of North Carolina (UNC) in the Democratic Republic of Congo. The main objective of this study was to evaluate the feasibility and effectiveness of integrated treatment for TB and HIV delivered by nurses at the primary health center-level using a CD4-stratified timing strategy based 2006 WHO recommendation. The ITART study generated an electronic database that contains data on TB and HIV diagnosis and treatment outcomes, as well as a number of covariates of interest for a cohort of adult and children newly diagnosed with TB across 5 sites. Data were prospectively collected on patients at baseline/enrollment, monthly follow-up visits during TB treatment, and at 6 months post-TB treatment start.

This analysis will address two specific aims:

Aim 1: To identify demographic, clinical, and behavioral factors that predict "delayed ART initiation" among patients in a highly resource-limited setting; with delayed ART initiation defined as ART timing deviating from the CD4-stratified timing strategy.

Aim 2: To determine the impact of implementation fidelity to ART initiation per CD4-stratified strategy on mortality, in a highly resource-limited setting.

CHAPTER TWO: BACKGROUND AND SIGNIFICANCE

The Burden of TB

Tuberculosis (TB) is among the top fifteen causes of death in the world and is a major source of morbidity and mortality in limited-resource.[1] Tuberculosis (TB) disease is caused by infection of *Mycobacterium tuberculosis* (M.TB). A subset of those persons with M.TB infection will progress to TB disease, a state during which patients are symptomatic and infectious. TB disease is curable through a multi-drug treatment regimen that is typically given over the course of six months with close monitoring by health care providers. Despite this, in 2012, there were approximately 8.6 million new cases of TB and 1.3 million deaths from TB; the majority of which were in limited-resource countries.[1]

The Burden of HIV

HIV/AIDS ranks as the sixth leading cause of death in the world and is a major source of morbidity and mortality in limited-resource settings.[9] HIV is a virus that transmitted through a number of mechanisms, the most common of which globally, is unprotected sex. HIV infects the cells of the immune system. Persons with HIV-infection progress to AIDS, a disease state defined by one or more illnesses indicative of severe immunosuppression. By end 2012, there were approximately 35 million people infected with HIV, 2.3 million of which were

infected within the preceding year; and, 1.6 million deaths from AIDS.[3] Similar to TB, the majority of AIDS cases and deaths are in limited-resource countries.

The Burden of TB/HIV

HIV-infected persons are 20-30 times more likely to develop TB disease after M.TB infection and one-fourth of all HIV deaths are attributable to TB disease.[4] The HIV prevalence among patients with TB disease is typically higher than that in the general population; and as depicted in Figure 2.1, in some sub-Saharan countries, as high as 50-80%. In addition, higher levels of immunosuppresion are associated with extrapulmonary TB, disseminated TB, and rare atypical clinical presentations. The diagnosis of these types of TB disease is relatively complex and may result in treatment delay and subsequently, in increased morbidity and mortality.[10] Furthermore, laboratory research suggests that TB disease enhances HIV replication.[11, 12] This is supported by the finding of shorter survival among HIV-infected patients with TB disease compared with HIV-infected patients without TB disease in numerous observational cohorts.[13, 14]

Mortality Due to TB/HIV in Patients With and Without ART

A review conducted by Mukadi et al in 2001, found that during the pre-ART era, case-fatality rates in HIV-infected TB patients across several sub-Saharan African countries ranged from 16-35%.[5] Thereafter, a number of observational studies have compared mortality between patients with and without ART and found that ART reduced mortality in patients with TB disease by 54% to 95%.[6]

Two of these studies, conducted by Dheda et al in the United Kingdom from 1998 to 2001 and Haar et al in The Netherlands from 1993-2001, were similar. The investigators retrospectively reviewed medical records to identify HIV-infected patients who were diagnosed and treated for TB disease. The primary objective of these studies was to compare risk of death between patients who were treated for TB before and during the ART era. Dheda et al found a 72% reduction in mortality (adjusted-HR=0.28; 95% CI: 0.13, 0.63).[15] Similarly, Haar et al found a 54% reduction in mortality (adjusted-OR=0.46; 95% CI: 0.24, 0.89).[16]

Three of these studies, conducted by Manosuthi et al in Thailand from 2000 to 2004, Nahid et al in the United States from 1990 to 2001, and Velasco et al in Spain from 1996 to 2004, were similar. The investigators retrospectively reviewed medical records to identify HIV-infected patients diagnosed and treated for TB disease. The primary objective of these studies was to compare patients the risk of death between patients on and not on ART. Manosuthi et al found that patients not on ART had an increased risk of mortality (adjusted-HR=20.0; 95% CI: 8.62, 45.45).[17] Nahid et al found that patients on ART had a decreased risk of mortality (adjusted-RR=0.36; 95% CI: 0.14, 0.91).[18] Velasco et al found that patients on ART had a decreased risk of mortality (adjusted-HR=0.38; 95% CI: 0.20, 0.72).

Although these five studies provide evidence that ART reduces mortality among patients with TB disease, they are limited by retrospective study design, and subsequent potential for selection, information, and confounding biases, and reverse causality.

Studies conducted by Akkslip et al in Thailand from 2003 to 2004 and Varma et al in Thailand from 2005 to 2006 address this methodological limitation by using a prospective study design. Both of these studies prospectively collected data on HIV-infected TB patients to estimate the effect of ART on survival. Akkslip et al found that ART during TB treatment

reduced risk of mortality (adjusted-RR=0.20; 95% CI: 0.1, 0.4).[19] Varma et al found that ART during TB treatment reduced the risk of mortality (adjusted-RR=0.16; 95% CI: 0.07, 0.36).[20] These findings confirm the substantial effect of ART on survival among patients with TB disease.

Current Uptake of ART Among Patients with TB/HIV

Despite this evidence, the proportion of HIV-infected TB patients who initiated ART has only increased from 36% in 2005 to 57% in 2012.[2] One obstacle to ART initiation among HIV-infected TB patients is the need to refer patients from the TB clinic to the HIV clinic. TB treatment services are highly decentralized in most countries, while HIV treatment services are not. Implementation demonstrated that the more integrated HIV services are into TB clinical settings, the higher the uptake among TB patients.[7, 8] A second obstacle to ART initiation is the concern among healthcare providers that co-treatment for TB and HIV may result in drug-drug interactions, immune reconstitution inflammatory syndrome (IRIS), and poor adherence. These concerns may contribute to delay or failure to initiate ART during the course of TB treatment.

WHO Guidelines on Timing of ART Among Patients with TB/HIV

The WHO (WHO) 2004 "Interim Policy on Collaborative TB/HIV Activities" recommends that ART be offered to all TB patients who are eligible per national guidelines. This recommendation was vague and did not address health provider concerns around co-treatment. In 2006, WHO recommended that ART be initiated between 2 weeks and 2 months for patients with CD4<200, after 2 months for patients with CD4 200-350, or, after completion of TB treatment for patients with CD4>350. This recommendation weighs healthcare provider

concerns with the level of patient immunosuppression, and provides more concrete criteria on when to initiate ART during TB treatment. No formal evaluation of this recommendation was done to date. Consequently, the effectiveness of this this type of CD4-stratified strategy for timing of ART initiation is unclear.

In 2009, WHO recommended that ART be initiated in all TB patients within 2-8 weeks, regardless of CD4 count. This recommendation was based on only moderate evidence, and caters to settings in which CD4 count is not readily available. Based on the results of 3 recent RCT studies, 2012 WHO TB/HIV Policy for Collaborative TB/HIV Activities recommended that patients with CD4<50 initiate ART within 2 weeks; however, still suggest that all other patients be initiated within 8 weeks regardless of CD4 count. This reflects a shift back toward CD4-stratified timing of ART initiation.

Predictors of Delay of ART Initiation in TB Clinical Settings

Lawn et al conducted a retrospective observational study among HIV-TB patients from one site in South Africa from 2002 to 2008. The purpose of this study was to quantify and explore determinants of time delay between TB treatment start and ART initiation. Patients were categorized by whether they received integrated TB/HIV care, defined as diagnosis and treatment of TB within the HIV clinic; or non-integrated TB/HIV care, defined as diagnosis and treatment of TB within the TB clinic and referral to HIV clinic for ART. Competing risk regression and time to event analyses were used to account for the possibility of death or loss-to-follow-up before ART initiation. The proportion of patients who initiated ART during TB treatment was similar (86% and 89%) across patients who did and did not receive integrated TB/HIV care. However, the median time to ART initiation was lower among patients who were

receiving integrated TB/HIV care, than among those who were not (41 days versus 116 days; p-value <0.0001).[21] The proportion of patients who initiated ART within 8 and 12 weeks was significantly lower among patients receiving non-integrated TB/HIV care (19% and 24%, versus, 59% and 77%; p<0.001). Recent calendar period, lower CD4 cell count, smear-positivity, extrapulmonary TB, and integrated TB/HIV care, were each independently statistically associated with shorter time to ART initiation; while, age, gender, and new/recurring TB status, were not. Although successful in quantifying the time to ART initiation, especially between patients who did and did not receive integrated TB/HIV care; this study falls short in identifying predictors of time to ART initiation. The number and scope of predictor variables assessed, especially those that would be potentially modifiable, were limited; and there is a need for more comprehensive study.

Lawn et al conducted a similar second study in South Africa from 2002 to 2008 with the same primary objective. This study included patients from across three non-integrated TB/HIV care sites and used accelerated failure time modeling. In this study, the mean time to ART initiation was 2.66 months (IQR: 1.58, 4.17).[22] Lower CD4 count, more recent calendar year, and clinic site, were each independently significantly accelerated time to ART initiation; while, age, gender, new/reoccurring TB status, and type of TB were not. This study has similar limitations as the other study by Lawn et al, and additionally, does not account for competing risks to ART initiation including death and loss-to-follow-up.

Chilton et al conducted a retrospective observational study of factors contributing to delay in ART initiation, defined as counter to the 2006 WHO recommendations described above, among HIV-infected patients with TB disease in an urban site in the United Kingdom from 1998 to 2007. Overall, of the 83 patients who should have initiated ART, 63 (76%) had delay in ART

initiation. The reasons for delay varied by patient CD4 category.[23] The reason for delay in patients with CD4<100 seems to be clinically-related and physican-determined; while in patients with CD4>200 seems to be non-clinical and patient-determined. A major limitation of this study was that reason for delay was collected from existing medical records, which limits the ability to obtain specific information and also creates potential for information bias. In addition, this study was conducted in the United Kingdom and the reasons for delay may not translate to limited-resource settings.

Although these studies provide some insight in to factors that may predict timely ART initiation, they are few and all have limitations that justify Aim1 of the proposed study.

Timing ART Initiation in HIV Patients in General

There is extensive body of published research that examines the optimal time to initiated ART in HIV-infected patients in general. All of these studies examine the impact of initiating ART at different time points corresponding to a patient's CD4 cell count fall below a specified value. Early randomized controlled trials conducted in severely immuno-compromised patients (with CD4<200 cells/mm^3) showed that ART initiation can reduce the rate of AIDS or death by half.[24, 25] Based on this evidence, WHO issued guidance in 2004 that recommended ART initiation in patients with CD4<200. The question of whether the benefit of ART would hold in HIV-infected patients with higher CD4 counts remained unanswered. Since then, several observational and randomized controlled trials have examined this question.

There have been four major observational studies conducted among HIV-infected patients (both with and without TB) that have aimed to examine whether initiation of ART when a patient's CD4 cell count is <350 cells/mm^3 provides clinical benefit. Investigators from the

When to Start Consortium conducted a retrospective observational study of 21,247 HIV-infected patients who initiated ART at CD4 cell count <550 cells/mm^3 across 15 cohorts in the United States and Europe between 1998 and 2006.[26] The primary finding from this study was that initiating ART at CD4 cell count 251-350 cells/mm3, compared to initiating at CD4 cell count 351-450 cells/mm3, resulted in increased risk of incident AIDS or death (HR=1.28; 95% CI: 1.40 - 1.57). Kitahata et al conducted an retrospective observational study that included parallel analyses of 8362 patients from 1996 to 2005 with baseline CD4 cell count 351-500 cells/mm3 and 9155 patients with baseline CD4 cell count >500 cells/mm3.[27] The primary findings of this study were that deferment (v. immediate initiation) of ART was associated with a higher risk of death in both groups of patients. In patients with baseline CD4 cell count 351-500 cells/mm3, the RR=1.69; 95% CI: 1.26 - 2.26; in patients with baseline CD4 cell count >500 cells/mm3, the RR=1.94; 95% CI: 1.37 – 2.79). Kaplan et al conducted an observational study of 4976 patients from 1996 to 2002 who initiated ART across a range of baseline CD4 cell counts.[28] The primary finding from this study was that initiation of ART at lower CD4 cell count was associated with an increased hazard of death (HR=6.3, 3.5, 1.7, 1.5; for initiation at CD4 cell count of 0-49, 50-199, and 200-349, 350-499, compared to the referent of ≥500 cells/mm3). Jonsson Funk et al conducted an observational study of patients in the CASCADE collaboration (composed of multiple clinical cohorts in Europe) from 1996 to 2009.[29] The investigators used sequential nested cohorts to examine the effect of starting ART within a given month within specified CD4 strata. The primary finding of this study was that compared with deferring ART within a given month, ART initiation at CD4 cell count <500 cells/mm^3 was associated with lower disease progression (HR range: 0.32-0.75). Taken together, all of these observational

studies suggest that initiating at either <350 or <500 CD4 cell count is warranted in the general HIV population.

Two RCTs have aimed to examine whether initiation of ART when a patient's CD4 cell count is <350 cells/mm^3 provides clinical benefit. In the first of these, the multi-site international Strategies for Management of Antiretroviral Therapy (SMART) RCT, investigators randomized patients with CD4<350 cells/mm^3 to two treatment groups; one in which ART was initiated immediately and one in which ART was initiated when CD4 cell count fell to <250 cells/mm^3.[30] The primary finding of this study was that patients who initiated ART at CD4<250 cells/mm^3 (compared to <350 cell/mm^3) had higher risk of death or opportunistic infection (HR=3.47; 95% CI: 1.26-9.56). In the second, conducted in Haiti by Severe et al, HIV-infected patients with 200<CD4<350 cells/mm^3, were randomized to received immediate ART or ART delayed until CD4 fell below 200 cells/mm^3 and followed up for the primary endpoint of death. This study was stopped by the DSMB at interim analysis due to substantially higher mortality in the delayed ART group (HR=4.0; 95% CI: 1.6-9.8).[31]

Based on the evidence from these studies, in 2009 the WHO recommendation rais5ty56ed the recommended CD4 cell count threshold for ART initiation from 200 to 350 cells/mm^3.

Timing of ART Initiation in Patients with TB/HIV

The SAPiT RCT by Abdool Karim et al in South Africa, examined the effect of ART initiation after 1 month (early-integrated arm), 2 months (late-integrated arm) , or 6 months (sequential arm) of starting TB treatment, on mortality and AIDS. The study population consisted of 642 HIV-infected patients aged 18 or older, with baseline CD4<500, and smear-

positive, pulmonary TB disease. Patients were followed-up for a maximum of 2 years. An interim analysis was conducted approximately three months after the end of study recruitment. The main finding of this analysis was that ART initiation under either of the integrated arms, reduced mortality by 54% (HR=0.44; 95% CI: 0.25-0.79).[32] Based on the results of this analysis, the data safety and monitoring board (DSMB) required that all patients in the sequential arm who had not initiated ART, be offered ART as soon as possible. The subsequent analysis compared the early-integrated and late-integrated arms, with stratification across baseline CD4 categories. The main finding from this analysis was that ART in the early integrated arm reduced the risk of mortality or AIDS, however, only among patients with baseline CD4<50 (stratum-specific HR=0.32; 95%: 0.07-1.13).[33] Patients in the early integrated arm had a higher incidence of IRIS and this effect was inversely proportional to baseline CD4 [among CD4>50, IRR=2.16 (1.12, 4.47); among CD4<50, IRR=4.71 (1.48, 19.64)]. The findings of the study did not suggest that earlier ART had any effect on adherence or outcome of TB treatment. A major limitation of this study is that was not originally powered for stratified analysis using the CD4 50 cut-point. There were only 35 patients with CD4<50, and this may have contributed to the lack of statistical significance for several of the key outcomes.

The STRIDE RCT by Havlir et al in multiple countries, examined the effect of ART initiation within 2 weeks (earlier ART) or between 2 and 3 months (later ART), on mortality or AIDS. The study population consisted of 806 HIV-infected patients aged 13 years or older, with baseline CD4 cell count<250, and confirmed or probable TB disease based on either smear or culture or clinician assessment. Patients were followed-up for a maximum of 2 years. The main finding of this study was that earlier ART reduced the risk of mortality or new AIDS-defining illness, however, only among patients with CD4<50 cells/mm^3 (15.5% v. 26.6%; p=0.02).[34] The

risk of IRIS was higher in the earlier ART group, compared to the late ART group, regardless of baseline CD4 cell count (11% v. 5%; p<0.001), however; none of the deaths that occurred were attributable to IRIS. In contrast to the SAPiT study, the STRIDE study was powered for stratified analysis by baseline CD4 cell count ≥ or <50 and patients. A major limitation of STRIDE, however, was that the results are presented as an absolute risk (not rate or hazard), and does not take into account actual person-time at risk. For example, it is not clear how the 62 patients who were lost to follow-up or withdrew from the study were analyzed, since they were not at risk for the entire 48 week period. If the person-time at risk varied across the two study groups, this may potentially impact the main study finding; however, this is not addressed by the investigators.

The CAMELIA RCT by Blanc et al in Cambodia, examined the effect of ART initiation at 2 weeks (earlier ART) or at 8 weeks (later ART), on mortality. The study population consisted of 661 HIV-infected patients aged 18 years or older, with CD4 cell count<200, and smear-positive TB disease. Patients were followed-up for a minimum of 50 of weeks. The main finding of this study was that earlier ART reduced the risk of mortality [HR=0.62; 95% CI: (0.44-0.86)].[35] The risk of IRIS was higher in the earlier ART group, compared to the later ART group [HR=2.51; 95% CI: (1.78-3.59)]. There was no difference in adherence to or outcome of TB treatment across the two study arms. A prime difference between CAMELIA and the other two RCTs, is that the study population was much more immunosuppressed. This is partly due to the enrollment criteria, which limited the study population to patients with CD4 cell count <200, however; even among those patients who met this criteria, the majority (71%) had CD4 cell count <50. Whether this study population is representative of HIV-infected patients

with TB disease, especially in other geographic settings such as sub-Saharan Africa, is questionable.

The TIME RCT by Manosuthi et al in Thailand, examined the effect of ART initiation at 4 weeks or at 12 weeks, on the primary endpoint, mortality at 1-year. The study population consisted of 156 HIV-infected patients aged 18-65 years, with CD4 cell count<350, clinically or bacteriologically diagnosed active TB, no previous ART, and normal serum creatinine and aspartate aminotransferase and alanine aminotransferase (ALT) levels. Additionally, pregnant women were excluded from the study. The intended follow-up period was 96 weeks, however, because this study was prematurely ended on May 2011, some patients had shorter or insufficient follow-up and consequently, power was only 70%. The primary finding of this study was no statistically significant association between early ART initiation and mortality at 1-year (RR=0.85; 95% CI: (0.25 – 2.89).[36] In contrast to previous studies, this finding did not change after stratification by CD4 count; in fact, although still non-significant, the direction of the effect changed from protective to harmful [RR=1.59; 95% CI: (0.40 – 6.40) among CD4 count <50 cells/mm^3 and RR=1.24; 95% CI: (0.34 – 4.54), among CD4 count <100 cells/mm^3].

A retrospective observational study by Manosuthi et al in Thailand, examined the effect of initiating ART at 2, 4, 6, 9 and 12 months (versus not) on mortality. The study population consisted of 411 HIV-infected patients, aged 15 years or older, and, diagnosed and treated for TB between January 2000 and December 2004. The main finding from this study was that ART initiation within 6, 9, and 12 months were associated with reduced mortality (HR range: 2.37-2.68; $p<0.05$); while, ART initiation within 2 or 4 months, was not.[17] This finding is consistent with integrated v. sequential analysis from the SAPiT trial, but is counter to the early v. late analyses within the 6 months of TB treatment from all three RCTs. There are several potential

methodological reasons for this discrepancy. For example, it is not clear what sample sizes in each exposure category were generated by each of the timing cut-points. Since this study was not randomized, if only a small proportion of patients initiated ART within 2 months or 4 months, this may have negatively affected power to detect a difference using these cut-points. Furthermore, it is not clear whether the investigators adjusted for covariates in the models used to generate the HR estimates for the timing analyses; a necessary step to address potential confounding bias.

A prospective observational study by Varma et al in Thailand, examined the effect of initiating ART within various timepoints following start of TB treatment (versus not) on survival. The study population for timing analyses consisted of 200 HIV-infected patients, aged 17 or older, receiving treatment for TB disease for <4 weeks between May 2005 and Sep 2006, who initiated ART during the period of TB treatment. The main finding from this study was that ART initiation within 4 months (versus not), improved survival among bacteriologically confirmed cases of TB disease (HR=9.0; 95% CI: 1.1-73.0). Overall, there appeared to be a linear relationship between time to ART initiation and mortality; the longer ART was delayed, the higher the risk of mortality. Although, a Cox proportional hazards model was used to generate these estimates, because of the relatively small sample size, the investigators only adjusted for the severity of TB disease and baseline CD4 count. In addition, the incidence of IRIS was not studied according to ART timing status; therefore, the potential trade-off associated with early initiation was not apparent.

A retrospective observational study by Velasco et al in Spain, examined the effect of initiating ART within 2 months v. at 3 months or more on mortality. The study population consisted of 313 HIV-infected adults, diagnosed with TB disease between 1996 and 2004, who

initiated ART between time of TB diagnosis and the end of the study period. The main finding of this study was that ART initiation within 2 months reduced mortality, particularly in the time soon after TB diagnosis [adjusted-HR: for 6 months=0.15 (0.03-0.59); 1 year=0.33 (0.14-0.78); full follow-up period=0.37 (0.17-0.66)].[37] A major limitation of this study is the retrospective design and subsequent potential for selection, information, and confounding biases, and reverse causality. In addition, because this study was conducted in Spain, the results may not be generalizable to limited-resource settings.

A retrospective observational study by Franke et al in Rwanda, utilized advanced modeling methods to examine the effect ART initiation at various timepoints during TB treatment on survival. The study population consisted of 308 HIV-infected patients, aged 15 years or older, with baseline CD4 cell count <350, who were treated for TB disease at one of 5 ART sites between January 2004 and February 2007. The probability of survival was modeled to simulate a hypothetical scenario in which all patients with baseline CD4 cell count of 50, 100, 200 or 300 cells/mm^3, initiate ART at 15, 30 60, 180 days, or not at all, after start of TB treatment. Survival probabilities for ART initiation at 30, 60, 180 days and not at all, were statistically lower than for 15 days, but only when baseline CD4 cell count was set to 50 or 100 cells/mm^3.[38] A major limitation of this study is use of advanced modeling methods that make additional assumptions beyond those in typical regression modeling that is used in epidemiological studies. A key assumption is that the model that was used to generate survival probabilities was correctly specified and provided accurate predictions. The reliance on retrospective observational data, may contribute to violation of this assumption. In addition, this study population was receiving both TB treatment and ART services at ART clinic; a scenario which is not the norm in most sub-Saharan African settings, and may not be representative.

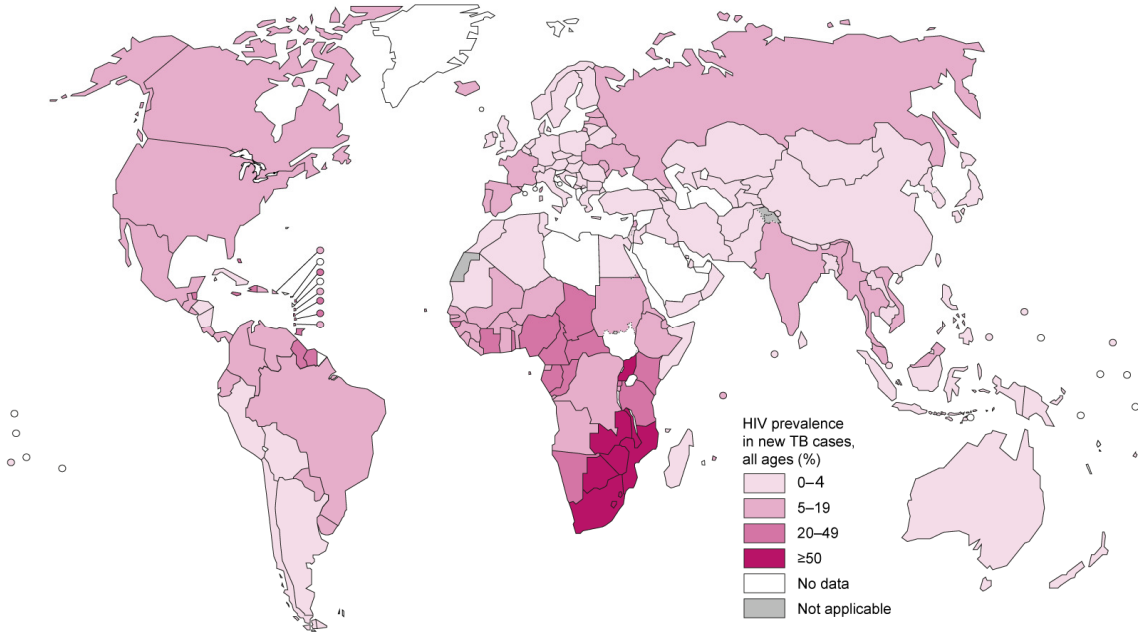
As summarized in Table 2.1, taken together, these studies suggest that ART initiation early during the course of TB treatment may offer a survival benefit; however, only among patients with severe immunosuppression. For that reason, a CD4-stratified timing strategy for ART initiation, such as the one suggested in the 2006 and 2012 WHO recommendations, may be appropriate.

Translation of RCT Findings into Real-World Practice: Implementation Fidelity

Findings from RCTs are not always replicable in less strictly controlled settings; this is particularly likely at the primary care level in resource-limited settings. Observational studies from sub-Saharan Africa have found that most patients initiate ART late, after 8 weeks of TB treatment.[6-10] Lack of integration of TB and HIV treatment services has been identified as one of the key contributors to this delay.[9, 10] Interventions to integrate TB and HIV services have been shown to reduce, but not eliminate delay in ART initiation.[11, 12]

Implementation fidelity, defined as the degree to which an intervention is implemented as intended, is a potential modifier of the relationship between an intervention and its intended outcome and is an important component of translating evidence-based recommendations into clinical practice.[13, 14] Achievement of high implementation fidelity is one of the best ways to replicate the success an intervention has achieved in RCT studies.[13] Low fidelity to interventions or guidelines can explain differences between outcomes observed in routine clinical settings and those achieved in RCT studies. The relatively low coverage of timely ART initiation in patients with TB suggests that implementation fidelity to the 2012 WHO guidelines for timing of ART initiation in TB patients may be a challenge in routine care settings, even when HIV and TB services are integrated. Data on implementation fidelity in resource limited settings and its effects on desired outcomes are limited.

Figure 2.1 Estimated HIV Prevalence in New Cases of Tuberculosis Disease, 2012



Source: Global Tuberculosis Report 2013, World Health Organization 2013.

Table 2.1 Summary of Studies of Effect of ART Timing Among HIV-infected Tuberculosis Patients on Clinical Outcomes of Interest

Study	Country/ Design	Study Population	ART Timing Exposure	Outcome	
				Death	AIDS or death
SAPiT – Abdool Karim et al, 2010 & 2011	S. Africa/ RCT	• HIV+ • ≥18 • smear+ PTB • CD4<500 • (N=642)	Within 2 months v. after 6 months (ref)	HR=0.44 (0.25, 0.79)	Not studied.
			Within month v. within 2-3 months (ref)	Death: NS, but, few events, and small sample of patients with CD4<50 (N=37)	Among patients with CD4<50, IRR=0.32 (0.07, 1.13)
ACTG 5221 – Havlir et al, 2011	Multiple/ RCT	• HIV-infected • ≥13 yrs • confirmed or probable TB • CD4<250 • (N=806)	Within 2 weeks v. between 8 and 12 weeks (ref)	Not studied as an outcome separate.	Among patients with CD4<50, proportion with outcome= 15.5% v. 26.6%; (1.5 – 20.5%; p=0.02
CAMELIA – Blanc et al, 2011	Cambodia/ RCT	• HIV-infected • ≥18 yrs • smear+ TB • CD4≤200 • (N=661)	At 2 weeks v. at 8 weeks (ref)	HR=0.62 (0.44-0.86)	Not studied.
TIME – Manosuthi et al, 2012	Thailand/ RCT	• HIV-infected • 18-65 yrs • Clinical or bacteriology confirmed TB • CD4<350 • (N=156)	At 4 weeks v. at 12 weeks (ref)	RR=0.85 (0.25 - 2.89)	Not studied, but hospitalization was: RR=1.14 (0.59 – 2.22)

Manosuthi et al, 2006	Thailand/ retrospective observational	• ART-naïve • HIV-infected • ≥15 yrs • Dx and Tx TB between Jan 2000 and Dec 2004 • treated for TB • (N=411 on ART)	Binary using 2 month cutpoint	Survival: HR=2.04 (0.69-6.00)	Not studied
			Binary using 4 month cutpoint	Survival: HR= 1.97 (0.83-4.72)	Not studied
			Binary using 6 month cutpoint	Survival: HR=2.65 (1.15-6.10)	Not studied
			Binary using 9 month cutpoint	Survival: HR=2.68 (1.26-5.68)	Not studied
			Binary using 12 month cutpoint	Survival: HR=2.37 (1.11-5.06)	Not studied
Varma et al, 2009	Thailand/ Prospective observational	• HIV-infected • Receiving TB Tx <4 weeks at enrollment between May 2005 and Sep 2006 • >17 years • (N=200); restricted timing analysis to patients who initiated ART during TB treatment	Binary using various timing cutpoints	Survival: 4 month cutpoint, among bacteriologically confirmed TB cases, HR=9.0 (1.1-73.0) Other cutpoints: NS	Not studied
Within 2 months v. at ≥3 months (ref)	Spain/ Retrospective observational	• HIV-infected • Adult • Dx TB between 1996-2004 • ART naïve at TB Dx, but on ART • (N=313)		Over full follow-up period: HR=0.37 (0.17-0.66) At 6 months follow-up: HR= 0.15 (0.03-0.59) At 1 year follow-up HR=0.33 (0.14-0.78)	Not studied
Franke et al	Rwanda/ Retrospective observational and modeling	• HIV-infected • >15 years • CD4≤350 • ART naïve • TB Tx Jan 2004 Feb 2007 • (N=308)	ART at 15 (ref), 30, 60 or 180 days after start TB Tx	Survival probabilities for 30, 60, 180 days were statistically lower than for 15 days, but only when baseline CD4 was set to 50 or 100	Not studied

CHAPTER THREE: STUDY DESIGN AND METHODS

Overview of the ITART Study

UNC has provided programmatic and technical support to the School of Public Health at the University of Kinshasa to implement programmatic and research activities to strengthen and expand HIV prevention, care, and treatment services in DRC, since 2001. These activities include scaling up HIV testing and access to ART among patients diagnosed with TB, who have a higher HIV prevalence than the general population in DRC. In 2008, Van Rie et al published the results from a preliminary study conducted among 1238 TB patients across three UNC-supported clinics.[8] This study aimed to identify the best model for implementation of HIV testing among TB patients. The primary finding from this study was that a provider-initiated counseling and testing (PITC) model in which TB nurses offer HIV testing in the TB clinic resulted in high uptake of HIV testing (98%) compared to the traditional model of referral to off-site VCT (69%). The HIV prevalence among TB patients who accepted HIV testing was 19% (N=205), which is consistent with national estimates for DRC. Of these 205 patients who were HIV-infected, only 21 were able to access ART, of whom 9 were already receiving ART before they were diagnosed with TB and of who 2 started ART after TB treatment were completed. This study provided evidence for integration of HIV services into TB clinical settings and rationale for integration of HIV care and treatment services, including provision of ART.

Study Design of the ITART Study

The ITART study was a prospective observational cohort study consisting of patients diagnosed with TB disease and identified as HIV-positive as a part of TB diagnosis, who were offered ART within the TB clinic under a nurse-driven integrated model. The study enrolled 599 patients from August 2007 to November 2009. Inclusion criteria for the study were: 1. initiation of treatment for TB disease at one of 5 participating health centers, and, 2. confirmation of HIV-infection, and, 3. willingness and ability to provide informed consent. Exclusion criteria for the study were: 1. treatment for TB at non-participating health center, or, 2.on ART at time of TB diagnosis and preference to continue ART at a non-participating health center. Patients were informed of study by the on-site UNC counselor/nurse and asked for written informed consent to participate. The study was approved by institutional review boards at both UNC at Chapel Hill and the University of Kinshasa.

At enrollment, all patients were administered a baseline questionnaire that included demographic, clinical, and behavioral data deemed relevant to the study aims. In addition, laboratory testing including CD4, alanine aminotransferase (ALT), hemoglobin, and a physical exam were conducted. Patients were assessed for ART initiation at enrollment. A CD4-stratified based timing strategy in line with the 2006 WHO recommendation was used by health care providers to decide when to initiate ART after TB treatment start. In this algorithm, patients should be initiated on ART at 1 month after TB treatment start, if baseline CD4<100 cells/mm^3 or baseline WHO clinical stage 4 condition other than extrapulmonary TB, or 2 months after TB treatment start, if baseline CD4 100-350 cells/mm^3. Patients with baseline CD4>350 were reassessed at month 5 of TB treatment; ART was initiated if and when CD4 fell below 350 and/or WHO clinical stage 4 conditions were present.

Patients on ART were scheduled to receive CD4 and viral load testing every 6 months following ART initiation. Patients were scheduled for follow-up visits weekly during the first two month of TB treatment, monthly from the third month to the end of TB treatment, at the end of TB treatment, and semiannually after the end of TB treatment. During follow-up visits, in addition to any scheduled laboratory testing, a physical exam and questionnaire on adherence, sexual behavior, and disclosure status were administered. Patients who were more than 3 days late for a scheduled follow-up visit were traced using TB clinic nurses. If necessary, study staff contacted patients by phone or by home visit on 2 attempts before being regarded as "lost-to-follow-up". Other reasons for inactivation from the study including death, loss-to-follow-up, and voluntary withdrawal were also documented. Per study strategy, patients received ART in the TB clinic during the period of TB treatment, and then were transferred to the ART clinic on-site within the same facility. In addition, based on patient preference, some patients were transferred-out of the study to continue their TB treatment and/or ART at a non-ITART clinic. Patients were also administratively censored at the end the ITART study (in February 2010).

The overall (descriptive) cohort analyses for pediatric and adult patients in ITART have already been conducted, and provide valuable preliminary data for this dissertation study.

Analytical Population

The specific aims were achieved through secondary analysis of the ITART study data. As depicted in Figure 3.1, additional inclusion/exclusion criteria were applied to the larger ITART study population. The analytical population for Aim1 included patients who were: 1. aged ≥13 years at enrollment, 2. ART naïve at enrollment, 3. enrolled in ITART within 1 month of their TB treatment start date, and 4. had baseline CD4 count (within 30 days of and closest to TB

diagnosis date, prior to ART initiation) available. The analytical population for Aim2 used the analytical population for Aim1 as a starting point and additionally excluded patients who were not eligible to initiate ART at 1 month or 2 months based on baseline CD4 and/or WHO staging criteria e.g. CD4>350 and no WHO stage 4 condition. Based on this, the analytical population for Aim1 included 492 patients, and the analytical population for Aim2 included 395 patients.

Exposure/Outcome Assessment

For Aim1, there was not a primary exposure since it focused on identifying predictors of the main outcome, which is binary: delayed ART initiation (or not). To define timing of ART eligibility, we compared the ART initiation date with TB treatment start date. To accommodate scheduling limitations, clinic closure on weekends and holidays, and on-site availability of consulting physician, patients eligible for ART initiation at 1 month of TB treatment who were expected to, but did not initiate ART within 1 month plus 5 days of TB treatment, were classified as experiencing delayed ART initiation. Similarly, a 5 day window was applied to define delayed ART initiation among patients eligible for ART initiation at 2 months and completion of TB treatment. Patients who died or were LTFU prior to the time they were eligible plus 5 days who did not initiate ART, were not categorized as experiencing delayed ART. Patients with both baseline and follow-up CD4 count >350 cells/mm^3 who did not experience a WHO stage 4 condition during TB treatment, were also categorized as not experiencing delayed ART, since they were not scheduled to initiate ART during TB treatment.

For Aim 2, the main exposure was binary: ART initiation per CD4-stratifed timing strategy (or not). The main outcome was all-cause mortality at 6-months from the start of TB treatment, the duration of first-line TB treatment. Data on if and when a patient died during the follow-up

period were systematically collected on the patient inactivation form. LTFU was defined as in the ITART study protocol. Patients who were lost to follow up (LTFU) prior to six months were assigned a missing outcome.

Covariate Assessment/Definitions

For Aim1, all covariates hypothesized as potential predictors of delayed ART initiation based on subject matter knowledge and previously published literature were assessed. For Aim2, covariates were selected for inclusion in a directed acyclic graph (DAG) of the association between ART timing per CD4-stratified strategy and mortality at 6-months (Figure 3.4), based on published literature and subject matter knowledge. Descriptive statistics, distribution of association across sub-categories, substantive area standards, and clinical/programmatic relevance will be considered to define and categorize each covariate as detailed in Table 3.1.

Analytical Approach – Aim1

For Aim1, we used a logistic regression model to identify covariates that are predictive of delay in ART initiation (main outcome as defined above) in the analytical population (N=492). This model was selected because the predictor variables include a combination of both continuous and categorical variables and the outcome variable will be modeled as a categorical, and in this instance we are not attempting to estimate "risk"'; rather, we are attempting to estimate the odds or " predicted probability" of experiencing delay in ART initiation.

We selected demographic, behavioral, and clinical covariates that may be predictive of delayed ART initiation based on previously published literature and subject matter knowledge. We first ran a full logistic model containing all selected covariates. Subsequently, we used a backwards elimination stepwise method to generate a final (reduced) predictive model. Covariates were

assessed in order from highest to lowest Wald chi-square and eliminated from the model based on the likelihood ratio test using an alpha of 0.05.

In addition, we stratified patients by expected timing (1 month, 2 months, end of TB treatment, or deferred TB treatment) and then categorized observed timing as: 1. initiating ART before or at the expected time, 2. initiating ART after the expected time, or 3. not initiating ART. The median and interquartile range of delay was calculated with each stratum of patients whose ART initiation was after expected.

Analytical Approach – Aim2

For Aim 2, logistic regression and the parametric g-formula was used to estimate the difference in mortality risk under observed and complete implementation fidelity to the strategy for CD4-stratified timing of ART initiation used in the ITART study.

We used a logistic regression model to assess baseline covariates, including timing of ART initiation, as potential predictors of mortality. We first ran a full logistic model containing all selected covariates. Subsequently, we used a backwards elimination stepwise method to generate a final (reduced) predictive model. Covariates were assessed in order from highest to lowest Wald chi-square and eliminated from the model based on the likelihood ratio test using an alpha of 0.10. We estimated crude (OR) and adjusted (adjOR) odds ratios with 95% confidence intervals (CI).

In implementation science, implementation fidelity is defined as the degree to which programs are implemented as intended, with a focus on content or frequency of the intervention.[43] In this study, we defined implementation fidelity to the CD4-stratified ART initiation strategy as the

proportion of individuals who initiated ART timely, i.e. according to *a priori* defined CD4 criteria. We calculated each patient's timing of ART initiation, by comparing the ART start date with TB treatment start date. We categorized timing of ART initiation as per CD4-count strategy (*per strategy*) or deviating from CD4-count strategy (*not per strategy*); similar to how delayed ART initiation was defined in Aim 1. To accommodate for scheduling limitations, clinic closure on weekends and holidays, and limited availability of consulting physician, a five-day grace period was added to the one or two month of TB treatment to define ART initiation *per strategy.* Participants who initiated ART prior to the time they became eligible were classified as *per strategy*. Participants who died or were LTFU prior to eligibility for ART and had not initiated ART were categorized as initiating ART *per strategy*, since not initiating ART prior to death or LTFU did not constitute deviation from the CD4-stratified strategy. In sensitivity analyses, we explored the impact of narrowing the definition of ART initiation *per strategy* to exclude patients who were LTFU prior to time of ART eligibility, a subset of patients who could have started timely ART had they been retained in care.

Differences in proportions and medians of baseline characteristics between patients initiating ART *per strategy* and those initiating *not per strategy* were assessed using chi-square or Fisher's exact tests and Kruskal-Wallis tests, respectively.

In our study observed timing of ART initiation likely deviated from assigned timing of ART initiation in a proportion of patients, resulting in incomplete implementation fidelity. To estimate the causal effect of implementation fidelity, we need to compare mortality in the study population under observed intervention fidelity with mortality in the study population with complete implementation fidelity (depicted in Figure 1).[44][45] Due to the observational nature of

our study, we did not have a comparison group and were not able to directly observe mortality in a population of patients with complete implementation fidelity to CD4-stratified timing of ART initiation. We overcame this by using the parametric g-formula to estimate mortality in the cohort under the counterfactual scenario of complete implementation fidelity.[46-48] Figure 3.3 provides a step-by-step overview of this methodological approach.[46]

We built a logistic regression model to assess the association between initiating ART *per strategy* and mortality (step 1), including baseline covariates hypothesized to be potential confounders (depicted in the directed acyclic graph in Figure 3.4). We then used parameter estimates from the model to calculate predicted probabilities of death for each patient based on their baseline covariates and observed ART timing (step 2). This modeling method imputes an outcome for each patient based on the average risk across patients with observed outcomes with the same baseline characteristics. Consequently, the outcome of participants who were LTFU is no longer missing, as these participants are assigned an outcome based on their baseline characteristics. By averaging these predicted probabilities of death across all participants, we estimated the risk of mortality in the full cohort under the observed, real-life level of implementation fidelity (step 3).

To estimate the causal effect of implementation fidelity, a (counterfactual) probability of death corresponding to what would have happened to each participant had he or she initiated ART *per strategy* is needed. We calculated this probability of death based on the outcomes of patients with similar baseline characteristics who did initiate ART *per strategy* (step 4). Note that for patients who actually did initiate ART *per strategy*, this predicted probability of death is the same as that calculated in step 2. By averaging these probabilities, we estimated the risk of mortality in the full cohort under a scenario of complete (100%) implementation fidelity (step 5).

We then calculated the risk difference (RD) by subtracting this mortality risk estimate in the cohort with complete fidelity from the mortality risk estimate in the cohort with observed fidelity (step 6). Bootstrapping was used to generate the 95% confidence interval around the risk difference. This was done by creating multiple (n=500) datasets through random selection of 395 individuals with replacement from the original ITART study population, followed by rerunning step 1 through step 6, and using the standard error across all the risk differences estimates (step 7). Finally, we estimated the preventable fraction by dividing the RD by the estimated risk in the cohort under observed, real-life implementation fidelity (step 7). This measure is interpreted as the fraction of mortality that could be prevented if 100% implementation fidelity is achieved.

As a worked example, we apply this method for Patient X, who is 32 year old male, whose ART initiation was *not per strategy*, was tolerating TB treatment, had a CD4 of 45 cells/mm^3, did not have any contraindication to ARVs, was diagnosed with smear-positive pulmonary TB, was WHO stage 3, and underweight (BMI <18.5).The logistic regression model that includes a variable corresponding to whether the patient initiated ART per strategy and covariates included in Table 1generated regression parameters such that:

Ln[P(D=1|X=*x*)]= -2.6590 + 0.0305(age) + -0.6731(female) + -0.9064(per_strategy) + -2.5365(tb_tx_tolerated) + 2.0222(cd4_less_50) + 0.6315(cd4_50_99) + 0.7738(cd4_200_350)+ 0.2346(contraindication) + 0.3727(smear_neg) + 0.0296(extrapulmonary) + 0.3744(who_4) +0.8832(underweight)

Scenario 1: Observed fidelity to CD4-stratified strategy for timing of ART initiation

As shown in the calculation below, for Patient X, the (factual) predicted probability of death under his observed fidelity to ART timing was 21%:

Ln[P(D=1|X=*x*)]= -2.6590 + 0.0305(32) + -0.6731(0) + -**0.9064(0)** + -2.5365(1) + 2.0222(1) + 0.6315(0) + 0.7738(0)+ 0.2346(0) + 0.3727(0) + 0.0296(0) + 0.3744(0)+ 0.8832(1)

Ln [P(D=1|X=x)]= -1.3141

P(D=1|X=*x*)=[exp(-1.3141)]/[1+ exp(-1.3141)]=**0.21**

Scenario 2: Complete Fidelity to CD4-stratified timing of ART initiation

In order to estimate the (counterfactual) predicted probability of for Patient X under complete fidelity to ART timing, we used the same regression parameters and baseline characteristics, however, we enter a "1" instead of a "0" for the coefficient corresponding to the regression parameter for *ART per strategy*. As shown in the calculation below, for Patient X, the predicted probability of death under complete fidelity was 9.5%:

Ln[P(D=1|X=*x*)]= -2.6590 + 0.0305(32) + -0.6731(0) + -**0.9064(1)** + -2.5365(1) + 2.0222(1) + 0.6315(0) + 0.7738(0)+ 0.2346(0) + 0.3727(0) + 0.0296(0) + 0.3744(0)+ 0.8832(1)

Ln[P(D=1|X=*x*)]= -2.251

P(D=1|X=*x*)=[exp(-2.251)]/[1+ exp(-2.251)]=**0.095**

From this example, it is clear that for patients whose observed ART initiation was per strategy, the predicted probability under complete fidelity (Scenario 2) would be the same as the predicted probability under observed fidelity (Scenario 1).

Table 3.1 Definition of Key Covariates of Interest for Analyses of CD4-Stratified ART Timing

Covariate	Assessment in ITART Study	Definition Used in Analyses
DEMOGRAPHIC		
Gender	Patient self-reported gender at enrollment as either female or male	Female v. male
Age	Patient self-reported age in years at enrollment	Aim1: ≥40 years v. <40 years Aim2: age in years
Marital Status	Patient self-reported marital status as either married or unmarried at enrollment	Married v. unmarried
Education	Patient self-report of highest level of education completed as either none, primary, secondary, or higher education.	≥Secondary or <Secondary
Employment	Patient self-report of employment status as either employed or unemployed at enrollment.	Employed or unemployed
Mode of transport to clinic	Patient self-report of model of transport to clinic as walking, taking a taxi/bus, or other to be specified.	Walking v. vehicle
Travel time to clinic	Patient self-report of travel time in minutes to clinic.	≥30 minutes v. <30 minutes
TB VARIABLES		
Type of TB	Data on sputum-smear status as either positive or negative and site of TB disease as either pulmonary or extrapulmonary both were abstracted from the TB clinical records at baseline.	Smear-positive pulmonary v. smear-negative pulmonary v. extrapulmonary (including both)
History of TB	Data on history of TB as either new, relapse, failure, or return after default were abstracted from the TB clinical records at baseline.	History of TB (not new) v. no history of TB (new)
Toleration of TB treatment	Subjective provider assessment of the patient at the scheduled ART initiation visit as either tolerating (yes) or not tolerating TB treatment (no).	Intolerance of TB treatment (no) v. toleration of TB treatment (all other patients)

Disclosure of TB status	Patient self-report of either disclosing or not disclosing their TB diagnosis to other healthcare worker, partner, household member, friend, family member, employer, or other specified person.	Disclosure of TB (yes to one or more person) v. non-disclosure of TB
HIV VARIABLES		
Contraindication to ARV	Provider report of patient contraindication to any of the following ARVs that were available in ITART: EFZ, NVP, d4T, and AZT.	Contraindication to any ARV v. no contraindication to any ARV
HIV diagnosis at TB clinic	This variable was assessed based on whether the patient self-reported as HIV-positive at the time of TB diagnosis. These patients were re-tested as a part of the study to confirm their HIV diagnosis.	HIV diagnosis at TB clinic (newly diagnosed) v. HIV diagnosis prior to TB clinic
Baseline CD4 cell count	Blood draw for selected laboratory testing (including CD4 count) was done at enrollment visit and specimens were sent to a central laboratory for processing.	Baseline CD4 count was defined as the result available within 30 days of TB treatment start date, since this is the value that would have been considered in ART timing per strategy.
WHO clinical stage	WHO clinical staging was assessed by the study nurse at enrollment based on National Institute of Allergy and Infectious Disease, Division of AIDS (NIAD DAIDS) standard guidelines. All patients with pulmonary TB are WHO stage 3 by default. Patients with HIV wasting syndrome, pneumocystis pneumonia, recurrent bacterial pneumonia, herpes simplex, esophageal candidiasis, Karposi's sarcoma, cerebral toxoplasmosis, HIV-associated encephalopathy, or extrapulmonary TB were defined as WHO stage 4.	WHO stage 4 v. WHO stage 3

Disclosure of HIV status	Patient self-report of either disclosing or not disclosing their HIV diagnosis to other healthcare worker, partner, household member, friend, family member, employer, or other specified person.	Disclosure of HIV (yes to one or more person) v. non-disclosure of HIV
GENERAL CLINICAL VARIABLES		
Hospitalization in the prior year	Patient self-report at enrollment of whether or not they have ever been hospitalized. For patients who reported prior hospitalization, the year of hospitalization was also noted.	Hospitalization in the calendar year prior to the calendar year of TB treatment start v. not (all other patients)
Functional status	Health provider assessment of the patient's functional status at enrollment as: no limitations, ambulatory, in bed more than usual, bedridden).	Poor (in bed more than usual or bedridden) v. good (no limitations or ambulatory)
Body mass index (BMI)	Height and weight were assessed and recorded by the provider at enrollment. BMI is calculated as weight in kilograms divided by height in meters squared.	Standard BMI categories of: underweight <18.5, normal 18.5-25, overweight 25-30 overweight, >30 obese.
Alcohol use	Patient self-report on two alcohol-related variables that ask about frequency of drinking during a given week and the quantity of alcohol consumed in one sitting. There were few patients who met the definition of heavy drinking (frequent and high quantity).	Any alcohol use v. no alcohol use.

Figure 3.1 Application of Inclusion/Exclusion Criteria to Generate Analytical Populations

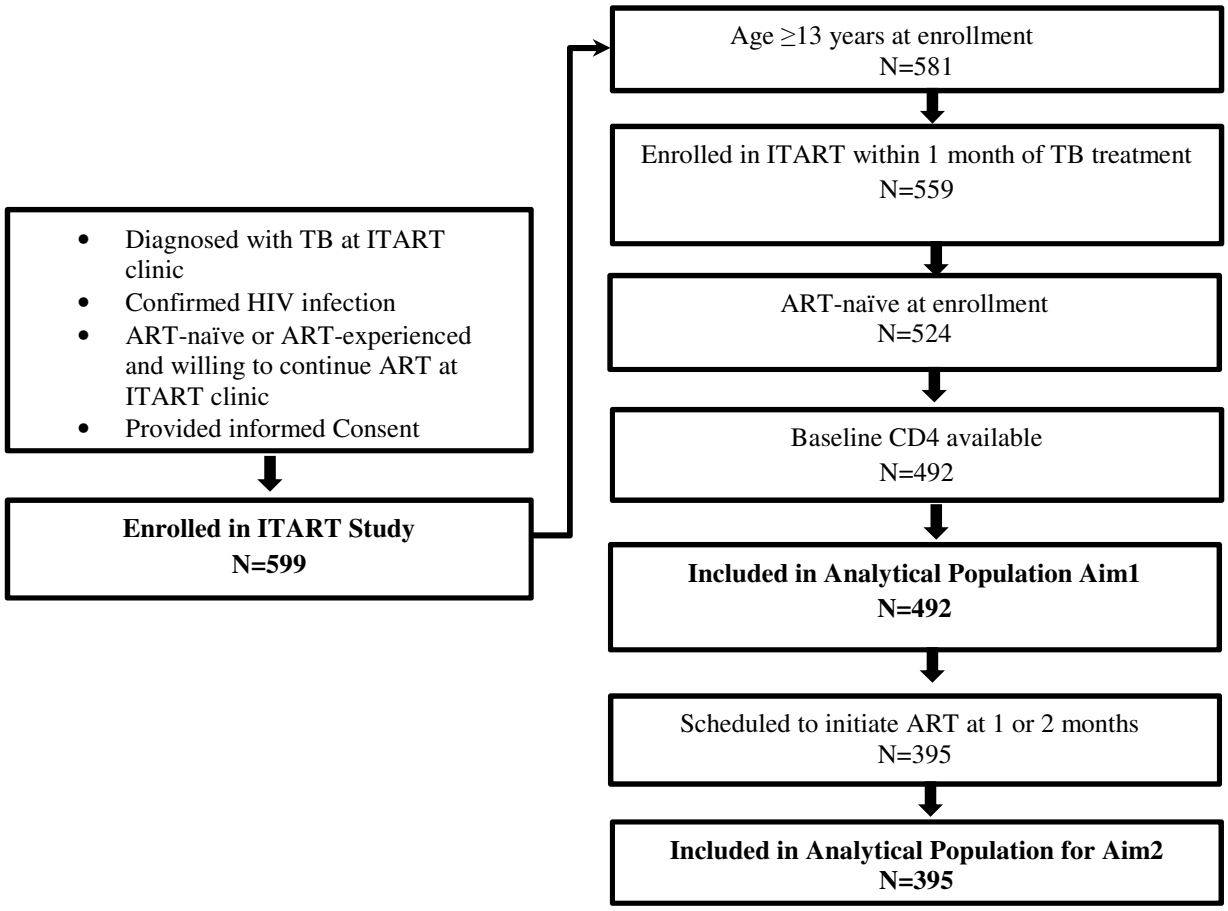


Figure 3.2 Graphical depiction of analytical approach to examine the effect implementation fidelity to CD4-stratified timing of ART initiation on mortality

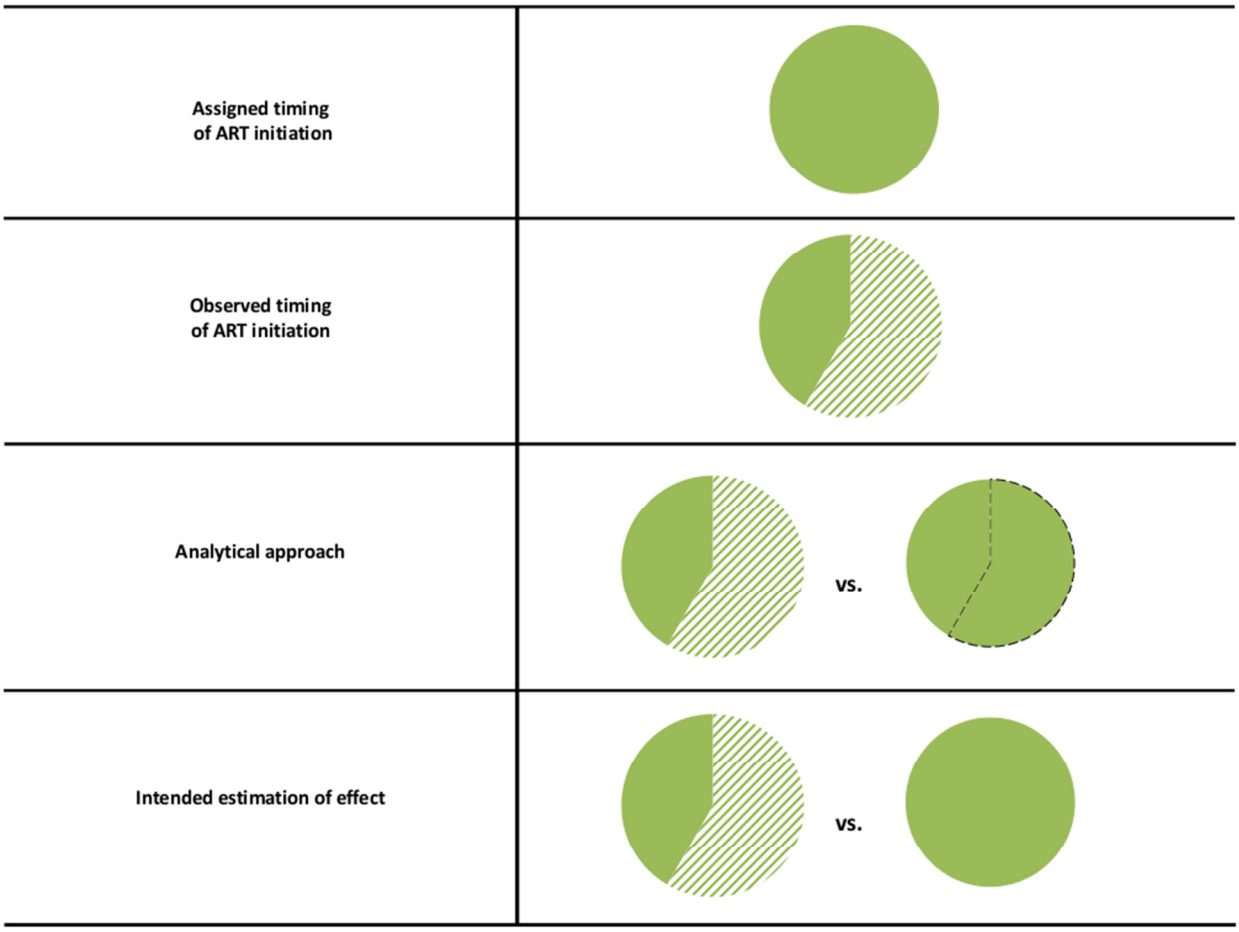


Figure 3.3 Steps of the parametric g-formula method to estimate the risk difference between the ITART population under observed and complete implementation fidelity to timing of ART initiation per CD4-stratified strategy

1. Build a logistic regression model of the association between initiating ART per strategy and mortality.

2. Use parameter estimates from model to calculate predicted probabilities of death for each patient based on their baseline factors (summarized in Table1) and observed ART timing.

3. Estimate the risk of death in the ITART population with observed fidelity to ART timing per CD4-stratified strategy, by taking the average of the predicted probabilities across all patients.

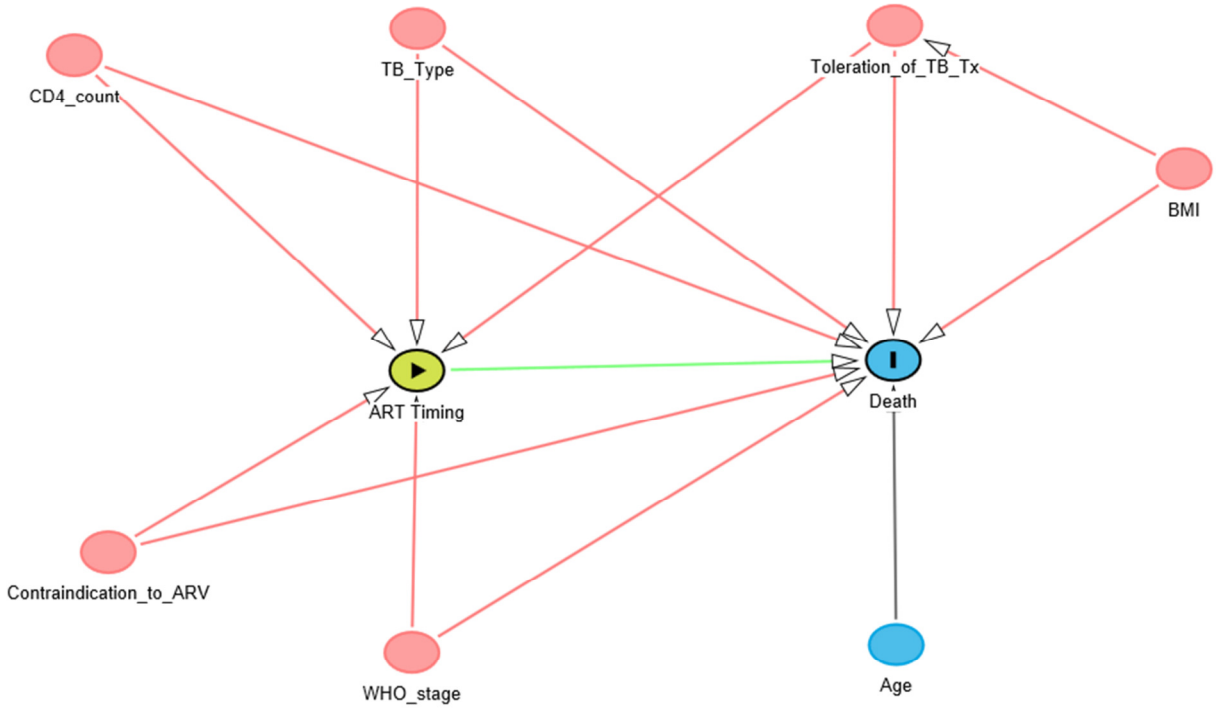
4. Recalculate predicted probabilities for each patient under scenario of complete fidelity to ART timing per CD4-stratified strategy.

5. Estimate the risk of death in the ITART population with complete fidelity to ART timing per CD4-stratified strategy by taking the average of the predicted probabilities of death calculated in step4 across all patients.

6. Estimate the risk difference by subtracting the risk of death in the ITART population with complete fidelity from the risk of death in the ITART population with observed fidelity.

7. Bootstrap the 95% confidence interval around the risk difference by creating multiple datasets through random selection with replacement from the original ITART population, rerunning steps 1-6, and using the standard error across all the risk differences estimates.

Figure 3.4 Direct Acyclic Graph (DAG) of Association between ART Initiation Per CD4-Stratified Timing and Mortality



CHAPTER FOUR: TIMING AND PREDICTORS OF DELAY OF ANTIRETROVIRAL THERAPY INITIATION AMONG PATIENTS RECEIVING INTEGRATED TUBERCULOSIS AND HIV TREATMENT

Characteristics at Baseline and Scheduled ART Initiation

Between August 2007 and November 2009, 599 patients diagnosed with TB and HIV were enrolled within the ITART study. Among them, 107 were sequentially excluded based on age<13 years (n=18), enrollment more than one month after TB treatment initiation (n=22), exposure to ART prior to enrollment (n=35), and lack of baseline CD4 count (n=32). The remaining 492 patients were included in the analysis.

Baseline characteristics of these patients are shown in Table 4.1. Participants were 60.0% female, 44.5% married, and had a median age of 38 years (IQR 32-45). Most (92.3%) patients were of low SES, less than half (46.3%) were employed, and 61.6% had at least completed secondary education. The majority (75.4%) of patients walked to the clinic, with about half (48.6%) spending over 30 minutes to get to the clinic. Of patients with pulmonary TB (79.7%), half were smear-negative. Overall 25.4% had a prior history of TB treatment. Most (95.3%) patients were newly diagnosed with HIV as part of the TB diagnostic process.

At baseline, median CD4 count was 168 cells/mm^3 (IQR 84-307) and 22.4% of patients were WHO clinical stage 4. Only 15.7% of patients had been hospitalized in the prior year, and few (5.5%) of patients had poor functional status. The median BMI was 17.9 (IQR 16.5-19.9) and over half (57.5%) of patients were underweight. At baseline, nearly all (89.2%) patients had

disclosed their TB status, while less than half (40.0%) had disclosed their HIV status. At their scheduled ART initiation visit, 145 (29.5%) of patients were assessed by the health provider as not tolerating TB treatment. In total, 26 (5.3%) of patients had contraindication to one or more antiretroviral drug. Reasons for contraindication included: pre-existing peripheral neuropathy for stavudine (n=22), pregnancy for efavirenz (n=1), and anemia for zidovudine (n=2).

Timing of ART Initiation

A total of 143 (29.1%) patients were eligible for ART initiation at 1 month of TB treatment, nearly all (n=141) because of CD4<100 cells/mm^3. Another 252 (51.2 %) patients were eligible for ART initiation at 2 months of TB treatment, based on CD4 100-350 cells/mm^3. The majority (58.8%, n=57) of patients with baseline CD4 >350 (n=97) were eligible for ART initiation at completion of TB treatment based on a CD4 ≤350 cells/mm^3 at month 5 (n=18), lack of follow-up CD4 count (n=32), or incident WHO stage 4 condition (n=7).

Overall, ART initiation was delayed in 235 (47.8%) patients (details in Figure 4.1). Patients scheduled to initiate ART at 2 months were less likely to experience delay in ART compared to patients scheduled to initiate ART at 1 month (45.2% v. 58.7%, p=0.010) and compared to patients scheduled to initiate ART at TB treatment completion (45.2% v. 64.9%, p=0.007). Among the 235 (47.8%) who experienced delay in ART, 171 (72.8%) initiated ART late, after a median of 12 days (IQR 4-27) beyond the time of eligibility plus 5 days and 64 (27.2%) never initiated ART. ART initiation was delayed by the provider in five patients for a median of 13 days (IQR 13-19) due to untreated oral candidiasis (n=4) and short-term travel outside of Kinshasa (n=1).

Of the 86 patients who never initiated ART, eight died a median of 20 days (IQR 8-30) before the time of eligibility for ART initiation. Of these, two had baseline CD4 count <100 cells/mm^3, five had baseline CD4 count 100-350 cells/mm^3, and one had baseline CD4 count >350 cells/mm^3. Fourteen patients were lost-to-follow-up prior to the date they became eligible for ART initiation. One patient was considered ineligible for ART due to the presence of a terminal illness at their scheduled ART initiation visit and died thereafter. Three patients refused ART as they did not feel ready to start long-term therapy. Two patients who never initiated ART self-reported suboptimal adherence to TB treatment (at least one missed dose within the prior four days) at the scheduled time of ART initiation.

Predictors of Delay in ART Initiation

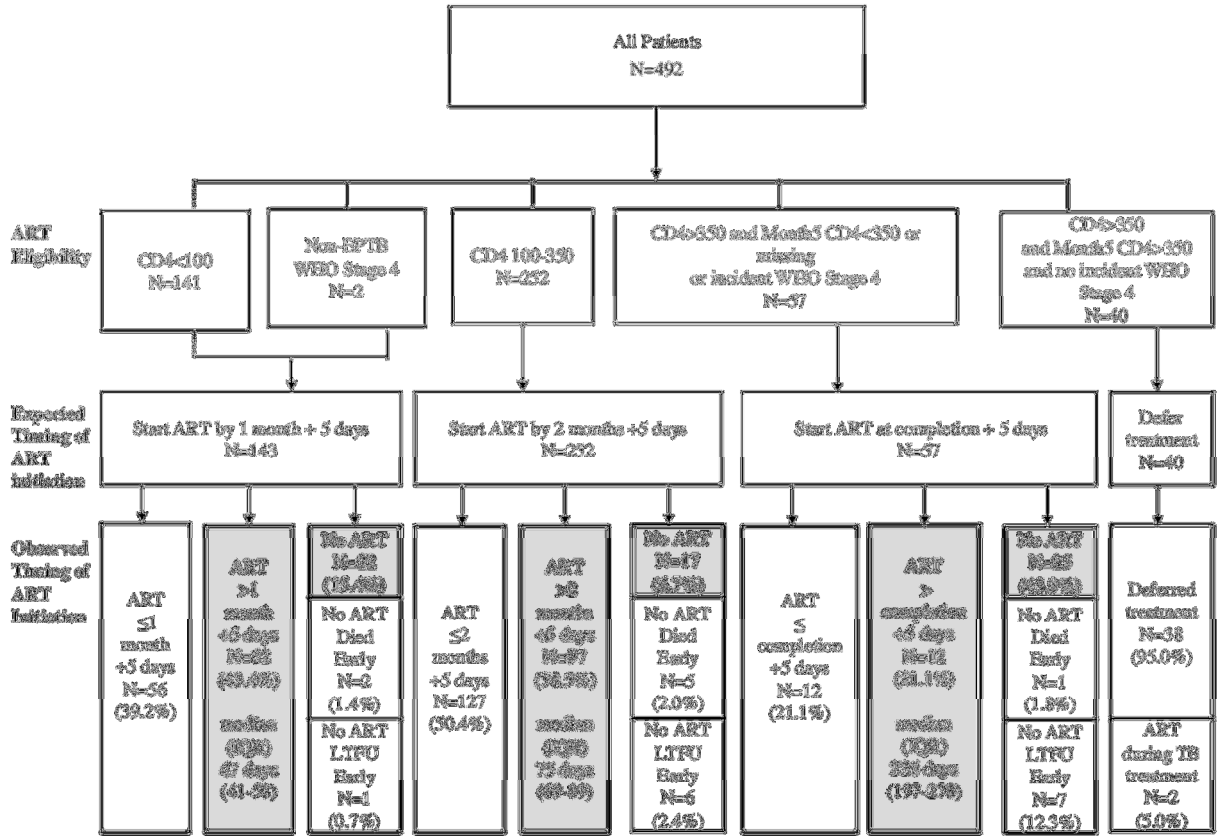
Crude and fully adjusted model results are shown in Table 4.2. After backwards elimination, the final model indicated that lower CD4 count, lack of disclosure of HIV status, contraindication to one or more ARV drugs, and intolerance of TB drugs were predictive of delay of ART initiation. Patients with contraindication to at least one antiretroviral drug were more likely to experience delayed ART initiation (adjOR 2.91, 95% CI 1.22-6.95). Lower CD4 count at baseline was associated with 20% higher odds of delayed ART initiation (adjOR 1.20, 95% CI 1.08-1.33 per 100 CD4 cell/mm^3). Patients who did not tolerate their TB drugs were nearly 2 times as likely to experience delay in ART initiation (adjOR 1.93, 95% CI 1.23-3.02). Failure to disclose HIV status, was associated with delayed ART initiation (adjOR 1.50, 95% CI 1.03-2.18). These predictors were robust to sensitivity analysis in which the definition of delayed ART initiation was expanded to include patients who were LTFU prior to the time eligibility. The full model results suggest that not being married (adjOR 1.46, 95% CI 0.99-2.16) and smear-negative pulmonary TB (v. smear-positive pulmonary TB) (adjOR 1.52, 95% CI 0.98-2.34) may be

predictive of delayed ART initiation, although they did not meet the *a priori* criteria for retention in the final model.

Table 4.1 Characteristics at baseline of 492 patients participating in the Integrated Tuberculosis and Anti-Retroviral Treatment (ITART) study at five primary care clinics in Kinshasa, Democratic Republic of Congo

Characteristic		n	%
Female		295	60.0
Age	<30	94	19.1
	30-39	182	37.0
	40-49	184	33.3
	≥50	52	10.6
Married		219	44.5
Completed secondary education		303	61.6
Employed		228	46.3
Low socio-economic status		454	92.3
Walk to clinic		371	75.4
Travel time to clinic (minutes)	<10	18	3.7
	10-19	141	28.7
	20-29	94	19.1
	30-39	136	27.6
	≥40	103	20.9
TB type	Smear + Pulmonary	193	39.2
	Smear – Pulmonary	199	40.5
	Extrapulmonary	100	20.3
History of TB		125	25.4
HIV diagnosis at TB clinic		469	95.3
CD4 count (cells/mm^3)	<50	77	15.6
	50-99	64	13.0
	100-199	136	27.6
	200-350	118	24.0
	>350	97	19.7
Hospitalization in Prior Year		77	15.7
Poor Functional Status		27	5.5
BMI	Underweight (<18.5)	283	57.5
	Normal (18.5-24.9)	194	39.4
	Overweight (25.0-29.9)	12	2.4
	Obese (≥30)	3	0.6
Alcohol use (any)		201	40.9
Disclosure of TB status		439	89.2
Disclosure of HIV status		197	40.0

Figure 4.1 Distribution of ART eligibility, expected timing of ART initiation, and observed timing of ART initiation in 492 patients receiving integrated TB and HIV treatment at five primary care clinics in Kinshasa, Democratic Republicof Congo.*



TB=tuberculosis; ART=antiretroviral therapy; CD4=CD4 count in cells/mm^3; shading=delayed ART; median (IQR)=median and interquartile range of time to ART initiation from TB treatment start. *Expected timing of ART is at 1 month or 2 months from the start of TB treatment or at completion of TB treatment with a +5 day window around these timepoints to accommodate scheduling limitations, clinic closure on weekends and holidays, and on-site availability of consulting physician.

Table 4.2 Predictors of Delayed ART Initiation in 492 patients participating in the Integrating Tuberculosis and Anti-Retroviral Treatment (ITART) study at five primary care clinics in Kinshasa, Democratic Republic of Congo

	Crude OR Univariate Model (95% CI)	**Adjusted OR Full Model (95% CI)**	**Adjusted OR Reduced Model (95% CI)**
Female	1.00 (0.70-1.44)	0.97 (0.65-1.45)	--
Age >40 years	1.06 (0.74-1.52)	1.01 (0.68-1.51)	--
Married	0.73 (0.51-1.04)	0.69 (0.46-1.01)	--
Secondary Education	0.88 (0.61-1.27)	0.94 (0.64-1.40)	--
Employed	0.88 (0.62-1.26)	1.04 (0.69-1.58)	--
Low SES	0.57 (0.29-1.12)	0.59 (0.29-1.22)	--
Walk to Clinic	0.80 (0.53-1.20)	0.83 (0.54-1.16)	--
Travel Time >30 min	0.85 (0.59-1.20)	0.79 (0.54-1.16)	--
TB Type Smear + Pulmonary	referent	referent	referent
Smear – Pulmonary	1.42 (0.95-2.12)	1.52 (0.98-2.34)	--
Extrapulmonary	1.15 (0.71-1.87)	1.03 (0.61-1.72)	--
History of TB	0.85 (0.57-1.28)	0.87 (0.56-1.35)	--
HIV diagnosis at TB clinic	1.20 (0.52-2.79)	0.89 (0.36-2.20)	--
CD4 count (per 100 cells/mm^3 decrease)	1.12 (1.02-1.23)	1.18 (1.06-1.32)	1.20 (1.08-1.33)
Prior Hospitalization	1.01 (0.62-1.65)	0.92 (0.54-1.54)	--
Poor Functional Status	1.19 (0.55-2.59)	1.42 (0.60-3.33)	--
Underweight (BMI<18.5)	0.90 (0.63-1.29)	0.89 (0.60-1.33)	--
Alcohol use (any)	0.97 (0.67-1.39)	0.96 (0.65-1.43)	--
Non-disclosure of TB status	1.15 (0.65-2.04)	0.87 (0.46-1.64)	--
Non-disclosure HIV status	1.46 (1.02-2.10)	1.60 (1.05-2.44)	1.50 (1.03-2.18)
Contraindication to ART*	2.58 (1.10-6.05)	2.90 (1.17-7.20)	2.91 (1.22-6.95)
Intolerance of TB Drugs*	1.36 (0.92-2.00)	1.93 (1.21-3.07)	1.93 (1.23-3.02)

OR=odds ratio; CI=confidence interval, SES=socio-economic status. *determined at scheduled ART initiation date (see text for details)

CHAPTER 5: CHAPTER FIVE: EFFECT OF CD4-STRATIFIED TIMING OF ANTIRETROVIRAL THERAPY INITIATION ON MORTALITY IN PATIENTS DIAGNOSED WITH TUBERCULOSIS IN A HIGHLY RESOURCE-LIMITED SETTING

Baseline Characteristics of Analytical Cohort

Between August 2007 and December 2009, 599 participants enrolled in the ITART study. Among them, 204 were sequentially excluded based on age<13 years (n=18), lack of baseline CD4 count (n=88), enrollment more than one month after TB treatment initiation (n=1), exposure to ART prior to enrollment (n=0), and CD4>350 (n=97). The remaining 395 participants were included in the analysis.

Baseline characteristics of patients are presented in Table 5.1. Most (80%) participants were diagnosed with pulmonary TB. Type of TB was smear-positive pulmonary in 35%, smear-negative pulmonary in 45%, and extrapulmonary in 20% of patients. Just over half (59%) were female and median age was 38 (IQR: 32-45). Most patients were underweight (median BMI 17.8, IQR: 16.5-19.7; 59.2% BMI<18.5). Patients presented late in the HIV disease process, with a median CD4 count of 131 cells/mm^3 (IQR: 63-224). Of the 143 (36%) patients eligible for ART at 1 month, 77 (54%) had CD4<50 cells/mm^3, 64 (45%) had CD4 50-99, and 2 (1%) had CD4>100 and WHO stage 4. Of the 252 (64%) patients eligible for ART at 2 months, 136 (54%) had CD4 100-199 and 116 (46%) had CD4 200-350. Few (n=24, 6%) of patients had contraindication to one or more antiretroviral drugs.

Implementation Fidelity to CD4-stratified Timing of ART Initiation

Overall, 183 (46%) participants initiated ART *per strategy*. Among the 212 (54%) participants who initiated ART *not per strategy*, 53 (25%) never initiated ART and 159 (75%) initiated ART with a median delay of 11 days (IQR: 4-24). Median delay did not differ by time of eligibility (12 days for 1 month v. 10 days for 2 months, p=0.62). Patients whose timing of ART initiation was *per strategy* had higher CD4 count (151 v. 113 cells/mm^3, p=0.002) and higher frequency of tolerating their TB drugs (96% v. 70%, p<0.0001) at the scheduled time of ART initiation than patients whose timing of ART initiation was *not per strategy*.

Predictors of Mortality in First Six Months of TB Treatment

Results of predictive modeling of mortality are presented in Table 5.2. In a series of crude (unadjusted) models, CD4 count <50 cells/mm^3 (OR 6.0, 95% CI: 2.2-16.4), and TB treatment intolerance (OR 12.3, 95% CI: 5.6-27.3) were predictive of mortality. In the final (reduced) model, TB treatment intolerance (adjOR 12.7, 95% CI: 4.8-33.2), CD4 count <50 cells/mm^3 (adjOR 7.3, 95% CI: 2.3-23.3), and male gender (adjOR 2.4, 95% CI: 1.0-5.6) were predictive of mortality. In addition, underweight (adjOR 2.2, 95% CI: 0.9-5.6), and not initiating ART per strategy (adjOR 2.5, 95% CI: 0.9-6.6) doubled the risk of mortality, although not statistically significantly.

Mortality under Observed Implementation Fidelity to CD4-stratified ART initiation

During the first six months of TB treatment, 33 participants died and 47 (11.9%) were LTFU. Among the 348 (88.1%) patients with an observed outcome, the six-month mortality risk was 9.5% (6.4-12.6%). The majority (n=26) of these deaths occurred in participants whose timing of ART initiation deviated from the CD4-stratified strategy.

When estimating the risk in the full cohort (i.e. including those LTFU) using the predicted outcome probabilities from the logistic regression model, the six-month mortality risk under observed implementation fidelity was 12.0% (95% CI: 8.2-15.7%).

Causal effect of implementation fidelity on six-month mortality

As shown in Table 4.3, complete fidelity to the CD4-stratified timing strategy for ART initiation in this population was estimated to result in a six-month mortality risk of 7.8% (95% CI: 2.4-12.3%), corresponding to a -4.2% risk difference (95% CI: -8.1, -0.3%). The preventable fraction of mortality due non-fidelity to the CD4-stratified ART initiation strategy was 35.1% (95% CI: 2.9-67.9%), suggesting that just over one third of observed mortality is preventable by fidelity to CD4-stratified ART initiation. These mortality estimates were robust to sensitivity analyses in which the definition of ART initiation *per strategy* was narrowed to exclude patients who were LTFU prior to the time eligibility (RD -4.1%, 95% CI: -7.8, -0.5% and preventable fraction 34.6%, 95% CI: 3.8-65.3%).

Table 5.1 Baseline characteristics of patients in the Integrating Tuberculosis and Anti-Retroviral Treatment (ITART) study analytical population by timing of ART initiation per CD4-stratified strategy (N=395)

Baseline Characteristic	All Patients		Timing of ART initiation per CD4-stratified strategy		Timing of ART initiation deviating from CD4-stratified strategy		p-value
	n	%*	n	%*	n	%*	
Total	395	100	183	46.3	212	53.7	--
Female	231	58.5	103	56.3	128	60.4	0.41
Age (years), median (IQR)	38	32-45	38	32-45	38	32-45	0.97
<30	65	16.5	32	17.5	33	15.6	0.61
30-39	158	40.0	73	39.9	85	40.1	0.97
40-49	129	32.7	56	30.6	73	34.4	0.42
≥50	43	10.9	22	12.0	21	9.9	0.50
TB type Smear + Pulmonary	139	35.2	71	38.8	68	32.1	0.16
Smear – Pulmonary	176	44.6	77	42.1	99	46.7	0.36
Extrapulmonary	80	20.3	35	19.1	45	21.2	0.60
CD4 count (cells/mm^3), median (IQR)	131	63-224	151	77-243	113	60-190	0.002
<50	77	19.5	33	18.0	44	20.8	0.50
50-99	64	16.2	21	11.5	43	20.3	0.02
100-199	136	34.4	61	33.3	75	35.4	0.67
200-350	118	29.9	68	37.2	50	23.6	0.003
BMI, median (IQR)	17.8	16.5-19.7	17.7	16.4-19.6	17.8	16.6-19.8	0.35
Underweight (<18.5)	234	59.2	114	62.3	120	56.6	0.25
Normal (18.5-24.9)	148	37.5	64	35.0	84	39.6	0.34
Overweight (25.0-29.9)	11	2.8	4	2.2	7	3.3	0.56
Obese (≥30)	2	0.5	1	0.6	1	0.5	1.0
Toleration of TB drugs	322	81.5	175	95.6	147	69.3	<0.0001
Contraindication to any ARV drug	24	6.1	7	3.8	17	8.0	0.09
WHO stage	90	22.8	41	22.4	49	23.1	0.87

*percentage reported unless noted otherwise; IQR=interquartile range; ART=antiretroviral therapy; ARV=antiretroviral

Table 5.2 Predictors of mortality in 395 HIV-infected patients diagnosed with TB with observed implementation fidelity to CD4-Stratified timing of ART initiation

		Crude OR Univariate Model (95% CI)	Adjusted OR Full Model (95% CI)	Adjusted OR Reduced Model (95% CI)
Gender	Male	1.78 (0.86-3.65)	1.97 (0.76-5.09)	2.37 (1.00-5.61)
	Female	1	1	1
Age (years)	<30	1.36 (0.44-4.17)	1.26 (0.32-4.96)	-
	30-39	1	1	-
	40-49	1.72 (0.71-4.14)	1.86 (0.64-5.37)	-
	≥50	2.40 (0.82-7.07)	2.96 (0.77-11.4)	-
Type of TB	Smear + Pulmonary	1	1	-
	Smear – Pulmonary	2.01 (0.81-4.98)	1.43 (0.48-4.24)	-
	Extrapulmonary	2.05 (0.71-5.91)	1.11 (0.07-17.79)	-
CD4 count (cells/mm3)	<50	5.99 (2.19-16.36)	7.66 (2.27-25.8)	7.30 (2.29-23.3)
	50-99	1.81 (0.53-6.19)	1.92 (0.47-7.86)	1.94 (0.49-7.59)
	100-199	1	1	1
	200-350	1.36 (0.44-4.17)	2.19 (0.58-8.20)	1.95 (0.56-6.79)
BMI category	Underweight (<18.5)	1.92 (0.87-4.27)	2.46 (0.94-6.2)	2.21 (0.89-5.62)
	Not Underweight (≥18.5)	1	1	1
TB treatment intolerance	Yes	12.33 (5.56-27.31)	12.75 (4.69-34.71)	12.65 (4.82-33.20)
	No	1	1	-
Contraindication to any ARV	Yes	0.90 (0.20-4.04)	1.43 (0.48-4.24)	-
	No	1	1	-
WHO stage	4	1.34 (0.59-3.01)	1.28 (0.10-16.1)	-
	3	1	1	-
ART initiation	Not per strategy	4.25 (1.79-10.07)	2.52 (0.93-6.86)	2.47 (0.93-6.55)
	Per strategy	1	1	1

OR=odds ratio; TB=tuberculosis; BMI=body mass index; ARV=antiretroviral; WHO=World Health Organization; ART=antiretroviral therapy

Table 5.3. Six-month mortality risk in the Integrating Tuberculosis and Anti-Retroviral Treatment (ITART) study population (N=395)under observed and complete implementation fidelity to timing of ART initiation per CD4-stratified strategy

	Estimate (95% CI)
Observed Risk in ITART population *	0.095 (0.064-0.126)
Estimated Risk in ITART population with observed implementation fidelity of the CD4-stratified ART initiation strategy	0.120 (0.082,0.157)
Estimated Risk in ITART population with complete fidelity to the CD4-stratified ART initiation strategy	0.078 (0.024, 0.123)
Estimated Risk Difference in mortality between observed and complete implementation fidelity	-0.042 (-0.081, -0.003)
Estimated Preventable fraction of mortality under complete implementation fidelity	0.351 (0.029, 0.679)

*excludes 47 patients missing six-month mortality outcome.

CHAPTER SIX: DISCUSSION

Despite full integration of TB treatment and ART by the same provider at the same primary health care TB clinic space, delay in ART initiation was common, with only half of all HIV-infected TB patients initiated ART per CD4-stratified timing strategy. This finding of low implementation fidelity suggests that the 2012 WHO recommendation that calls for CD4-stratified timing of ART initiation [49], may be challenging to implement in resource-limited routine clinical care.

Our finding of delay in ART initiation despite full integration of TB and HIV treatment confirms the finding of two studies in South Africa. In a before-after study, Kershberger et al. found that integration reduced median time to ART initiation from 147 to 75 days after TB treatment start. Half of all patients still initiated ART after 75 days; a timeframe that is late given a median CD4 cell count 84 cells/mm3 (IQR 32-158) in the study population.[40] In the Starting Antiretroviral Therapy at Three Points in Tuberculosis (SAPiT) study, a randomized controlled trial of ART timing in patients diagnosed with TB, 16.7% of patients assigned to early ART (within 4 weeks of TB treatment start) and 28.7% of patients assigned to late ART (within 4 weeks of the continuation phase) did not initiate ART per study protocol. [33]

Our study is the first to evaluate patient-level factors predictive of delayed ART initiation in an integrated TB/HIV treatment setting. Delayed ART initiation was more likely among those with lower CD4 count, no HIV disclosure, intolerance of TB drugs, and contradiction to an ARV drug. Two prior studies of delay in ART initiation among patients diagnosed with TB were

performed in a non-integrated setting. [21, 22] Similar to our findings, these studies found that smear-negative pulmonary TB was associated with delay in ART initiation, which may be related to the additional complexity and time required for a final diagnosis of this form of TB.[50] In contrast to our findings, these studies found that lower CD4 count was associated with less delay. This contradictory finding may be due to the fact that the outcome in these studies was absolute time to ART, without consideration of baseline CD4 count or clinical stage and that these studies only included patients who reached the ART clinic and initiated ART. The outcome in our study was delayed ART initiation, defined based on the study protocol, which recommended earlier ART initiation for patients with lower CD4 count. Consequently, our finding of lower CD4 count as a predictor, may in fact, reflect the challenge of early ART initiation due to a number of factors including patient readiness to immediately start life-long ART, potential for immune reconstitution inflammatory syndrome[33, 34][51], non-adherence due to increased pill burden, and overlapping drug toxicities.[52] Additionally, we found that intolerance of TB treatment was predictive of delayed ART initiation, suggesting that both providers and patients may be reluctant to initiate ART until TB treatment is tolerated. Our finding that non-disclosure of HIV status was predictive of delayed ART initiation is consistent with other studies that have found an association between non-disclosure of HIV status and lack of readiness to initiate ART, which may be related to social support and fear of stigma; factors that may also explain why being non-married was a potential predictor in our study [53-55].

Our finding of patient-level factors associated with delayed ART can inform additional policy and programmatic interventions. Patient-level factors can influence both patient and health provider perceptions and decision-making about ART initiation; therefore, interventions should be designed accordingly. Training of health providers and health communication messaging to

patients should emphasize the importance of early ART initiation, particularly among patients presenting with low CD4 count. Availability of more than one first-line antiretroviral regimen could help expedite ART initiation in patients with one or more drug contraindications. Furthermore, since severity of TB drug intolerability may vary substantially, clear guidance to health providers on the severity of TB drug intolerance that warrants delay of ART is needed. Finally, provider-initiated testing and counseling for HIV should emphasize and facilitate safe HIV disclosure by patients; an essential component of the HIV prevention package that was associated with timely ART initiation in our study.

Under the condition of low implementation fidelity to the CD4-stratified ART initiation strategy, the six-month mortality estimate risk in the full cohort was 12.0%. This estimated risk was higher than the observed risk (9.5%) because patients who were LTFU and missing an outcome, had a higher risk of mortality based on their baseline characteristics. Although not unexpected given that many of the risk factors for LTFU are also risk factors for mortality; this finding suggests that risk of mortality would have underestimated in a complete-case analysis. This finding also suggests that even if these patients had been retained in care, a relatively large proportion may have died. Under the scenario of 100% fidelity to the CD4 count stratified ART initiation strategy, we found a 4.2% (95% CI 0.03% to 8.1%) reduction in the six-month mortality risk, representing a 35.1% preventable fraction of mortality in this population. There were ten patients whose predicted probability of death was greater than 50% even under the scenario of complete fidelity to timing of ART initiation per CD4-stratified timing. All ten had baseline CD4 count <50 cells/mm^3 and did not tolerate TB treatment, suggesting that additional interventions beyond timely ART initiation may be necessary to prevent death in these patients. Since the definition of TB treatment intolerance is inherently non-specific, patients with severe

immunosuppression who are assessed as not tolerating TB treatment may in fact have other underlying clinical conditions that may contribute to mortality and require attention beyond that available at the primary care level.

Few studies have quantified implementation fidelity to international guidelines for resource limited settings, including those for integrated TB/HIV care and treatment and we are not aware of any studies that have attempted to estimate the gain that can be achieve by reaching 100% implementation fidelity. This is an important limitation as it is only by evaluating the fidelity with which an intervention has been implemented that a viable assessment can be made of its contribution to desired outcomes.[43] As such, it remains unclear based on current monitoring of most international public health programs whether any positive outcomes following implementation of new guidelines might be improved further if greater attention was paid to implementation fidelity. It has therefore been suggested that research of these interventions and their outcomes should involve an evaluation of implementation fidelity if the true effect of the intervention is to be discerned.[43]

Our study provides one of the first examples of using modern epidemiological methods to measuring the impact of implementation fidelity to international guidelines in resource limited settings on desired outcomes. The parametric g-formula generates unbiased effects of the causal effect of an intervention using observational data when assumptions of exchangeability (no uncontrolled confounding or uncontrolled selection bias), positivity (patients in each treatment group across all strata of each covariate), and consistency (for a given treatment, the counterfactual outcome is equivalent to the observed outcome for each patient) are met. This allows investigators to extend the analysis of observational study to estimate the population attributable fraction that contrasts scenarios of intervention fidelity beyond the traditional all

versus none. We used this approach to study the risk difference between different levels of implementation fidelity to one intervention; however, this approach could also useful to compare effects across multiple or complex interventions.[46, 48, 56] We believe that the application of the parametric g-formula has great potential to contribute to the field of implementation science.[56, 57]

Our study has several limitations that must be considered in the interpretation of our findings. First, transport for CD4 count specimens/results was provided as a part of the study to help ensure CD4 count was available for decision-making on timing of ART initiation. Our findings may not be generalizable to settings in which availability of CD4 count is limited. Second, our study provided additional training and support of nurses to provide integrated ART and TB treatment and more frequent and extensive clinical visits beyond those available in routine care. Consequently, our findings may not be fully generalizable to routine care provided in primary care clinics by nurses who do not receive this level of support. While these differences may impact the timing of ART in patients diagnosed with TB, we do not believe that they are likely to substantially influence patient characteristics associated with delayed ART initiation. Monitoring of ART timing and patient factors associated with delayed ART in routine settings will thus be important. Third, the nurses making decisions on when to initiate ART were trained and closely monitored as part of the research study. The degree of implementation fidelity in routine settings may be even lower and the causal effect of implementation fidelity may have been underestimated. Fourth, similar to most studies of implementation fidelity, our assessment focused exclusively on the adherence component by assessing whether ART was initiated on time. We did not evaluate other components of implementation fidelity, such as intervention complexity, facilitation strategies, quality of delivery and patient responsiveness, factors that can influence or moderate the level of adherence by health care workers to guideline

implementation.[43] Fifth, we focused on reduction in short-term mortality, which is only one of the desired outcomes of TB/HIV care. Other potentially relevant outcomes could be reduction in long-term mortality and treatment success for HIV (adherence to ARV medications and virologic suppression) and TB (adherence to TB medications and confirmed cure).

Despite fully integrated TB/HIV treatment, nearly half of all HIV-infected patients diagnosed with TB experienced delay in ART initiation during TB treatment, such that timing of ART initiation was not per CD4-strategy. Strategies to achieve high implementation fidelity to CD4-stratified timing of ART initiation for patients with TB at primary care clinics in resource limited settings need to be developed as greater implementation fidelity has the potential to reduce mortality. Several patient-level factors that predicted delayed ART initiation can inform the development and implementation of such strategies to specifically target patients identified as at-risk of delayed ART initiation.

There are several next steps for research on timing of ART in patients with TB based on our study findings and limitations. First, research is needed to compare outcomes between CD4-stratified ART timing and early ART timing regardless of CD4. As discussed, the three major RCT studies assigned patients to either early or late ART timing regardless of CD4 count. In contrast, all patients in our study were assigned to CD4-stratified ART timing. To date, there has not been a comparison between CD4-stratified ART timing and early timing regardless of CD4 count. This would be a helpful comparison to inform further development of WHO guidelines. A pooled analysis of ITART and one or more of the RCT studies that used a model-based standardization approach would allow for a comparison of ITART under the scenario of complete fidelity to CD4-stratified ART timing and ITART under the scenario of early ART timing regardless of CD4. Second, as countries implement 2012 WHO guidelines for CD4-

stratified ART timing, research on additional outcomes of TB/HIV treatment such as long-term mortality, TB treatment adherence, TB cure, ART adherence, HIV viral load suppression, etc. are necessary. This type of research could be done in a pre/post intervention design, particularly if implementation of the new WHO guidelines is implemented across regions or sites gradually over time. Third, given our finding that several patient-level characteristics predicted delayed ART in an integrated TB/HIV setting, additional research that studies the impact of interventions that target these factors would be helpful to inform wide-scale implementation.

REFERENCES

1. World Health Organization. (2013) Factsheet: The top 10 causes of death. Factsheet N310. 1/18/12.

2. World Health Organization. (2013) Global tuberculosis report 2013. WHO/HTM/TB/2013.11.

3. Joint United Nations Programme on HIV/AIDS (UNAIDS). (2013) Global report: UNAIDS report on the global AIDS epidemic 2013. UNAIDS/JC2502/1/E.

4. Corbett EL, Watt CJ, Walker N, Maher D, Williams BG, et al. (2003) The growing burden of tuberculosis: Global trends and interactions with the HIV epidemic. Arch Intern Med 163(9): 1009-1021. 10.1001/archinte.163.9.1009.

5. Mukadi YD, Maher D, Harries A. (2001) Tuberculosis case fatality rates in high HIV prevalence populations in sub-saharan africa. AIDS 15(2): 143-152.

6. Lawn SD, Kranzer K, Wood R. (2009) Antiretroviral therapy for control of the HIV-associated tuberculosis epidemic in resource-limited settings. Clin Chest Med 30(4): 685-99, viii. 10.1016/j.ccm.2009.08.010.

7. Centers for Disease Control and Prevention (CDC). (2008) Provider-initiated HIV testing and counseling of TB patients--livingstone district, zambia, september 2004-december 2006. MMWR Morb Mortal Wkly Rep 57(11): 285-289.

8. Van Rie A, Sabue M, Jarrett N, Westreich D, Behets F, et al. (2008) Counseling and testing TB patients for HIV: Evaluation of three implementation models in kinshasa, congo. Int J Tuberc Lung Dis 12(3 Suppl 1): 73-78.

9. World Health Organization. (1/18/12): 5.

10. De Cock KM, Soro B, Coulibaly IM, Lucas SB. (1992) Tuberculosis and HIV infection in sub-saharan africa. JAMA 268(12): 1581-1587.

11. Hoshino Y, Tse DB, Rochford G, Prabhakar S, Hoshino S, et al. (2004) Mycobacterium tuberculosis-induced CXCR4 and chemokine expression leads to preferential X4 HIV-1 replication in human macrophages. J Immunol 172(10): 6251-6258.

12. Zhang Y, Nakata K, Weiden M, Rom WN. (1995) Mycobacterium tuberculosis enhances human immunodeficiency virus-1 replication by transcriptional activation at the long terminal repeat. J Clin Invest 95(5): 2324-2331. 10.1172/JCI117924.

13. Straetemans M, Bierrenbach AL, Nagelkerke N, Glaziou P, van der Werf MJ. (2010) The effect of tuberculosis on mortality in HIV positive people: A meta-analysis. PLoS One 5(12): e15241. 10.1371/journal.pone.0015241.

14. Whalen C, Horsburgh CR, Hom D, Lahart C, Simberkoff M, et al. (1995) Accelerated course of human immunodeficiency virus infection after tuberculosis. Am J Respir Crit Care Med 151(1): 129-135.

15. Dheda K, Lampe FC, Johnson MA, Lipman MC. (2004) Outcome of HIV-associated tuberculosis in the era of highly active antiretroviral therapy. J Infect Dis 190(9): 1670-1676. 10.1086/424676.

16. Haar CH, Cobelens FG, Kalisvaart NA, van Gerven PJ, van der Have JJ. (2007) HIV-related mortality among tuberculosis patients in the netherlands, 1993-2001. Int J Tuberc Lung Dis 11(9): 1038-1041.

17. Manosuthi W, Chottanapand S, Thongyen S, Chaovavanich A, Sungkanuparph S. (2006) Survival rate and risk factors of mortality among HIV/tuberculosis-coinfected patients with and without antiretroviral therapy. J Acquir Immune Defic Syndr 43(1): 42-46. 10.1097/01.qai.0000230521.86964.86.

18. Nahid P, Gonzalez LC, Rudoy I, de Jong BC, Unger A, et al. (2007) Treatment outcomes of patients with HIV and tuberculosis. Am J Respir Crit Care Med 175(11): 1199-1206. 10.1164/rccm.200509-1529OC.

19. Akksilp S, Karnkawinpong O, Wattanaamornkiat W, Viriyakitja D, Monkongdee P, et al. (2007) Antiretroviral therapy during tuberculosis treatment and marked reduction in death rate of HIV-infected patients, thailand. Emerg Infect Dis 13(7): 1001-1007.

20. Varma JK, Nateniyom S, Akksilp S, Mankatittham W, Sirinak C, et al. (2009) HIV care and treatment factors associated with improved survival during TB treatment in thailand: An observational study. BMC Infect Dis 9: 42. 10.1186/1471-2334-9-42.

21. Lawn SD, Campbell L, Kaplan R, Little F, Morrow C, et al. (2011) Delays in starting antiretroviral therapy in patients with HIV-associated tuberculosis accessing non-integrated clinical services in a south african township. BMC Infect Dis 11: 258. 10.1186/1471-2334-11-258.

22. Lawn SD, Campbell L, Kaplan R, Boulle A, Cornell M, et al. (2011) Time to initiation of antiretroviral therapy among patients with HIV-associated tuberculosis in cape town, south africa. J Acquir Immune Defic Syndr 57(2): 136-140. 10.1097/QAI.0b013e3182199ee9.

23. Chilton D, Edwards SG, Pellegrino P, Miller RF. (2008) Factors influencing delay in initiating antiretroviral therapy among HIV infected patients coinfected with tuberculosis. Thorax 63(10): 935-936. 10.1136/thx.2008.104232.

24. Hammer SM, Squires KE, Hughes MD, Grimes JM, Demeter LM, et al. (1997) A controlled trial of two nucleoside analogues plus indinavir in persons with human immunodeficiency virus infection and CD4 cell counts of 200 per cubic millimeter or less. N Engl J Med 337(11): 725-733.

25. Gulick RM, Mellors JW, Havlir D, Eron JJ, Gonzalez C, et al. (1997) Treatment with indinavir, zidovudine, and lamivudine in adults with human immunodeficiency virus infection and prior antiretroviral therapy. N Engl J Med 337(11): 734-739.

26. When To Start Consortium, Sterne JA, May M, Costagliola D, de Wolf F, et al. (2009) Timing of initiation of antiretroviral therapy in AIDS-free HIV-1-infected patients: A collaborative analysis of 18 HIV cohort studies. Lancet 373(9672): 1352-1363. 10.1016/S0140-6736(09)60612-7.

27. Kitahata MM, Gange SJ, Abraham AG, Merriman B, Saag MS, et al. (2009) Effect of early versus deferred antiretroviral therapy for HIV on survival. N Engl J Med 360(18): 1815-1826. 10.1056/NEJMoa0807252.

28. Kaplan JE, Hanson DL, Cohn DL, Karon J, Buskin S, et al. (2003) When to begin highly active antiretroviral therapy? evidence supporting initiation of therapy at CD4 lymphocyte counts< 350 cells/µL. Clinical Infectious Diseases 37(7): 951-958.

29. Writing Committee for the CASCADE Collaboration. (2011) Timing of HAART initiation and clinical outcomes in human immunodeficiency virus type 1 seroconverters. Arch Intern Med 171(17): 1560-1569.

30. Strategies for Management of Antiretroviral Therapy (SMART) Study Group, Emery S, Neuhaus JA, Phillips AN, Babiker A, et al. (2008) Major clinical outcomes in antiretroviral therapy (ART)-naive participants and in those not receiving ART at baseline in the SMART study. J Infect Dis 197(8): 1133-1144. 10.1086/586713.

31. Severe P, Juste MA, Ambroise A, Eliacin L, Marchand C, et al. (2010) Early versus standard antiretroviral therapy for HIV-infected adults in haiti. N Engl J Med 363(3): 257-265. 10.1056/NEJMoa0910370.

32. Abdool Karim SS, Naidoo K, Grobler A, Padayatchi N, Baxter C, et al. (2010) Timing of initiation of antiretroviral drugs during tuberculosis therapy. N Engl J Med 362(8): 697-706. 10.1056/NEJMoa0905848.

33. Abdool Karim SS, Naidoo K, Grobler A, Padayatchi N, Baxter C, et al. (2011) Integration of antiretroviral therapy with tuberculosis treatment. N Engl J Med 365(16): 1492-1501. 10.1056/NEJMoa1014181.

34. Havlir DV, Kendall MA, Ive P, Kumwenda J, Swindells S, et al. (2011) Timing of antiretroviral therapy for HIV-1 infection and tuberculosis. N Engl J Med 365(16): 1482-1491. 10.1056/NEJMoa1013607.

35. Blanc FX, Sok T, Laureillard D, Borand L, Rekacewicz C, et al. (2011) Earlier versus later start of antiretroviral therapy in HIV-infected adults with tuberculosis. N Engl J Med 365(16): 1471-1481. 10.1056/NEJMoa1013911.

36. Manosuthi W, Mankatitham W, Lueangniyomkul A, Thongyen S, Likanonsakul S, et al. (2012) Time to initiate antiretroviral therapy between 4 weeks and 12 weeks of tuberculosis treatment in HIV-infected patients: Results from the TIME study. J Acquir Immune Defic Syndr 60(4): 377-383. 10.1097/QAI.0b013e31825b5e06.

37. Velasco M, Castilla V, Sanz J, Gaspar G, Condes E, et al. (2009) Effect of simultaneous use of highly active antiretroviral therapy on survival of HIV patients with tuberculosis. J Acquir Immune Defic Syndr 50(2): 148-152. 10.1097/QAI.0b013e31819367e7.

38. Franke MF, Robins JM, Mugabo J, Kaigamba F, Cain LE, et al. (2011) Effectiveness of early antiretroviral therapy initiation to improve survival among HIV-infected adults with tuberculosis: A retrospective cohort study. PLoS Med 8(5): e1001029. 10.1371/journal.pmed.1001029.

39. Henegar C, Behets F, Vanden Driessche K, Tabala M, Bahati E, et al. (2012) Mortality among tuberculosis patients in the democratic republic of congo. The International Journal of Tuberculosis and Lung Disease 16(9): 1199-1204.

40. Kerschberger B, Hilderbrand K, Boulle AM, Coetzee D, Goemaere E, et al. (2012) The effect of complete integration of HIV and TB services on time to initiation of antiretroviral therapy: A before-after study. PLoS One 7(10): e46988.

41. Huerga H, Spillane H, Guerrero W, Odongo A, Varaine F. (2010) Impact of introducing human immunodeficiency virus testing, treatment and care in a tuberculosis clinic in rural kenya. Int J Tuberc Lung Dis 14(5): 611-615.

42. Breitenstein SM, Gross D, Garvey CA, Hill C, Fogg L, et al. (2010) Implementation fidelity in community-based interventions. Res Nurs Health 33(2): 164-173. 10.1002/nur.20373; 10.1002/nur.20373.

43. Carroll C, Patterson M, Wood S, Booth A, Rick J, et al. (2007) A conceptual framework for implementation fidelity. Implement Sci 2: 40. 10.1186/1748-5908-2-40.

44. Hernan MA, Robins JM. (2014 (forthcoming)) Casual inference. In Press.

45. Westreich D. (2013) From exposures to interventions: An example in pregnancy and response to HIV therapy. Am J Epidemiol 177 (suppl 11): S1-S181(S1-S181.): S135.

46. Petersen ML, Wang Y, van der Laan, Mark J, Bangsberg DR. (2006) Assessing the effectiveness of antiretroviral adherence interventions: Using marginal structural models to replicate the findings of randomized controlled trials. JAIDS 43(Supplement 1): S96-S103.

47. Robins J. (1987) A graphical approach to the identification and estimation of causal parameters in mortality studies with sustained exposure periods. J Chronic Dis 40: 139S-161S.

48. Westreich D, Cole SR, Young JG, Palella F, Tien PC, et al. (2012) The parametric g-formula to estimate the effect of highly active antiretroviral therapy on incident AIDS or death. Stat Med 31(18): 2000-2009. 10.1002/sim.5316; 10.1002/sim.5316.

49. World Health Organization. (2012) WHO policy on collaborative TB/HIV activities: Guidelines for national programmes and other stakeholders. WHO/HTM/TB/2012.6.

50. Dimairo M, MacPherson P, Bandason T, Zezai A, Munyati SS, et al. (2010) The risk and timing of tuberculosis diagnosed in smear-negative TB suspects: A 12 month cohort study in harare, zimbabwe. PloS One 5(7): e11849.

51. Lawn SD, Myer L, Bekker LG, Wood R. (2007) Tuberculosis-associated immune reconstitution disease: Incidence, risk factors and impact in an antiretroviral treatment service in south africa. AIDS 21(3): 335-341. 10.1097/QAD.0b013e328011efac.

52. McIlleron H, Meintjes G, Burman WJ, Maartens G. (2007) Complications of antiretroviral therapy in patients with tuberculosis: Drug interactions, toxicity, and immune reconstitution inflammatory syndrome. J Infect Dis 196(Supplement 1): S63-S75.

53. Wong LH, Van Rooyen H, Modiba P, Richter L, Gray G, et al. (2009) Test and tell: Correlates and consequences of testing and disclosure of HIV status in south africa (HPTN 043 project accept). J Acquir Immune Defic Syndr 50(2): 215.

54. Abaynew Y, Deribew A, Deribe K. (2011) Factors associated with late presentation to HIV/AIDS care in south wollo ZoneEthiopia: A case-control study. .

55. Tessema B, Biadglegne F, Mulu A, Getachew A, Emmrich F, et al. (2010) Magnitude and determinants of nonadherence and nonreadiness to highly active antiretroviral therapy among people living with HIV/AIDS in northwest ethiopia: A cross-sectional study. AIDS Research and Therapy 7(1): 2.

56. Ahern J, Hubbard A, Galea S. (2009) Estimating the effects of potential public health interventions on population disease burden: A step-by-step illustration of causal inference methods. Am J Epidemiol 169(9): 1140-1147.

57. Taubman SL, Robins JM, Mittleman MA, Hernán MA. (2009) Intervening on risk factors for coronary heart disease: An application of the parametric g-formula. Int J Epidemiol 38(6): 1599-1611.