OPTIMIZATION OF TB/HIV CO-TREATMENT IN ETHIOPIAN PATIENTS

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OPTIMIZATION OF TB/HIV CO-TREATMENT IN ETHIOPIAN PATIENTS

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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This thesis work is first and foremost dedicated to my most loving family: Wzo Seble Deneke (my dear wife), Amanuel, Kaleb and Dawit Wondwossen (my three sons), for inspiring me, to their continued support as well as prayers. I sincerely thank my lovely queen who is my most beloved wife and my three handsome princes who are my kids for the unconditional love you provided me, teaching me endurance and sacrificing your time as well as interests for my tireless academic interests. May God bless you all in his abundant grace and keep the family’s unity always safe.

It is also dedicated to the living memory of my sister and brother, Wzo Tiruwork Amogne (Yeshum as I call you) and Ato Tewodros Amogne (Gezye as I call you). Both died at their productive age from tragic car accidents but their memory keeps on actively surviving inside me.
Abstract

Tuberculosis (TB) and HIV infection act with deadly synergy. HIV is the most important risk factor for latent TB reactivation and active TB progression following exposure or reinfection while TB accelerates HIV progression. TB is the most frequent cause of morbidity and mortality in HIV infection. Anti-TB therapy (ATT) must precede initiation of combination Antiretroviral Therapy (cART), TB being the most immediate threat. Undoubtedly cART benefits; however, important clinical challenges emerge when cART is initiated during TB therapy. Optimization TB and HIV cotreatment is therefore required.

Paper II: We hypothesized that by initiating efavirenz (EFV)-based cART earlier than the second week of ATT in patients with CD4 counts < 200 cells/µL; overall survival can be improved at 48 weeks. The study hypothesis was tested with randomized, open-label, clinical trial comparing efficacy and safety of EFV-based cART one week, four weeks and eight weeks after ATT in coinfected patients with baseline CD4 count < 200 cells/µL. The results showed that cART one week after TB therapy doesn’t improve overall survival at 48 weeks. All-cause mortality in subgroups with CD4 count below 50 cells/µL versus above was lowest in week one. However, compared with week four and eight, first line ATT interruption from severe Drug-Induced Liver Injury (DILI) was highest in week one deaths (P=0.03) and in the CD4 subgroup < 50 cells/µL (P=0.02). The key question for CD4 category < 50 cells/µL is striking the optimal balance between the potential survival benefits if cART is initiated one week after TB therapy as opposed to the increased morbidity and mortality due to DILI and risk of ATT interruption.

Paper I and IV: We investigated DILI during TB/HIV cotreatment and HIV-treatment with EFV-based cART. DILI is the most important treatment limiting factor for continuation of both ATT and/or cART. Multiple evidences show that TB/HIV coinfected patients experience higher rate of adverse drug reactions than those without HIV. Both are prospective cohort studies and analysis was made with multivariate Cox regression model. Outcome measures were incidence rates for DILI, ATT and/or cART interruptions as well as assessment of risk factors. Paper I on EFV-based cART in HIV-infected patients with baseline CD4 counts < 200 cells/µL revealed high plasma EFV levels and CYP 2B6*6 genetic polymorphism predict DILI events in addition to baseline transaminitis, low CD4 count, low serum albumin and platelet values. Paper IV specifically addressed severe form of DILI during TB/HIV cotreatment compared with only TB treatment in HIV-negative patients as well as EFV-based cART in HIV-infected patients with baseline CD4 counts < 200 cells/µL inclusive of isoniazid preventive therapy. The findings from this study are: severe DILI risk is increased several folds with TB/HIV coinfection whereas concurrent
cART timing doesn’t alter the risk. Incidence rate of ATT interruption is higher with CD4<50 cells/µL. Independent risk factors for severe DILI in addition to TB/HIV coinfection were abnormal alanine aminotransferase and bilirubin values at baseline, CD4 < 50 cells/µL and positive HCV antibody result. In summary, the result concur earlier initiation of EFV-based cART during TB/HIV coinfection, though DILI remains as the most important challenge. It is more related to ATT than EFV-based cART and not affected by the timing of EFV-based cART within the first 8 weeks of TB therapy. 

**Paper III** evaluated Ethiopian HIV-1 subtype C virus (HIV-1CET) at near full length genome level for phylogenetic analysis, genotypic drug resistance and viral tropism. The results showed high diversity among HIV-1CET strains compared to other geographical locations suggesting introduction of HIV-1C in the country occurred in early phase of HIV-1C epidemic. Primary drug resistant mutations were identified in < 5% of the cases and 95% of the viral strains had R-5 tropism.
LIST OF SCIENTIFIC PAPERS

The thesis is based upon the following studies, which will be referred to by their Roman numerals:


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LIST OF ABBREVIATIONS

TB               tuberculosis
cART             combination antiretroviral therapy
ATT              Antituberculosis therapy
DILI             Drug-Induced Liver Injury
EFV              efavirenz
TB-IRIS          Tuberculosis associated immune reconstitution inflammatory syndrome
WHO              World Health Organization
MDR-TB           multidrug resistant tuberculosis
ALT              alaninie aminotransferase
AST              aspartate aminotransferase
ALP              alkaline phosphatase
HIV-1C<sub>E</sub> HIV subtype C (Ethiopian strain)
IPT              isoniazid preventive therapy
PTB              pulmonary tuberculosis
SP-PTB           smear-positive pulmonary tuberculosis
SN-PTB           smear-negative pulmonary tuberculosis
EPTB             extra pulmonary tuberculosis
ULN              upper limit of normal
RH               rifampicin and isoniazid
RHZE             rifampicin, isoniazid, pyrazinamide and ethambutol
OIs              opportunistic infections
HAART            highly active antiretroviral therapy
aHR              adjusted hazard ratio
SD               standard deviation
IQR              interquartile range
NFLG             near full length genome
GRT              genotypic resistance testing
GTT              genotypic tropism testing
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>PLHIV</td>
<td>people living with HIV</td>
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<tr>
<td>ADI</td>
<td>AIDS defining illness</td>
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<td>RIF</td>
<td>rifampicin</td>
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<tr>
<td>CNS</td>
<td>central nervous system</td>
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<td>DOT</td>
<td>directly observed therapy</td>
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<td>NNRTIs</td>
<td>nonnucleoside reverse transcriptase inhibitors</td>
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<tr>
<td>NVP</td>
<td>nevirapine</td>
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<tr>
<td>MAC</td>
<td>mycobacterium avium complex</td>
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<td>LMICs</td>
<td>low and middle-income countries</td>
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1. INTRODUCTION TO THE THESIS

This thesis focuses on tuberculosis (TB) and HIV coinfection in a high TB and HIV prevalent setting. Globally TB and HIV are the leading causes of death from infectious diseases. Each one is deadly and their intersection is deadlier. TB is the most frequent cause of morbidity and mortality in HIV-infected patients. According to the World Health Organization (WHO) in 2013, an estimated 9 million people developed TB worldwide and 1.1 million had HIV co-infection of whom 30% died. According to this report, Ethiopia ranks 8th from the 22 of the high TB burden countries. In addition, is one of the high TB/HIV and MDR-TB burden countries.

TB and HIV cotreatment has potential benefits and risks. The optimal timing for combination antiretroviral therapy (cART) undoubtedly needs further definition. Guidelines in general recommend initiation of cART as early as the second week of antituberculosis therapy (ATT) and earlier than two weeks when the CD4 count is < 50 cells/µL. Nevertheless the evidences for earlier initiation are strong only for CD4 < 50 cells/µL. The optimal time for cART initiation is a function of the interaction between efficacy and safety parameters as well as the level of immunosuppression. The clinical trial in this thesis assessed different cART timings within the first two months of ATT with respect to measurable endpoints after 48 weeks of enrollment.

HIV-1 virus subtype C\textsubscript{ET} dominates the Ethiopian HIV-epidemic accounting for 99% of viral subtype in circulation unlike other neighboring countries. This is based on earlier studies using smaller gene fragments. We have characterized the virus at full-length genome level for phylogenetic analysis, baseline genotypic resistance testing and assess its tropism.

In the background section of this thesis, the current knowledge of TB/HIV coinfection and the Ethiopian HIV-1 subtype C virus are described. Synopses of the known and unknown facts about the TB/HIV coinfection, evidence-based review for the need of optimizing TB and HIV cotreatment and drug induced liver injury (efavirenz–based and ATT-induced) are discussed. Previous studies about the HIV-1CET viral strain are reviewed. The study rational including the objectives for the various studies is explained. The study methods are described in detail, the results discussed and last recommendations are made.
Tikur Anbessa (Black Lion) Hospital in Addis Ababa, the main collaborating Medical Center for this thesis
2. BACKGROUND

2.1. Tuberculosis and HIV coinfection
Tuberculosis (TB) and HIV are the two most deadly communicable diseases worldwide. In high income countries, all forms of TB are regarded AIDS-defining illness (ADI) while the World Health Organization (WHO) clinical staging differentiates pulmonary TB (stage 3) from extrapulmonary TB (WHO clinical stage 4 or equivalent of AIDS) [1]. Tuberculosis and HIV (TB/HIV) coinfection is the most frequent cause of morbidity and mortality in people living with HIV (PLHIV) [2,3]. In this thesis unless stated, “tuberculosis” refers to the active disease rather than latent form of infection. The global HIV associated TB mortality had its peak in 2004 around 540,000 deaths. It declined to 360,000 in 2013, equivalent to 25% of all TB deaths (HIV-infected and uninfected) and a quarter of an estimated 1.5 million of HIV-associated deaths in the same year[4]. Treatment with combination Antiretroviral Therapy (cART) and TB/HIV collaborative activities are possible explanations for this steady decline of mortality starting from 2004. In the world there were approximately 35 million PLHIV and 9 million new TB incident cases by the end of 2013; 70% of them were living in the sub-Saharan Africa[4]. The toll of TB/HIV pandemic has been in sub-Saharan Africa where there are high incidence rates of active TB and HIV infection per capita. The two infections killed almost 3 million people altogether by the end of 2013 [4]. The risk of developing tuberculosis among PLHIV is 30 times higher compared with HIV-uninfected [4]. For the aforementioned reasons, it is not surprising that TB/HIV coinfection becomes major public health problem in sub-Saharan Africa. Unlike other opportunistic infections active TB occurs at any CD4 count whereas the current CD4 count of an individual determines the incidence and clinical presentations of the disease. Furthermore, the interaction between risk of developing TB and the CD4 count is reciprocal. The risk of TB increases as CD4 cell count decreases [5,6]. Likewise, the mortality rate associated with TB is high in persons with the low CD4 counts [7]. TB case fatality ratios (CFR) in African and Asian patients not receiving cART or whom it was deferred for 8 weeks ranges from 16-35% [8,9,10] whereas without cART up to 50% of PLHIV who are diagnosed with TB die during the first 6-8 months of TB treatment[11,12,13].

2.1.1. Epidemiology of tuberculosis and HIV epidemics
Globally, one-third of HIV-infected persons are estimated to have concomitant (usually latent) infection with M. tuberculosis [14]. Thirteen percent of the 9.0 million people who developed TB in 2013 worldwide were HIV-positive. Sub-Saharan Africa, epicenter of the TB/HIV coepidemic, hosts 80% of the estimated TB/HIV coinfect ed cases [4]. The
prevalence of HIV coinfection among TB patients is highest in the African region. Of the 1.1 million TB patients tested for HIV within 44 countries, 41% tested positive in 2013.

Among the high TB/HIV burden countries, this ranged from 7% in Mali to 74% in Lesotho and Swaziland [4]. The presence of HIV makes one more vulnerable to develop active TB, and having TB in turn accelerates HIV disease progression. The HIV epidemic in sub-Saharan Africa is driving the global TB burden whereas the incidence of TB remained stable or declining in other regions. At present one of four deaths from TB is HIV related[4]. These annual incidence rates are extremely high compared to the estimated 10% lifetime risk of active TB following latent TB in people without HIV coinfection. The risk of active disease in HIV-uninfected is estimated to be approximately 5% in the 18 months after initial infection and then approximately 5% for the remaining life time[15] but in those infected the risk for active disease (localized or disseminated) rises to approximately 8-10% per annum[16]. Among HIV-infected individuals, the risk of acquiring TB is 9 to 16 times that of HIV-uninfected [17,18]. Annual incidence rates of about 10% were described in HIV-infected patients in South Africa[6]. Autopsy studies in PLHIV without TB diagnosis revealed high rates of TB (21%-52%), even after receiving cART for up to 10 months. Almost half of this disease remains undiagnosed at the time of death [19].

Ethiopia ranks among the top ten of 22 high TB burden countries 4]. The annual TB incidence rate in high burden countries is 100 cases per 100,000 populations or more. Eighty percent of all estimated TB cases in the world reside in these countries. Furthermore, Ethiopia is one of the high TB/HIV and MDR TB burden countries. The prevalence and incidence of all forms of TB (includes HIV-positive) are 211 and 224 per 100,000 populations, respectively [4]. Tuberculosis remains one of the leading causes of mortality. Excluding HIV related deaths; in 2013 TB mortality was estimated to be 32 per 100,000 of the population. About 13% of all new TB cases are also HIV coinfected [4]. Mortality rate because of HIV associated tuberculosis is estimated to be 5.9 per 100,000 populations. Among TB patients with known HIV status, about 11% are HIV co-infected [4]. According to the national surveillance, 1.6% of new TB cases (95% CI 0.9-2.8) and 12% of previously treated TB cases (95% CI 5.6-21) were estimated to be MDR TB cases [4].

2.1.2. Interactions between HIV, TB and cART

2.1.2.1. Effect of HIV on TB
Tuberculosis risk increases after HIV acquisition, doubling within the first year due to rapid depletion of TB-specific T-helper cells. This occurs soon after HIV infection [20,21].
Subsequently, the risk of active TB disease progressively increases with declining immunity [5,6]. In contrary to the previous considerations, immune activation and dysregulation occurs soon after HIV infection and it will be profound. Immediately after HIV is acquired, generalized immunosuppression occurs. It will be marked with diminished responsiveness to previously encountered antigens [22,23]. This T-helper cell dysfunction is qualitative disorder and does not correlate with the absolute CD4 cell counts. It could last for several months [23]. It is a possible explanation to allow early progression to TB disease after infection with HIV. HIV-infection increases the risk of reactivating latent M. tuberculosis infection, placing HIV-positive persons at increased risk for developing TB[24]. HIV infection also increases the risk of rapid TB progression after primary M. tuberculosis acquisition or reinfection. HIV-infected patients have an increased risk of recurrent TB after successful therapy, usually due to exogenous reinfection [25]. A systematic review of 32 studies and studies from Brazil, South Africa and the USA confirmed that TB/HIV coinfected patients are at an increased risk of recurrent disease, especially those with low CD4 counts [26]. Consistent with an earlier study, molecular epidemiologic data from South African goldmines suggested that exogenous reinfection accounts for approximately two-thirds of recurrent disease [27]. This contrasts with data from low-TB burden setting in the USA where the relapse rate among HIV-infected patients was substantially reduced by increased duration of TB treatment, suggesting the importance of relapse in this setting[28]. In summary HIV increases risk of active TB by accelerated progression of TB following acquisition or reinfection, reactivation of latent infection and increased recurrence even after successful treatment [16,29,30].

2.1.2.2. Effect of TB on HIV
TB may accelerate the progression of HIV disease via immune activation and is associated with a higher mortality rate and shorter survival in HIV-positive persons [31]. TB hastens HIV progression risk to AIDS and mortality risk markedly increases despite ATT [32,33]. Plasma HIV RNA level significantly increases when TB intersects [34]. As it is true with other opportunistic infections, HIV RNA level usually drops after initiation of successful TB treatment [35]. However, for unknown reasons some patients from Ethiopia persistently had high HIV RNA levels in spite of effective ATT [36, 37]. Effective TB therapy leads to reduction in T-cell activation markers and stabilization of CD4 cell counts [38]. Generalized immune activation, due to TB infection, may increase the proportion of CD4 cells that are preferential targets for HIV. Increased expression of the HIV coreceptors CCR5 and CXCR4 occurs in HIV infected patients with TB coinfection [39]. This might lead to enhanced viral replication. Major drug-drug interactions, particularly associated with rifampicin, complicate the management of TB/HIV coinfection when cART is started. Rifampicin is the cornerstone of anti-tuberculosis therapy (ATT) in TB endemic settings and an important component of first-line TB treatment. Rifampicin vigorously induces the cytochrome P450 3A (CYP 3A) enzyme system resulting in markedly lower concentrations
of cART drugs metabolized through this pathway. Conversely, isoniazid also part of first-line ATT, is competitive inhibitor that specifically targets enzymes CYP2C19 and CYP 3A. Efavirenz (EFV) is the preferred non-nucleoside reverse transcriptase inhibitor (NNRTI) component of the first-line cART regimen according to the WHO guideline particularly during TB/HIV coinfection. Metabolism of EFV entails 8-hydroxylation by CYP 2B6 with subsequent glucuronidation. It is also known to induce CYP3A4 as well as its own metabolism. If EFV plasma concentrations are decreased due to interaction with enzyme inducers, this may increase the potential for therapeutic failure and the development of drug resistance. There are currently conflicting reports in the literature regarding the nature of the interaction with some studies reporting increased metabolism of EFV in the presence of RIF and others reporting just the opposite[108].

2.1.2.3. Effect of cART on TB
The risk of TB among HIV-infected persons not receiving cART is high. On a population level, there has been a remarkably consistent 80% decrease in TB risk among persons who receive cART [40,41,42]. However, even after several years of cART, the risk of TB remains higher than in HIV-uninfected persons, suggesting that immune restoration is not complete[16,29]. This finding is also an important reminder that HIV-infected persons receiving stable cART remain at increased risk of TB, compared with HIV-uninfected persons. Without cART up to 50% of PLHIV who are diagnosed with TB die during the first 6-8 months of TB treatment [43,44]. Studies of PLHIV in sub-Saharan Africa have also documented high rates of TB among PLHIV initiating ART; however, TB frequently remains undiagnosed[19]. Although ART is known to reduce the risk of TB among PLHIV cohorts by a mean of 67% (95% CI 61-73%), the rate of TB among PLHIV with sustained high CD4 counts on ART for longer than 5 years remains more than 4 times the rate of TB in HIV-uninfected individuals[32,33]. WHO recommends implementation of the 3 I’s (Intensive case finding, Isoniazid preventive therapy, TB Infection control), along with early initiation of cART as core strategies for reducing HIV-associated TB.

Although immune reconstitution because of cART has long-term benefits with regard to risk of TB, it can also unmask TB not clinically recognized before cART initiation [45,46]. Such antiretroviral associated TB can sometimes be associated with the immune reconstitution inflammatory syndrome (IRIS). Consistent with this phenomenon, risk of TB is particularly high during the first 3 months of cART[47]. It is unclear whether cART is associated with an initial increase in risk of TB, compared with persons not receiving cART.

2.1.3. Clinical aspects of TB in HIV–infected patients
HIV associated TB differs from HIV-uninfected TB in multiple aspects [5]. The risk of TB
and the clinical as well as radiographic manifestations of the disease are primary examples[48,49,50]. Antiretroviral therapy has a profound effect on lowering the risk of TB in HIV-infected persons, but it can also be associated with immune reconstitution inflammatory disease and unmasking of previously subclinical disease. There are also differences in treatment of HIV associated TB because of overlapping drug toxicities and drug-drug interactions between cART and ATT. The clinical manifestations of TB in the HIV-infected patient are heavily influenced by the degree of immunosuppression [51,52]. The classic symptoms of TB (cough, fever, weight loss and night sweats) have wide range differential diagnosis in HIV infection. Mycobacterial infections are the most common cause of fever of unknown origin in HIV infection, with TB being overwhelmingly the most common cause in developing countries[53]. There are two key factors that affect the clinical presentation of TB in HIV-infected adults: the degree of immune suppression and the rate of disease progression.  

Pulmonary tuberculosis (PTB) – PTB presents as same as HIV-uninfected patients when immunity is relatively preserved (CD4 > 200 cells/µL). In this case the typical adult pattern with upper lobe predominance and cavitation is seen. However, in patients with severe immunosuppression (CD4 < 200 cells/µL), PTB generally presents without cavities as lower and mid-lung zones infiltrates [54,55]. Hemoptysis which is associated with cavitation is unusual in HIV-infected patients with advanced immune suppression. Disseminated TB also is more common with severe immunosuppression. The second factor affecting clinical presentation is the fact that TB tends to progress more rapidly in HIV-infected individuals [56,57]. A study of ambulatory miners in South Africa revealed that TB disease duration was three fold shorter when HIV existed [57]. Consequently TB during HIV is more sub acute than a chronic disease. The clinical implication is such that it is important to diagnose TB early in order to reduce morbidity and mortality related with it. Cough of any duration should trigger work-up for pulmonary tuberculosis. On the flip side, the shorter disease duration in addition to higher proportion of smear negativity with HIV infection lowers risk of TB transmission from HIV-infected patients.

HIV-infected PTB patients often have smear-negative PTB because of low prevalence of cavitory disease. Because sputum smear is the principal means of diagnosing TB in high TB/HIV prevalent settings, smear-negative patients are often treated late, if at all. The mortality rate is higher among such patients than among HIV-infected patients with smear positive TB (because of delays in TB diagnosis in the former) and higher than among HIV-uninfected persons with smear-negative disease (because of HIV infection)[58].

Subclinical disease: Asymptomatic, subclinical TB, with normal chest radiography but positive sputum culture results, is a common feature of HIV-associated tuberculosis. It may account for 10% of cases in high TB/HIV prevalent settings [59,60,61]. It is recognized when sputum cultures are done either intensive case finding or in prevalence surveys. The natural history of subclinical TB in HIV is inadequately understood[62]. In one study from South Africa on 1429 (54% on cART) HIV clinic attendees; sputum positive cultures for
M. tuberculosis were seen in 8.8%. Although TB prevalence was lower in people already on cART compared to not, the proportion of people with positive TB sputum cultures and no symptoms of TB were higher [63].

**Extrapulmonary tuberculosis (EPTB)** - EPTB and disseminated form of TB are more seen in HIV-infected patients with advanced immunosuppression [6,64,65,66,67]. EPTB occurs alone or in association with PTB in 40-60% of cases. Commonest sites for extrapulmonary involvement are the lymph nodes and pleura, but virtually any site can be involved [52,68,69]. A study of 464 patients with extrapulmonary TB, HIV-infected patients (n=199) were more likely than control patients to have either mediastinal adenopathy or disseminated, genitourinary, or intrabdominal TB[68]. Involvement of the genitourinary system in HIV infection is usually a manifestation of disseminated disease. A case-control study of 3019 patients with TB found that pleural involvement was more common among HIV-infected patients than HIV-uninfected patients (11 versus 6 percent)[70]. Disseminated TB may present as prolonged fever without pulmonary symptoms and is a common cause of death among hospitalized HIV-infected patients[71,72].

### 2.1.4. Management of TB/HIV coinfection

#### 2.1.4.1. General principles

The discussion that follows is about drug-susceptible tuberculosis in HIV-infection. The approach towards optimal management of TB in HIV coinfected patients is complicated with the need for cART, drug-drug interactions, over-lapping toxicities and immune reconstitution inflammatory syndrome [73,74]. Recommendations for selecting antituberculosis treatment regimens in HIV-infected adults follow the same general approach as for adults without HIV infection. The treatment of tuberculosis in HIV requires multiple first-line ATT drugs. The standard is to use four drugs during the first eight weeks, called the intensive phase. The drugs used are rifampicin (rifamycin derivative often used), isoniazid, pyrazinamide and ethambutol. The following four months of treatment (continuation phase) is with two drugs and these are rifampicin and isoniazid. Total of six months of treatment is the standard in pulmonary TB, but other forms like CNS involvement with TB would require up to 12 months of treatment. However, intermittent dosing of ATT is not recommended for HIV-infected patients, particularly when the CD4 count is < 100 cells /µL. Vitamin B-6 (pyridoxine) should be supplemented to prevent peripheral neuropathy because of isoniazid. The usual dose recommended is 50 mgs daily. In addition cotrimoxazole needs to be given as OI preventive therapy in TB/HIV coinfected
patients. Directly observed therapy (DOT) facilitates adherence and helps to prevent the development of drug resistance.

2.1.4.2. The challenges
The clinical scenario is often complicated requiring administration of multiple drugs: combination therapy for treating TB, prophylaxis and possible treatment for intervening OIs and cART. In resource limited settings, the patients often present late to care with severe protein energy malnutrition and other comorbidities. Therapy for susceptible TB is often as favorable as HIV-uninfected patients [75,76]. The duration of treatment for the HIV-infected patient with active TB is discussed elsewhere. Treatment for HIV infection appears to be as effective in the HIV/TB coinfected patient as it is in patients with HIV alone when attention is paid to potential drug-drug interactions and over-lapping toxicities. Careful review for drug interactions is essential when considering ATT in patients taking antiretroviral therapy or vice versa. This is discussed in another section of this thesis. The other challenges which are drug-induced liver injury and immune reconstitution inflammatory syndrome are discussed separately.

3. OPTIMIZATION OF TUBERCULOSIS AND HIV CO-TREATMENT

Drug susceptible TB during HIV infection responds as favorably as in HIV-uninfected patients. This is on the basis of rates of sputum culture conversion rates and adherence to first-line TB treatment [75,76]. On the contrary, all-cause mortality rate is higher among HIV-infected than uninfected [48]. Tuberculosis treatment in HIV-infected patients is the same as HIV-uninfected. Standard first-line TB treatment for two months with four-drug regimen (intensive phase), followed with four months of treatment with two-drug regimen (continuation phase) is highly effective in patients with HIV associated drug-susceptible tuberculosis [28]. However, management of TB/HIV coinfection poses multiple clinical challenges: defining the optimum duration of TB treatment and cART timing in relation to starting TB therapy, concerns of pill burden, drug-drug interactions and complications of the cotreatment, such as drug toxicities and immune reconstitution inflammatory syndrome (IRIS). Mortality risk was reduced by 56% with cART initiated while on TB treatment rather than at completion [77]. Majority of deaths during TB/HIV cotreatment occur within the first two months of TB therapy[9]. The optimal time to start cART during ATT depends on the following factors: potential benefits, possible risks and the level of immunosuppression.

Early reports of treatment outcomes in patients with HIV associated TB revealed pretty good initial outcomes, but poor long term results from HIV related mortalities [43]. At
present because of potent and safe cART regimens, long term outcomes of TB therapy have improved. Mortality during the first two months of TB therapy in HIV coinfected patients are more likely TB-related than from other HIV-associated comorbidities [43,78,79]. Consequently, anti-tuberculosis therapy should be initiated first with rifamycin-based combination therapy (if drug-susceptible) and drug related adverse events like hepatotoxicity recognized early to be managed accordingly. Optimum TB/HIV coinfected case management requires trimethoprim-sulfamethoxazole prophylaxis and early initiation of cART[80].

3.1. Duration of TB treatment in TB/HIV coinfection

Because initial responses to therapy are mostly excellent in both HIV-infected and uninfected patients, the optimum duration of TB treatment is determined by the risk of TB relapse. Current treatment guidelines (The American Thoracic Society, Infectious Disease Society of America and the WHO) recommend that the duration of TB therapy be the same for both HIV-infected and uninfected persons [76,81,82]. For drug-susceptible PTB, a 6-month course of rifamycin-based therapy is the standard of care [76]. This is because of comparable rates of TB relapse in persons receiving 6-month regimens of rifamycin-based therapy (e.g. rifampicin or rifabutin)[43,83,84,85]. Nevertheless, the evidences are not sufficiently strong as most of the studies which showed equal efficacy had small sample size and were non-randomized. Therefore, some experts suggest longer duration of TB therapy. Systematic reviews and meta-analysis done in 2010 and 2012[26,86] evaluated the duration of TB treatment, dosing schedule of rifamycin-based TB therapy, and effect of cART on TB treatment outcomes in HIV-infected patients. More than 5000 citations, six randomized trials and 21 cohort studies were reviewed. In conclusion, the findings suggest relapses occur more commonly with regimens employing two months rifamycin therapy compared with at least eight months (adjusted Hazard ratio, aHR=5, 95% CI 1.9-13.2) and lower with regimens using rifamycin for more than 9 months than six months (pooled risk difference -9.1%; 95% CI -16.5 to -1.8 %). Compared with daily therapy during the intensive phase of TB therapy, thrice weekly regimen was associated with higher risk of treatment failure (aHR==4.0; 95% CI 1.5-10.4) and relapse (aHR=4.8; 95% CI 1.8-12.8). In fact, the data have limitations in terms of design and the number of randomized trials. Thus, randomized controlled trials comparing efficacy and safety of six versus nine months of TB treatment in TB/HIV coinfected patients are priority.

3.2. The optimal timing of cART in HIV-infected adults with TB

Without cART among TB/HIV coinfected patients and CD4 count <200 cells/µL, the mortality rate is as high as 91% [43,44,87]. Initiation of cART is associated with improved overall survival in HIV-infected patients including those with TB/HIV coinfection [88,89,90,91,92]. It is therefore recommended that HIV-infected patients receive treatment
for both diseases, regardless of their CD4 cell counts. On the other hand, the optimal timing of cART initiation in relation to the timing of TB therapy requires further definition. TB case fatality ratios (CFR) in African and Asian patients not receiving cART or in whom it was deferred for 8 weeks ranges from 16-35%[8,9,10]. In settings where HIV prevalence is high, the CFR was highest in the first two months of TB therapy suggesting cART should begin early. Mortality risk was reduced by 56% with cART initiated while on TB treatment rather than at completion[77]. The optimal balance between the potential benefits and risks directs cART timing during TB therapy. The potential benefit of starting cART before two weeks is earlier immune restoration and improved survival. Whereas the potential risks are augmented toxicities, TB-IRIS, drug interactions and pill burden that complicate treatment outcome and patient care.

Current consensus guidelines including the WHO (2013) recommend that cART be started in all TB/HIV coinfected patients (irrespective of CD4 cell counts) within the first eight weeks of TB treatment as soon as it is possible (strong recommendation, low quality evidence). The recommendation is based on emphasis given to overall survival and reduced mortality risk than increased incidence of IRIS and its consequences or drug related adverse events. A systematic review and meta-analysis of the scientific literature was conducted to assess the optimal timing for cART initiation following TB therapy [93]. The outcome measures were minimum severe adverse events, incidence of IRIS, death, AIDS defining events and lost to follow-up. Only RCTs evaluating early versus delayed cART (1 to 4 weeks vs. 8 to 12 weeks after initiation of TB treatment) were used for the main analysis of benefits and harms. Eight trials were included (n=4568) that were conducted in Africa, Asia, and the United States. The selected studies generally had low risk of bias for the assessed domains. Overall, early cART reduced mortality compared with delayed cART (relative risk [RR], 0.81 [95% CI, 0.66 to 0.99]; I²=0%). In a prespecified subgroup analysis, early cART reduced mortality compared with delayed cART among patients with baseline CD4 counts < 50 cells/µL (RR, 0.71 [95% CI 0.54 to 0.93]; I²=0%). However, a mortality benefit from early cART was not found among those with CD4 counts > 50 cells/µL (RR, 1.05[95% CI 0.68 to 1.61]; I²=56%). Early cART was associated with higher incidence of TB-IRIS than delayed cART (RR, 2.31 [95% CI, 1.87 to 2.86]; I²=19%). Only few of the trials provided sufficient data for subgroup analysis. The conclusion from this systematic review and meta-analysis is that early cART in HIV-infected adults with newly diagnosed TB improves survival in those with CD4 cell counts < 50 cells/µL, although this is associated with a 2-fold higher frequency of TB-IRIS. In patients with CD4 counts greater than 50 cells/µL, the evidence is insufficient to support or refute a survival benefit conferred by early versus delayed cART initiation.

### 3.3. Overlapping drug toxicities

Hepatotoxicity is a common adverse effect of isoniazid, the rifamycins, and pyrazinamide. It also occurs frequently with nonnucleoside reverse-transcriptase inhibitors (NNRTIs) and
HIV-1 protease inhibitors. Underlying HIV infection may also contribute to the risk, and concomitant hepatitis C virus infection further increases the risk [94,95]. Other adverse effects, such as gastrointestinal upset and rash, are common with both types of medications. Although stavudine and didanosine are less frequently used for the treatment of HIV infection, they can cause peripheral neuropathy, as isoniazid.

3.3.1. Efavirenz-induced hepatotoxicity
Efavirenz-based cART is the preferred treatment regimen with rifampin-based TB therapy. It is also the preferred first-line cART in most guidelines. Use of EFV has been linked with severe hepatotoxicity, though less frequently than nevirapine (NVP) [96]. The cumulative incidence reaches up to 28.6% for NVP and 13.6% for EFV [96,97]. EFV is metabolized by liver enzymes, mainly by CYP2B6 and to a less extent by CYP3A4/5. UGT2B7 is also involved in direct EFV and 8-hydroxyefavirenz glucuronidation. Studies have reported influence of ABCB1 genetic variations on EFV kinetics [98]. Ethnicity and Pharmacogenetic variations are well documented to influence between patient variability in susceptibility toward EFV-based cART induced DILI. Severe DILI (defined as ALT>5xULN), occurs in 1.3-4.5% of patients receiving efavirenz based cART.

3.3.2. First-line TB drugs induced liver injury
In TB/HIV coinfection currently recommended treatment regimens for TB involve drugs which are potentially hepatotoxic. Of the commonly used first-line ATT, three are hepatotoxic (isoniazid, rifampicin and pyrazinamide) with increasing risk when used together. These drugs are often given in combination making it difficult to identify the culprit drug. Although many TB patients with or without concomitant HIV tolerate first-line ATT very well, the outcome is unfavorable when severe DILI occurs [99,100,101,102]. There is limited data about DILI in TB/HIV coinfected patients during the HAART era. In general, the reported incidences of DILI range from 4 to 27% and jaundice from 0 to 7% [103]. The studies have the following limitations: varying definitions of methodology, inclusion of mild cases which could just be adaptation, smaller sample size, not accounting for population, treatment and monitoring differences, lack of HIV negative control groups and unable to exclude other causes of liver injury. The exclusive roles of HIV and cART are difficult to evaluate in severe DILI during TB/HIV coinfection. However, it appears to be slight [103,104].

3.4. Drug interactions in the treatment of HIV-related tuberculosis
Antiretroviral regimens based on a NNRTI plus two nucleoside analogues combine
potency, low pill burden and safety. Interactions between the rifampicin (RIF) and EFV are therefore particularly important. A general point about drug-interactions due to rifampicin is that, while this agent causes many drug-drug interactions, its metabolism is not affected by other drugs (although it induces its own metabolism via a cytosolic enzyme)[105]. RIF, a cornerstone first-line ATT in resource-limited settings, is a potent inducer of drug metabolizing enzymes and transport proteins, and this may reduce plasma exposure of antiretroviral drugs including protease inhibitors and NNRTIs. Despite its long term auto-induction effect, EFV is the preferred NNRTI to be used with RIF based ATT because of its proved efficacy, simpler dosing schedule, proved better safety profile and reduced impact on its pharmacokinetics compared with nevirapine or protease inhibitors. EFV is metabolized by CYP2B6 and CYP3A. As a potent CYP3A inducer, rifampicin decreases EFV concentrations, although the magnitude of the interaction (10-20% decrease in the AUC and trough concentration) suggests that CYP2B6, which is not thought to be affected by rifampicin, is the major metabolic pathway [106,107]. CYP2B6, the major EFV-metabolizing enzyme, is induced by EFV and RIF. Therefore, coadministration of RIF with EFV may result in additive effect to induce CYP2B6 enzyme and this may further reduce systemic EFV exposure. We studied this interaction in Ethiopians and found out that RIF-based ATT has no significant influence on long-term EFV plasma exposure and efficacy. Therefore, increasing the standard dose of EFV (600 mgs daily) in adults is not required when coadministered with RIF-based ATT[108].

3.5. Tuberculosis associated immune reconstitution inflammatory syndrome (TB-IRIS)

The term “immune reconstitution inflammatory syndrome” describes collection of inflammatory disorders that manifests phenotypically. The features are paradoxical worsening of already existing infectious process/es and it follows initiation of cART. The infectious process may have been diagnosed and treated or it could be subclinical and later unmasked by the host’s restored immunity to mount inflammatory response [45,46]. The key concepts are: it is an inflammatory response to preexisting pathogens and manifests with worsening. It always follows the introduction of cART. There is no universally agreed upon definition for IRIS. However, it is generally accepted that the diagnosis of IRIS requires the worsening of a recognized (“paradoxical” IRIS) or unrecognized pre-existing infection (“unmasking” IRIS) in the setting of improving immunologic function. Nearly any pathogen can cause IRIS, but the most common are mycobacteria (TB and MAC) and fungi (especially Cryptococcus). The interval from cART to IRIS is 60% within 60 days with a reported range of 3-658 days [109]. Risk factors with the OI pathogen include: CD4 count < 50-100 cells/µL at initiation of cART, high HIV RNA levels at baseline, rapid fall in HIV RNA levels, treatment naive at the time of OI treatment and short interval between cART and OI treatment[109]. In the case of TB, two forms of IRIS are recognized. Paradoxical TB-IRIS occurs in patients diagnosed with TB and established on TB treatment before cART, who then manifest with recurrent or new TB symptoms and clinical
manifestations after ART initiation. Unmasking TB-IRIS occurs in patients who are not on TB treatment when they start cART, and who then have unusually inflammatory presentation of TB in the first three months of cART [110].

4. HIV INFECTION

4.1. HIV Virology
Human immunodeficiency virus (HIV) belongs to family of viruses known as retroviruses and genus lentiviruses. HIV-1 was first isolated in 1983, soon after the beginning of the AIDS epidemic, and HIV-2 was isolated subsequently in 1986. HIV-1 and HIV-2 represent two distinct epidemics. The two HIV types show 40-60% amino acid homology[111]. The HIV-1 epidemic started as a zoonotic infection acquired from chimpanzees in equatorial West Africa harboring a similar lentivirus known as simian immunodeficiency virus (SIVcpz) which evolved to HIV-1. HIV-2 is thought to have originated in a similar fashion in equatorial West Africa from sooty mangabey monkeys carrying a related simian immunodeficiency virus (SIVsmm).

Phylogenetic analysis of HIV samples has led to the classification of HIV-1 in to three genetic groups, M (major), O (outlier) and N (non-M, non-O). Most HIV-1 infections globally are caused by group M viruses. With group M, nine subtypes are recognized, designated by the letters A-D, F-H, J and K[112]. Although this classification was initially based on env sequences, it applies to all regions of the genome. Genetic variation within a subtype can be of the order of 15-20%, whereas variation between subtypes is approximately 25-35%, depending on the subtypes and genome regions examined [113].

HIV-1C has become responsible for nearly half of global infections and dominates now in low-and middle-income countries (LMICs), mainly regimented in southern African countries and India[114].

4.2. HIV-1 subtype C in Ethiopia (HIV-1CET)
HIV-1 subtype C (HIV-1CET) was first identified in Ethiopia in 1986[115]. Unlike many neighboring countries, the Ethiopian HIV-1 epidemic is dominated exclusively by HIV-1C viruses. Ethiopia shows HIV-1C prevalence of 99%, though the depth of sampling was very low [116,117]. Two genetically different subtype C strains designated C and C’, co-circulate roughly with similar prevalence and among the same risk groups and different geographical areas in Ethiopia [118]. Thomson et al. revealed that Ethiopian-C clade corresponds to one subtype C cluster similar to other eastern African countries including Burundi, Djibouti, Kenya, and Uganda; while the Ethiopian–C’ clade is assigned to be an
independent cluster associated to southern Africa[119].

A study conducted in Ethiopia showed that infection with clade CET is associated with initially lower HIV-1 RNA levels but more rapid onset of disease than infections with clade C’ET[120]. The authors proposed that the clade CET may be less efficiently transmitted than clade C’ET, which is consistent with epidemiological evidence that show that the strain C’ET has gained ground and surpassed the clade CET over time.
5. RATIONALE OF THE THESIS

Drug susceptible TB in HIV infection responds often as favorably as in HIV-uninfected patients. Nevertheless, there are defined potential benefits as well as established risks during TB/HIV cotreatment. Mortality risk reduces by almost 60% when cART is initiated during TB treatment rather than at completion. Therefore, it is an appropriate research question defining the optimal time to initiate cART during ATT. Most of the deaths related to TB/HIV coinfection occur within the first two months of TB therapy (intensive phase). Recent meta-analysis that included 8 clinical trials (n=4568) showed that early initiation of cART (one to four weeks after ATT) improves overall survival in those with CD4 counts < 50 cells/µL, though the data is insufficient to prove such benefit when the CD4 count is above. Our study hypothesis was that initiating cART earlier than second week of TB therapy in patients with CD4 counts < 200 cells/µL, will improve overall survival at 48 weeks (paper II). This study tries to answer a clinically relevant question during HIV infection with comorbidities, “Does earlier cART always mean better patient outcome?”

One of the most important treatment limiting adverse effects during TB/HIV cotreatment is drug-induced liver injury (DILI). TB/HIV coinfection and advanced immunosuppression are mentioned as risk factors for DILI. In addition, commonly used drugs for treatment and prevention of opportunistic infections in HIV such as trimethoprim-sulfamethoxazole and fluconazole are potentially hepatotoxic. The role of concurrent cART on first-line TB therapy induced DILI severity has not been adequately investigated. There is limited data about DILI in TB/HIV coinfected patients during the HAART era. In general, the reported incidences of DILI range from 4 to 27% and jaundice from 0 to 7%. The studies have the following limitations: varying definitions of DILI and methodology, inclusion of mild cases which could just be adaptation, smaller sample size, not accounting for population, treatment and monitoring differences, lack of HIV negative control groups and unable to exclude other causes of liver injury. Thus, we hypothesized that concurrent TB/HIV treatment increases severe DILI risk and interruption of first-line TB therapy (paper IV). To the best of our knowledge in the HAART era there has not been any prospective, controlled study (same population) with adequate sample size that evaluated severe DILI, the effect of concurrent EFV-based cART timing and validated Hy’s Law in TB/HIV coinfected patients with baseline CD4 counts below and above 200 cells/µL. Although EFV-based regimen is often the preferred first line cART regimen, hepatotoxicity data is limited in the African population. Efavirenz is metabolized mainly by CYP 2B6 and to a lesser extent by CYP3A4/5. It is possible that Pharmacogenetic variations affect exposure to EFV to affect susceptibility to DILI. Use of EFV has been linked with severe DILI events. Drug induced liver injury related to EFV-based cART was studied during HIV-infection (CD4 count< 200 cells/µL) without concomitant active TB (paper I). Potential risk factors are sought and
pharmacogenomics analysis performed to find out genetic markers.

Based on earlier studies in Ethiopia using smaller gene fragments, mainly partial pol, gag, env and LTR, a mixed circulation of HIV-1C<sub>ET</sub> (subtype C and C’) is suggested and their introduction into Ethiopia has been estimated in the late 1970s or early 1980s. Intra-HIV 1C recombination has been proposed. However, NFLG of HIV-1C from Ethiopian migrants in Israel identified a strong geographical clustering of the NFLG sequences, but showed a weak geographic cluster in small fragments with respect to HIV-1C<sub>ET</sub> strains. We evaluated the clustering pattern of HIV-1C<sub>ET</sub> strains in Ethiopia by analyzing the HIV-1C<sub>ET</sub> strains at the Near Full Length Genome (NFLG) level from our cohort of HIV infected patients (paper III). The presence of drug resistance mutations is assessed and viral tropism evaluated.

6. STUDY OBJECTIVES

6.1. General objectives:
The general objectives of this thesis are to find out the optimal timing to initiate EFV-based cART during TB/HIV cotreatment when the CD4 count is < 200 cells/µL, assess drug-induced liver injury because of EFV-based cART and TB/HIV cotreatment with first-line ATT and EFV-based cART. The Ethiopian HIV viral subtype (HIV-1CET) is characterized at near full length genome level.

6.2. Specific objectives:
A. Compare after 48 weeks of study enrollment in TB and HIV coinfected patients with baseline CD4 counts < 200 cells/µL initiated with cART at weeks one, four and eight (paper II):
   1) All-cause mortality rates
   2) Incidence rates of hepatotoxicity requiring interruption of first-line TB therapy
   3) Incidence rates of TB-IRIS
   4) Incidence rates of AIDS-defining illnesses
   5) Increases in the median number of CD4 counts
   6) Proportion of patients with undetectable HIV-RNA levels (i.e. <400 copies/mL)
   7) Proportion of smear positive PTB cases converted smear negative after 8 weeks.
B. In TB/HIV coinfected patients with active TB cases without HIV and HIV patients with CD4 count < 200 cells/µL (paper IV):

1) Compare risk of severe DILI events during TB/HIV co-treatment compared with TB treatment and HIV treatment with EFV-based cART and isoniazid preventive therapy.

2) Compare the risk of severe DILI events in subgroups of TB/HIV co-infected participants (CD4 category below 200 cells/µL versus equals to and above 200 cells/µL) started with EFV-based cART (one, four and eight weeks) subsequent to ATT.

3) Evaluate patient risk factors for severe DILI emerging from TB and/or HIV cotreatment

4) Characterize the different phenotypes of Liver injury seen in the severe DILI cases.

5) Define Time of onset of severe DILI in relation to the start of ATT.

6) Incidence rates of ATT and/or cART interruption

7) Compare severe DILI specific mortality ratio

8) Compare all-cause mortality rates before reintroduction of first-line ATT

9) Assess time to remission of severe DILI events upon stopping first-line ATT

10) Assess hospitalization rate because of severe DILI

11) Assess recurrence of liver injury upon re-exposure to first-line ATT

12) Evaluate Hy’s Law validity in cases treated for TB and/or HIV

C. Regarding the HIV-1C subtype in Ethiopia (paper III):

1) Characterize the strain at near full-length genome (NFLG) level

2) Perform baseline drug resistance testing (GRT)

3) Perform genotypic tropism testing (GTT)

D. Regarding EFV-based cART DILI (paper I):

1) Assess for genetic biomarkers

2) Assess for predictors of DILI
7. MATERIAL AND METHODS

7.1. Study design
A multicenter, open label prospective cohort study was undertaken in Addis Ababa, Ethiopia from June 2, 2008 until April 22, 2011. There are three groups of patients. The first group (Group one) are TB/HIV co-infected patients with baseline CD4 count below and above 200 cells/µL, the second group (Group two) HIV negative, TB cases on ATT and the third group (Group three) HIV-infected patients (CD4 < 200 cells/µL) screened negative for TB and treated with EFV-based cART and isoniazid preventive therapy (IPT). All study participants were followed by the same investigator team in the same setting and time interval. According to baseline CD4 counts and time to initiate efavirenz based cART subsequent to ATT, Group one (TB/HIV) is subdivided in to four subgroups: Subgroup one (CD4 count < 200 cells/µL, cART one week), Subgroup two (CD4 cell count < 200 cells/µL, cART four weeks), Subgroup three (CD4 cell count < 200 cells/µL, cART eight weeks) and Subgroup four (CD4 cell count > 200 cells/µL, cART eight weeks).

7.2. Ethical considerations
Ethical permission was obtained from the Institutional Review Board of College of Health Sciences at the Addis Ababa University, the Ethiopian Science and Technology Agency as well as Food, Medicine and Health Care Administration and Control authority of Ethiopia.

7.3. Settings and subjects
Study participants were recruited from four trial sites within Addis Ababa, Ethiopia. The main trial site was Tikur Anbessa Specialized referral hospital and others were health center. Eligible subjects were ambulatory with confirmed or probable new TB diagnosis and/or confirmed HIV infection. They were 18 years or older and written informed consent obtained on enrolment. The diagnosis of TB was based on WHO criteria for the diagnosis of smear positive pulmonary TB (SP-PTB), smear negative TB (SN-PTB) and extrapulmonary TB (EPTB). Miliary form of TB with pulmonary involvement (PTB) and EPTB manifested with PTB were classified as PTB. Exclusion criteria were TB of the central nervous system, Karnofsky score < 40. Serum ALT more than 3 times the upper limit of normal (ULN), total bilirubin > 2.5mgs/dL, hemoglobin < 8 gms/dL, previous antiretroviral or ATT and pregnancy. HIV diagnosis was made per the national guide line of two different positive rapid test results.
7.4. Interventions

Subjects in Group one (TB/HIV) and two (TB) received daily weight adjusted fixed dose combinations of rifampicin, isoniazid, ethambutol and pyrazinamide (RHZE) for 2 months (intensive phase) and subsequent fixed dose combinations of isoniazid and rifampicin (RH) for 4 months (continuation phase). In Group one, Subgroups one to three were randomly allocated while Subgroup four were series of cases with TB/HIV co-infection (baseline CD4 cell count of > 200 cells/µL) in whom cART was initiated at the 8th week of ATT. The cART regimen used in our study was EFV-based (600 mg once daily) plus lamivudine, and study site physician selection of zidovudine, stavudine or tenofovir. Time to initiate cART was deferred when ATT was interrupted because of DILI. The participants were assessed at enrolment (baseline), and subsequently in weeks 1, 2, 4, 6, 8, 12, 24, 36 and 48. At each study visit complete blood count, serum AST, ALT, ALP, Bilirubin (total and direct), urea and creatinine were measured. At baseline and 12 weeks interval for HIV infected participants, CD4 count and HIV RNA levels were determined until the 24th week and last at 48th week. Hepatitis B surface antigen (HBsAg) and Hepatitis C Antibody (HCV Ab) status were confirmed at baseline. Sputum for acid-fast bacilli (AFB) was examined with Ziehl-Neelsen stain when PTB was diagnosed. HIV positive participants were screened for active TB with standardized symptoms questionnaire, sputum exam with Ziehl-Neelsen staining and routine chest X-ray. In addition to the above investigations, additional blood samples were collected from 251 HIV infected patients, CD4 counts < 200 cells/µL, for genotype analysis and from 212 patients on the 4th week of EFV-based cART blood sample was collected for quantification of plasma EFV and 8-OH EFV concentrations (paper I).

All HIV infected study participants with baseline CD4 counts less than 350 cells/µL received cotrimoxazole 960 mgs daily or dapsone 100 mgs daily if proved allergic. Group three (HIV) participants received IPT for six months which was started after the 8th week of enrollment irrespective of TST status. Participants are considered to have completed IPT if they attended all six isoniazid prescription visits.
Figure 1: Study design and participants enrolment

TB diagnosis
N=754

With HIV
N=575
Group one

- CD4 < 200 cells/μL
  - cART one week
    - N=163
    - Subgroup one
  - CD4 < 200 cells/μL
    - cART four weeks
      - N=160
      - Subgroup two
  - CD4 < 200 cells/μL
    - cART eight weeks
      - N=155
      - Subgroup three
  - CD4 ≥ 200 cells/μL
    - cART eight weeks
      - N=97
      - Subgroup four

48 wks follow up
- Comp=210 (92%)
- LTF=10 (5%)
- Died=7 (3%)

Without HIV
N=219
Group two

HIV diagnosis
N=295

CD4 < 200 cells/μL
TB screen negative
N=295
Group three

48 wks follow up
- Comp=210 (74%)
- LTF=41 (14%)
- Died=39 (12%)

* Comp= Completed the 48 weeks of the study
** LTF= Lost To Follow up (LTF) is defined as can’t be reached or outcome traced.
8. RESULTS

A total of 1089 patients participated in the different studies. The study design, the numbers of participants enrolled, completed the study or Lost to follow-up are all shown in Figure 1. There were three main groups and four subgroups. Group one, with TB and HIV coinfection, comprised of 575 (52.8%) study participants. Group two, active TB cases without concomitant HIV infection, had 219 (20.1%) participants and Group three, HIV-infected (CD4 counts < 200 cells/µL) without concomitant TB, had 295 (27.1%) participants. Group one, depending on cART timing with respect to TB therapy (one, four and eight weeks) and baseline CD4 counts below and above 200 cells/µL, subgroup analysis was made. Subgroup one (CD4 < 200 cells/µL and cART at a median of one week) had 163 study participants, Subgroup two (CD4 count < 200 cells/µL and cART at a median of four weeks) had 160 study participants, Subgroup three (CD4 count < 200 cells/µL and cART at a median of eight weeks) had 155 study participants and Subgroup four (CD4 count > 200 cells/µL and cART at a median of eight weeks) had 97 study participants.

Demographic, clinical and laboratory characteristics of the study participants

The mean age (1SD) of the study participants was 35 years (10). The Female to Male sex ratio was one to one except in Group three where the females were 2.6 times more. At baseline, the BMI (mean, 1SD) was 19.2 kgs/m2 (3.1), Karnofsky score (median, IQR) was 80% (30%), Hemoglobin (mean, 1SD) was 12.1 (2.2) gms/dL, WBC count (median, IQR) was 5,500 (3,300) cells/µL, absolute neutrophil count (median, IQR) was 3,400 (2,900) cells/µL, Platelet count (median, IQR) was 283,000 (184,000) cells/µL, AST (mean,1SD) was 49 (39.5) IU/L, ALT (mean, 1SD) was 35 (22.4) IU/L, ALP (mean, 1SD) was 141.2 (95.2) IU/L, and serum albumin level (mean,1SD) was 3.7 (0.8) g/dL. Over all 172 (22%) had smear positive pulmonary TB (PTB+ve), 382 (48%) had smear-negative pulmonary TB (PTB-ve) and 239 (30%) had extrapulmonary tuberculosis (EPTB). Of the HIV-infected patients enrolled our study 773(89%) had CD4 count < 200 cells/µL.


The study was done on 285 participants from Group three. The participants received
daily cotrimoxazole prophylaxis 960 mgs tablets or dapsone 100 mgs tablets if allergic to cotrimoxazole, followed with efavirenz-based cART three to five days later. A total of 285 patients were studied. The incidence rate of any one DILI event (defined as ALT/AST > 2xULN, 50IU/L taken as ULN) was 27.9/100 person-years and severe DILI (defined as > 5xULN) was 6.13/100 person years. The median time for DILI development was two weeks and four weeks for severe DILI. None of the patients who had severe DILI subsequently died. Multivariate Cox regression analysis revealed the following risk factors significantly associated with EFV-based cART DILI: Lower baseline Platelet counts, plasma albumin levels, CD4 counts where as higher plasma HIV-RNA levels, baseline serum ALT, AST, ALP values, Plasma EFV levels as well as CYP 2B6*6.

8.2. Study II. Efficacy and Safety of Antiretroviral Therapy Initiated One Week after Tuberculosis Therapy in Patients with CD4 Counts < 200 cells/µL: TB-HAART Study, a Randomized Clinical Trial.

A total of 478 patients from Group one with baseline CD4 count < 200 cells were studied. They were randomized to start cART after initial first-line TB therapy at a median of one week (subgroup one, n=163), four weeks (subgroup two, n=160), and eight weeks (subgroup three, n=155). The primary endpoint was all-cause mortality rate at the end of the 48 weeks of study. The secondary endpoints were hepatotoxicity-requiring interruption of TB therapy, TB-associated immune reconstitution inflammatory syndrome (TB-IRIS), new AIDS defining illness (ADIs), CD4 counts, HIV RNA levels, and AFB smear conversion rates. All analyses were intention-to-treat. Sixty-four deaths (13.4%) occurred in 339.2 person-years. All-cause mortality rates at 48 weeks were 25 per 100 person-years in week one, 18 per 100 person-years in week four and 15 per 100 person-years in week eight (P=0.2 by the log-rank test). All-cause mortality incidence rate ratios with CD4 counts below 50 cells/µL versus above were 2.8 in week one (95% CI 1.2-6.7), 3.1 in week four (95% CI 1.2-8.6) and 5.1 in week eight (95% CI 1.8-16). Serum albumin < 3gms/dL (adjusted HR, aHR=2.3) and CD4 < 50 cells/µL (aHR=2.7) were independent predictors of mortality. Compared with similar subgroups from weeks four and eight, first-line TB treatment interruption because of severe DILI was high in week one deaths (P=0.03) and in the CD4 subgroup < 50 cells/µL (P=0.02). Cox-multivariate regression analysis showed that positive HCV Ab test (aHR=4.4, 95% CI 1.5-13) and CD4 count < 50 cells/µL (aHR=2.95, 95% CI 1.2-3.2) were independent predictors of grade 3 or 4 hepatotoxicity. The incidence rate of TB-IRIS was significantly higher in week one, but only one patient from 22 who had TB-IRIS subsequently died. There were 39 ADIs documented at a median of four weeks after ATT started. There was no significant difference in incidence rates among the three groups at week 48. The median number of CD4 cells gained, the proportion of
patients who achieved undetectable HIV RNA levels, rates of sputum smear conversions in the 48 weeks of the study were not significantly different among the various study groups.

8.3. Study III. Phylogenetic analysis of near full-length genome reveals high intra-HIV-1 subtype C diversity but a strong geographical cluster in Ethiopia abstain in sub-genomic region.

Plasma samples (n=150) from 478 TB/HIV coinfected participants with baseline CD4 count < 200 cells/µL were randomly selected (every third sample or fourth when missing). In 30 of the samples, HIV-1C near full length genome level (NFLG) analysis was performed amplifying two large amplicons of 5.5 kb and 3.7 kb, followed by Sanger sequencing with 17 primers. Genotypic Resistance Testing (GRT) and Genotypic Tropism Testing (GTT) were performed with in-house methods. The phylogenetic analysis at Near Full Length Genome identified two clusters of HIV-1C\textsubscript{ET}, though all strains still formed one large overarching cluster together. A greater diversity was found among HIV-1C\textsubscript{ET} strains compared to HIV-1C strains from other geographical locations. The geographic clustering was abstained in the small sub-genomic regions, pol and env. Ninety five percent of the subtype C viral strains had R5 tropism. Primary drug resistance mutation was identified in <5% of the isolates.

8.4. Study IV. Evaluation of severe antituberculosis Drug-Induced Liver Injury, the effect of concurrent antiretroviral therapy timing and Hy’s Law in Tuberculosis & HIV coinfected patients: A Prospective cohort study.

The study aims to assess risk of severe Drug-Induced Liver Injury (DILI), the effect of concurrent cART and validate Hy’s Law during TB and HIV cotreatment. Participants were TB/HIV coinfected, taking cART within the first eight weeks of TB therapy (Group one) compared with HIV negative TB cases (Group two) and HIV infected patients on efavirenz based cART and INH preventive therapy (Group three). Of 1089 patients enrolled, 575 (52.8%) were in Group one, 219 (20.1%) in Group two and 295(27.1%) in Group three. A total of 64 severe DILI events occurred within 817 Person-Years (PY) of follow up, incidence rate (IR) = 7.8/100 person-years (95% CI 6.1-9.9) and case fatality ratio=32.8%. The IR of severe DILI were 14.6/100 PY (Group one), 1/100 PY (Group two) and 0.5/100 PY (Group three). Subgroup analysis in Group one showed the following risks of severe DILI events: Subgroup one (23 cases, aHR=32.1, 95% CI 4.3-240), Subgroup two (18 cases, aHR=27.4, 95% CI 3.6-206),
Subgroup three (16 cases, aHR= 29.2, 95% CI 3.9-221) and Subgroup four (4 cases, aHR= 11.4, 95% CI 1.2-103), P value 0.08 by the log rank test. When baseline CD4 counts in TB/HIV coinfected participants were categorized below 50 cells/µL versus equals and above 50 cells, severe DILI risk was higher in the first group than latter (aHR=1.8, 95% CI 1.06-3.1). In multivariate analysis, independent risk factors for severe DILI in addition to TB/HIV coinfection were abnormal baseline ALT and T Bil values, CD4 < 50 cells/µL and positive HCV antibody result. Hepatocellular injury was the commonest phenotype of liver injury among patients with severe DILI. The median time for the onset of severe DILI is 14 days. All severe DILI cases interrupted first-line ATT. The rate of interruption was significantly higher in Subgroup one compared with other subgroups. Comparing subgroups with CD4 counts < 50 cells/µL versus equals or above, the incidence rate ratio (IRR) of interrupting first-line ATT was: Subgroup one (IRR=3.1, 95% CI 1.3-8.2), Subgroup two (IRR=1, 95% CI 0.3-3) and Subgroup three (IRR=2.2, 95% CI 0.7-6.7). Twenty one cases from those who discontinued first-line ATT died subsequently: 18 were from Group one, 2 were from Group two and 1 from Group three. Subgroup analysis showed 10 were in Subgroup one, 5 were in Subgroup two, 2 were in Subgroup three and 1 was in Subgroup four (P<0.001 by the log rank test).Total of 109 patients (10%) died during the study period. Of these cases 21 had severe DILI: 18 in Group one, 2 in Group two and 1 in Group three (P=0.003 by the log-rank test). Seven cases (10.9%) from the 64 who had discontinued first-line ATT died before reintroduced. All were in Group one (2 in Subgroup one, 3 in Subgroup two and 2 in Subgroup three).The severe DILI events took a median of 14 days (IQR 10-16 days) for ALT values to lower down below 2xULN. The range varies from 5 to 56 days. Total of 30 cases were hospitalized for a median of 15 days because of severe DILI.

None of the cases had recurrence of severe DILI when ATT reintroduced. Thirteen Hy’s Law cases, all Group one, identified whom three died of acute liver failure (ALF), supporting risk ratio of 12:10,000.
9. DISCUSSION

9.1. Overall findings

There are several important findings in our study regarding optimization of TB and HIV cotreatment from high TB/HIV prevalent setting. The most important research question addressed pertains to defining the optimal time to initiate EFV-based cART within the first eight weeks of ATT. The confirmed and probable TB cases depict the actual cases seen in high TB/HIV prevalent setting. The approach towards answering this research question is by trying to find out where to strike the balance optimally between the possible benefits and potential risks of TB/HIV cotreatment. Overall survival as a proxy indicator for the potential benefits and Drug-induced liver injury as proxy indicator for the safety of TB/HIV cotreatment are investigated. The study findings did not refute the benefits of earlier cART introduction; however, it is paradoxical to those who benefits most from cART introduction are simultaneously at risk of severe DILI. Thus earlier cART does not always mean better, unless properly monitored for possible untoward effects or adverse events.

9.2 Study I: High plasma efavirenz level and CYP 2B6*6 are associated with efavirenz-based HAART induced liver injury in the treatment of naive HIV patients from Ethiopia: a prospective cohort study.

Efavirenz-based cART regimen is among the most preferred regimens to initiate cART in treatment naïve HIV patients according to most guidelines including WHO. In our study the incidence of EFV-based cART induced DILI was found higher in Ethiopians compared with other studies; however, severe DILI incidence rate (defined AST/ALT > 5xULN) was 6.13 per 100 person years which is similar to other studies. Assessment of the effect of plasma pharmacokinetics in our study indicated that increased plasma efavirenz concentration was significantly associated with DILI. We also reported that patients with CYP 2B6*6/*6 genotype the risk of DILI is 2.7 times higher compared with homozygous wild-type (*1/*1). Our findings indicate high plasma EFV level as possible intermediate biomarker for the association of CYP2B6 genotype as a risk factor for DILI. The high EFV plasma level in patients who developed DILI may be the result of impaired EFV metabolism due to liver injury caused by other factors. However, the association of DILI with CYP2B6*6, a variant allele well known to cause increased plasma EFV levels, indicate involvement of direct toxicity by EFV and not by its metabolite as a possible mechanism for EFV-based cART induced liver injury in HIV patients.
9.3. Study II. Efficacy and Safety of Antiretroviral Therapy Initiated One Week after Tuberculosis Therapy in Patients with CD4 Counts < 200 cells/µL: TB-HAART Study, a Randomized Clinical Trial.

The results from the randomized clinical trial showed that initiation of cART one week after TB therapy was not associated with improved overall survival at 48 weeks in patients with CD4 count < 200 cells/µL compared with four and eight weeks. In contrast to our study hypothesis the mortality rate was higher in week one, albeit statistically insignificant. It is possible that our study was not adequately powered to reject the null hypothesis. Nevertheless, cART one week after TB therapy did not prove beneficial in terms of reducing all-cause mortality at week 48. Consistent with other studies, two thirds of the mortality events in our study occurred within the first two months of TB therapy. One-third of the mortality events occurred before cART was initiated. Post-hoc analysis showed cases with absolute CD4 count less than 50 cells/µL compared with more than or equals to 50 cells/µL are five times as likely to die within 48 weeks when cART was deferred for eight weeks. Overall absolute CD4 counts less than 50 cells/µL and serum albumin level < 3gms/dL while initiating TB therapy were independent predictors of mortality in our study. Similar results were reported from other cohort studies. Majority of the deaths in our study occurred during the first two months of TB therapy. Deaths the first two months of TB therapy in HIV coinfected patients are more likely TB-related than other HIV-associated morbidities. Therefore, the patient’s overall condition including other comorbidities is probably more important than the time to initiate cART in predicting outcome. Different studies including our have clearly shown CD4 count less than 50 cells/µL independently predicts mortality in TB/HIV coinfection. In our study, the mortality trend increased in this subset of patients as cART deferred from week one to eight. On the other hand, this same group of patients had the highest incidence of grade 3 and 4 hepatotoxicity and subsequent interruption of first-line TB therapy in week one. Thus the key practical question for CD4 subgroup < 50 cells/µL is striking the optimal balance between the potential survival benefit if cART is initiated one week after TB therapy as opposed to the increased morbidity and mortality due to hepatotoxicity and risk of TB treatment interruption.

Based on our findings, we recommend cART later than the first but earlier than the fourth week in subset of patients with baseline CD4 count less than 50 cells/µL.
9.4. Study III. Phylogenetic analysis of near full-length genome reveals high intra-HIV-1 subtype C diversity but a strong geographical cluster in Ethiopia abstain in sub-genomic region.

There is great diversity of HIV-1CET strains compared to HIV-1C strains from other geographical regions in and outside Africa, by analyzing near full-length genomes (NFLG), as indicated by longer HIV-1CET strains clustered together. It suggests early introduction of HIV-1C in to the country followed with long time diversification. Though sub-clusters of HIV-1CET were identified, all HIV-1CET strains formed an overarching large monophyletic cluster at whole genome level. Phylogenetic cluster interpretation based on the small gene fragments did not hold true at whole genome level.

9.5. Study IV. Evaluation of severe antituberculosis Drug-Induced Liver Injury, the effect of concurrent antiretroviral therapy timing and Hy’s Law in Tuberculosis & HIV coinfected patients: A Prospective cohort study.

DILI risk is considered to increase with TB and HIV coinfection; however, the studies have important limitations. Our study involved over 1000 patients and compared TB/HIV coinfected patients with HIV-negative TB cases and TB-screen negative HIV cases. Our study findings confirm that TB/HIV coinfection poses severe DILI risk compared with HIV uninfected TB cases taking first-line ATT and HIV positive patients receiving EFV-based cART and IPT started after the 8th week. Severe DILI risk increases 20 folds with TB/HIV co-infection. Concurrent cART timing doesn’t increase severe DILI risk or the IR of TB treatment interruption except in subgroup with CD4<50 cells/µL where the risk increased two folds. Though statistically insignificant, the aHR was highest in Subgroup one and lowest in Subgroup four. Between Subgroups two and three, the aHR difference is narrow. From wide 95% CI, it is apparent that the study lacks adequate power to test for these subgroup differences. Interestingly 38(62.3%) out of the 61 severe DILI cases in Group one occurred before EFV-based cART was initiated. The respective numbers are: Subgroup one (8 cases, 35%), Subgroup two (12 cases, 67%), Subgroup three (14 cases, 88%) and Subgroup 4(4 cases, 100%). The proportion of participants who had CD4 < 50 cells/µL was highest in Subgroup one (37.4%) compared with 31.3% in Subgroup two and 25.2% in Subgroup three. This occurred by chance because assignment to these groups was random. Had the differences been from the EFV-based cART initiation, most of the severe DILI cases would have occurred after cART initiated. Thus, our study results show limited role of EFV-based cART and its timing to TB therapy as severe DILI risk
factor. In multivariate analysis, independent risk factors for severe DILI in addition to TB/HIV co-infection were abnormal baseline ALT and T Bil values, CD4 < 50 cells/µL and positive HCV antibody result. Host genetics explains partly the severity of DILI events, even though selecting study groups from a similar population minimizes genetic variability. In our earlier study of TB/HIV co-infection, NAT2 slow acetylator genotype predominates (68%) among Ethiopians. Compared with rapid acetylator genotype, significantly higher DILI incidence occurred in slow acetylator Ethiopian coinfected patients.

10. CONCLUSIONS

With respect to the question of cART timing within the first two months of TB therapy, our study from low income and high TB/HIV prevalent setting showed cART one week after TB therapy does not improve all-cause mortality rate in coinfected patients with CD4 count < 200 cells/µL compared with four and eight weeks at 48 weeks of enrollment. Majority of the deaths in our study occurred during the first two months of TB therapy. Deaths the first two months of TB therapy in HIV coinfected patients are more likely TB-related than other HIV-associated morbidities. Therefore, the patient’s overall condition including other comorbidities is probably more important than the time to initiate cART in predicting outcome. Different studies including our have clearly shown CD4 count less than 50 cells/µL independently predicts mortality in TB/HIV co-infection. In our study the mortality trend increased in this subset of patients as cART deferred from week one to eight. On the other hand, this same group of patients had the highest incidence rate of grade 3 and 4 hepatotoxicity and subsequent interruption of first-line TB therapy in week one. The key question for CD4 subgroup < 50 cells/µL is striking the optimal balance between the potential survival benefit if cART is initiated one week after TB therapy as opposed to the increased morbidity and mortality due to hepatotoxicity and risk of TB treatment interruption.

TB/HIV co-infection markedly increases severe DILI risk. It is a composite end point of the interaction among different variables which consist of advanced immunosuppression, background hepatitis, host genetics, concomitant hepatotoxic drug use to prevent and treat OIs and possibly altered drug metabolism in critical illness. Concurrent EFV-based cART timing did not increase severe DILI risk or affect first-line TB treatment interruption except in subgroup with baseline CD4 counts < 50 cells/µL. Hy’s law predicts TB treatment known risk of ALF in TB/HIV coinfected patients. The incidence of EFV-based cART induced DILI was relatively higher among Ethiopian HIV patients. CYP 2B6*6 is a possible genetic marker, where as high plasma
EFV level is the intermediate biomarker for EFV associated DILI in Ethiopian HIV patients. Interestingly enough the median time for any DILI event was overlaps at two weeks both to EFV-based cART and ATT-induced. Another observation is that during TB/HIV cotreatment, severe DILI is more associated with ATT than cART. The phylogenetic analysis showed a greater diversity among HIV-1CET strains compared to HIV-1C strains from other geographical location by NFLG analysis. We therefore propose that HIV-1C strains were introduced in Ethiopia at an early stage of the HIV-1C epidemic, followed by long time diversification within the country. Though sub-clusters of HIV-1CET were identified, all HIV-1CET strains formed an overarching large monophyletic cluster at whole genome level. Phylogenetic cluster interpretation based on the small gene fragments did not hold true at whole genome level. Due to high genetic diversity, we observed a lower sensitivity of the GTT as well as heterogeneity of tropism prediction by the established algorithms.

11. RECOMMENDATIONS

- Despite increased mortality with CD4 < 50 cells/µL, we recommend cART later than the first week of TB therapy to avoid serious hepatotoxicity and treatment interruption.
- During TB/HIV cotreatment, we recommend close monitoring of ALT in the first 8 weeks particularly the first two weeks of ATT.
- Symptom based approach to managing DILI in TB/HIV coinfected patients is not advisable.
- Reintroduction of first-line TB therapy can be safely done with fixed dose formulations.
- The roles of chronic immune activation and altered drug metabolism in HIV with or without comorbidities need to be studied.
- A better understanding of HIV-1C genomics architecture at whole genome level with larger number of sequences and statistical power is needed to optimize the molecular diagnostics and monitoring strategies.
- CYP 2B6 genotyping of HIV patients in clinical practice needs to be encouraged not only to identify patients at risk of EFV-induced central nervous system toxicity, but also to identify patients who are at risk of developing.
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13. REFERENCES


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