

## **A Replication Plan For**

### **“Prevention of HIV-1 Infection with Early Antiretroviral Therapy”**

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## **1. Introduction**

Since September 2015, World Health Organization (WHO) has recommended that anyone infected with HIV should begin antiretroviral therapy (ART) as soon after diagnosis as possible. With its "treat-all" recommendation, WHO removed all limitations on eligibility for ART among people living with HIV, all populations and age groups are now eligible for immediate treatment. Before, the recommendation by WHO was to initiate ART based on an individual's CD4 cell count, with sicker individuals having lower counts. The threshold CD4 count for ART initiation in lower-resource countries was increased from 200 in 2006 to 350 in 2010 and to 500 in 2013. These changes were supported by the results of several randomized, controlled trials that showed improved outcomes with earlier treatment (Severe et al. 2010; INSIGHT START Study Group, 2015; TEMPRANO ANRS Study Group, 2015).

At the same time, a study known as HPTN052 was undertaken by Cohen et al. (2011) which aimed to assess whether, in addition to benefits to the person being treated, HIV treatment could lower the risk of an HIV-infected individual transmitting HIV to his or her non-infected partner. The study was halted prematurely due to overwhelming evidence that indicated that early ART was associated with a 96% lower risk of index-to-partner transmission. It was the first study to suggest that HIV treatment could prevent transmission and serve as a prevention method.

Since carefully implemented clinical trials in a controlled research environment may not reflect what would happen in a real-world setting, several studies have been initiated to test the treatment as prevention approach at population level (Hayes et al. 2014; Iwuji et al. 2013; Havlir 2013; Moore et al. 2013). As such, HPTN052 was a seminal study in suggesting treatment is prevention, and was the basis on which additional studies were designed.

The influence of the HPTN052 study and the magnitude of effort required to scale up universal access to ART underscores the importance of carefully reviewing, understanding, and verifying the study results.

Therefore, in this paper we will use the raw data from the original study to replicate methods used and to produce the results presented in the original paper. In addition, we will apply alternative analytical methods to assess the robustness of the authors' results. The rest of the plan is structured as follows. Section 2 presents the study selected for the replication. Section 3 presents a critical appraisal of the original paper and our proposed replication plan. Specifically, we present the plan for pure replication and the plan for the MEA. Section 4 concludes.

## **2. Presentation of the selected study**

Cohen et al. (2011) enrolled 1763 HIV serodiscordant (one partner is HIV-1-positive and the other is HIV-1-negative) couples at 13 sites in 9 countries (Gaborone, Botswana; Kisumu, Kenya; Lilongwe and Blantyre, Malawi; Johannesburg and Soweto, South Africa; Harare, Zimbabwe; Rio de Janeiro and Porto Alegre, Brazil; Pune and Chennai, India; Chiang Mai, Thailand; and Boston, USA). Enrollment took place from June 2007 through May 2010. Patients with HIV-1 infection were eligible if their CD4 count was between 350 and 550, they were in a

stable sexual relationship with their partner and they had received no previous antiretroviral therapy except for short-term prevention of mother-to-child transmission of HIV-1. After the enrollment, HIV serodiscordant couples were randomly assigned in a 1:1 ratio to either receive ART immediately (early therapy, n=886) or after a decline in the CD4 count (two consecutive measurements of 250 or less) or the development of an illness related to acquired immunodeficiency syndrome (delayed therapy, n=877). The primary prevention end point was genetically linked HIV-1 transmission in HIV-1–negative partners. The primary clinical end point was the earliest occurrence of pulmonary tuberculosis, severe bacterial infection, a WHO stage 4 event, or death.

The primary analysis to determine the impact of early therapy on the primary prevention endpoint was done with the Kaplan-Meier method to calculate event-free probabilities and person-year analysis for incidence rate for a given year. The original authors also used Cox regression to estimate relative risks, which were expressed as hazard ratios and 95% confidence intervals, and adjustment for potential prognostic factors, such as the infected participant's baseline CD4 count, baseline plasma HIV-1 RNA concentration, and sex. The same Cox analyses were performed for outcomes of linked transmissions (genetically proven to have come from the partner), any transmissions (any new infection, from primary partner or other), clinical events, and composite monitoring events. The original authors used chi-square tests to compare the frequencies of adverse events. A P-value of less than 0.05 was considered statistically significant. Lastly, interim analyses using the O'Brien-Fleming method with the Lan-DeMets spending function were planned for when approximately 25%, 50%, 75%, and 100% of a total 340 composite events were observed. All analyses presented above were done by intention-to treat analysis.

The authors find that early initiation of ART reduced sexual transmission of HIV-1 among HIV-1 negative partners. Specifically, the authors find that 39 HIV-1 transmissions occurred since enrollment. Of these, 28 were biologically linked to the HIV positive partners. Of the 28 linked transmissions, only 1 occurred in the early therapy group. This difference represents a relative reduction of 96% due to the early initiation of antiretroviral therapy ( $P < 0.001$ ). Furthermore, the authors find that for serious HIV-1–related clinical events (i.e. a WHO stage 4 event, severe bacterial infection or pulmonary tuberculosis, or death), observed in HIV-1–infected participants, 40 were in the early-therapy group and 65 in the delayed-therapy group (hazard ratio, 0.59; 95% CI, 0.40 to 0.88;  $P = 0.01$ ). Finally, the authors find that 246 HIV-1–infected participants had one or more severe or life-threatening adverse events (grade 3 or 4) representing 14% in the early-therapy group and 14% in the delayed-therapy group ( $P = 0.64$ ). The most frequently reported adverse events included infections, psychiatric and nervous system disorders, metabolism and nutrition disorders, and gastrointestinal disorders.

### **3. The proposed replication plan**

This study includes two standard objectives for 3ie-funded replication research (Brown et al. 2014). The first objective is to assess whether we can produce the same results as reported in the original paper, using the data and methods from the original paper, a pure replication. The second objective is to assess whether the results are consistent against pre-specified

robustness checks that use different statistical techniques or alternative ways to measure variables, what is called a measurement and estimation analysis (MEA).

### **3.1 Underlying rationale for the planned measurement and estimation analysis**

While the original study was quite thorough, we believe that there are a few areas that could be explored to see how they might affect the results. In this section we describe these areas.

#### *3.1.1 Differences by region (Africa or not)*

A study by Abu-Raddad et al. (2013) reported higher viral loads in patients with HIV-1 infection in sub-Saharan Africa than patients in other parts of the world. The study we are replicating found that a high viral load at baseline is associated with an increased risk of HIV transmission, and that 82% of the new HIV-1 transmissions occurred in Africa even though only 54% of all the study participants were enrolled from this region. This suggests that there may have been different baseline viral loads by region within the Cohen et al. (2011) study. It is possible that the protective effect of treatment could be different by region and specifically, by baseline viral load average.

#### *3.1.2 Clustering*

The study was conducted in 9 countries at 13 sites. Patients from the same site may be more alike than patients from other places (Kloek, 1981; Moulton, 1986). Although the authors used a multivariable Cox regression analysis stratified by site, in order to test the robustness of the original results, we will use an alternative approach to account for correlation within sites for an overall estimation.

#### *3.1.3 Circumcision*

In the last decade, three studies (Auvert et al. 2005; Bailey et al. 2007; Gray et al. 2007) found that medical male circumcision is effective in reducing the risk of heterosexually acquired HIV infection in men of by about 60%. These three studies were so influential that they led WHO and UNAIDS to recommend male circumcision as an efficacious intervention for the prevention of heterosexually acquired HIV infection in men (WHO & UNAIDS, 2007). The original study did not adjust for the circumcision status of HIV-negative males involved in the study even though 51% of the participants were men and the baseline imbalance in proportion of circumcised men in the early-therapy group and the delayed group is statistically significant. As circumcision status may significantly affect the hazard ratio for the effect of early ART on HIV transmission to uninfected men, we will control for it.

#### *3.1.4 Prior HIV treatment through PMTCT*

Patients with HIV-1 infection were considered eligible for this study if their CD4 count was between 350 and 550 (at the time, not yet eligible for ART, with 550 considered “normal”) and they had received no previous antiretroviral therapy. An exception was made for prior use of ART due to short-term use for prevention of mother to child transmission of HIV-1 (PMTCT). Among the HIV-1 infected participants, 27% participants in both arms were women who reported prior ART use for PMTCT. It is possible these women reacted differently to ART. In fact, there is evidence that suggests that women with prior exposure to ARVs through PMTCT, or women with ART interruption due to PMTCT, have poorer outcomes once on lifetime

treatment (Naidu et al. 2012). It could be beneficial to separately assess the primary effect for this sub-group.

### *3.1.5 Cox proportional hazards assumption*

The original study authors used Cox proportional hazards regression to estimate relative risks. Cox models are often used to estimate effects such as the length of time it takes for an event to occur—in this case, for someone to acquire HIV-1 or to have a clinical event. However, the validity of the Cox model relies on whether all predictors satisfy the proportional hazards (PH) assumptions. Violations of these assumptions can produce results that are not meaningful, and different statistical models would then be more appropriate to estimate the effect of the intervention (Lin et al. 1993). Since new HIV infection may be more likely to result in transmission than older infection, and treatment may lower transmission risk, it is likely that the assumption of constant relative hazard will not hold true.

### *3.1.6 Understanding how the intervention works*

Treatment is a prevention only whether treatment leads to a significant reduction of viral load. Thus, a low viral load will reduce the likelihood of anyone infected with HIV who initiates ART to transmit HIV another person. The original authors did not explicitly test whether over time, the viral load is significantly low in the early therapy group compared the delayed therapy group. In addition, the reduction of viral load requires a high level of adherence to ART. Good adherence to ART is a strong predictor of positive health and treatment outcomes for HIV positive patients (Ross-Degnan et al. (2010). We will assess whether the effect of early initiation of antiretroviral therapy on rates of sexual transmission of HIV-1 varies by the level of adherence to ART.

## **3.2. Methods**

### *3.2.1 Pure replication*

For the pure replication we will first reproduce all data driven tables (Tables 1-3) and figures (Figure 2). Figure 1 is a flow chart of enrollment, randomization, and follow-up and will not be included in the pure replication except to verify whether the final enrollment and follow-up numbers are similar using the original authors' data and described methods. The original authors kindly provided the data they used for the paper, however, the dataset we received from the original authors did not include any code. Therefore, as a first step, we will recode all of the constructed variables (e.g. age groups) as necessary and all the statistical analysis based on the methods described in the original paper..

We will make every effort to resolve any discrepancies that may arise, through analysis and communication with the original authors. In the event that discrepancies persist in our results, we will make every effort to understand and report the sources of the discrepancies.

### *3.2.2 Measurement and estimation analysis*

#### *1: Subgroup analyses*

We will conduct two separate sub-group analyses. First we will separate the sample in two groups, a sample of participants from African countries and a sample of participants from non-African countries, and we will replicate the methods used to produce Tables 2, 3, and Figure 2 for these two samples. Second we will separately estimate the tables and figure for women who

reported prior ART use for PMTCT and for women who did not. It is possible that the sub-sample of women with prior PMTCT treatment and their partners will be too small for a meaningful estimate, but the larger group of women without prior treatment should provide information about possible heterogeneity of effect.

*2: Taking into account the correlations among patients within the same countries or within the same sites*

We will use an alternative approach to take into account the correlations among patients within the same clinics. We will adjust the standard errors of the estimates of the main results (Table 2 and Table 3) for clustering, using the design effect (“cluster” option in Stata). Specifically, as suggested by Bertrand et al. (2004), to avoid potential biases in the estimation of the standard errors, we allow for an arbitrary variance covariance structure within sites by computing the standard errors clustered at the site level.

*3: Controlling for male circumcision*

Because medical male circumcision reduces the risk of male HIV acquisition, we will control for male circumcision status of HIV- males enrolled in the study. Specifically, for the subgroup analysis of men, we will use a dummy variable for circumcision status.

*4: Assessing the validity of Cox proportional hazard model*

The Cox proportional hazard model, used in the original study, assumes that the proportional hazard assumption is satisfied. We will explicitly test the proportional hazard assumption along several key variables, including age, education level, marital status, and sexual activity. We will assess Schoenfeld residuals over time (Grambsch and Therneau 2000; Schoenfeld 1982). In addition, we will test time-dependent covariates to evaluate whether the strength of association (for example, the treatment effect) changes over time (Grambsch and Therneau 2000). If the PH assumption is violated for some predictors including age, sex, education level, marital status, and sexual activity, an extended Cox model will be used.

*5: Assessing the effect of early therapy on viral load and on rates of sexual transmission of HIV-1 by the level of adherence to ART*

We will test whether viral load at median follow-up and at the longest follow-up (endline) is significantly lower in the early therapy group compared to the delayed therapy group for participants enrolled in the study. In addition, we will assess the effect of early therapy on transmission of HIV-1 by the level of adherence to ART. First, we will separate the sample in two groups, a sample of participants with a adherence of at least 95% (as measured by pill count) and a sample of participants with a adherence of less than 95% (as measured by pill count), and we will replicate the methods used to produce Table 2 and Figure 2 for these two samples. It is important to note that, in general, adherence was high among participants (79% of participants in the early-therapy group and in 74% of those in the delayed-therapy group have an adherence of least 95% as measured by pill count). The analysis on the sample of participants with adherence of less than 95% might suffer from a lack of statistical power.

## 4. Conclusion

In this study, we propose to replicate Cohen et al. (2011) also known as HPTN052. This study was the first to show that treating HIV infected people can be effective in reducing the risk of HIV transmission. We propose to conduct a pure replication to assess whether the original results are reproducible using the original data and methods. We then conduct an MEA. , First, we will test the validity of Cox proportional hazard model by looking at goodness of fit using Schoenfeld residuals, and testing time-dependent covariates for strength of association. We will conduct subgroup analyses distinguishing participants from non-African regions from other regions, and women who reported prior ART use for PMTCT or not. We will also control for medical male circumcision and the timing of HIV infection of the HIV-1 positive partner. In addition, we will take into account clustering at the site level by adjusting standard errors and through multilevel mixed effects parametric survival model. Finally, we will assess the effect of early therapy on viral load and on rates of sexual transmission of HIV-1 by the level of adherence to ART. This replication will provide more insights regarding the study that has shifted the prevention and treatment of HIV to where treatment is now considered as prevention.

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