STATISTICAL ANALYSIS PLAN

Couples ART Adherence Intervention for PWID in Kazakhstan

National Clinical Trial (NCT) Identified Number: NCT03555396

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1. INTRODUCTION

The SMART Couple II study was designed to adapt a couples-based antiretroviral therapy (ART) adherence intervention for HIV-positive people who inject drugs (PWID) in Kazakhstan and their treatment support partners. The adapted intervention was then pilot tested among 66 HIV-positive PWID and their treatment support partners (n=66) to assess the feasibility and acceptability of the intervention and obtain preliminary estimates of adherence outcomes in the intervention arm versus the standard of care arm.

This document outlines the statistical analysis plan that will be followed to assess the adherence outcomes.

Primary Outcome: To evaluate the difference in ART adherence as measured through an electronic monitoring device (EMD) throughout the six-month follow-up between the intervention and standard of care arms.

Secondary Outcome 1: To evaluate the difference in biological measures of ART adherence (e.g., viral load, hair samples, dried blood spot testing) between the intervention and standard of care arms.

Secondary Outcome 2: To evaluate the difference in self-reported ART adherence as measured through a validated three-item adherence scale[1] at the six-month follow-up between the intervention and standard of care arms.

Secondary Outcome 3: To evaluate the difference in uptake of opioid substitution therapy (OST) at the six-month follow-up between the intervention and standard of care arms.

2. HYPOTHESES

The **primary** null (H_0) and alternative (H_A) statistical hypotheses are:

H_{P0}: ART adherence as measured through an EMD throughout the six-month follow-up period will not differ between the intervention and standard of care arms.

H_{PA}: ART adherence as measured through an EMD throughout the six-month follow-up period will be higher in the intervention arm compared to the standard of care arm.

The **first secondary** null (H_0) and alternative (H_A) statistical hypotheses are:

H_{S1O}: ART adherence as measured through biological measures will not differ between the intervention and standard of care arms.

H_{S1A}: ART adherence as measured through biological measures will be higher in the intervention arm compared to the standard of care arm.

The **second secondary** null (H_0) and alternative (H_A) statistical hypotheses are:

H_{S20}: Changes in self-reported ART adherence from baseline to the six-month follow-up period will not differ between the intervention and standard of care arms.

H_{S2A}: Changes in self-reported ART adherence from baseline to the six-month follow-up period will be greater in the intervention arm compared to the standard of care arm.

The **third secondary** null (H_0) and alternative (H_A) statistical hypotheses are:

H_{S30}: Uptake of OST at the six-month follow-up will not differ between the intervention and standard of care arms.

H_{S3A}: Uptake of OST at the six-month follow-up will be higher in the intervention arm compared to the standard of care arm.

3. METHODS

This section contains information about the study design and statistical analysis that will be performed to assess the hypotheses.

3.1. Study Design

We conducted a randomized controlled pilot trial of the adapted couple-based intervention. 66 couples were randomized into two arms – 33 couples into the intervention arm and 33 couples into standard of care (SOC). SOC consisted of an appointment with an AIDS Center nurse at baseline and three months and six months later. Under current SOC in Almaty, no behavioral intervention is provided. Participants obtain prescription refills and give blood for viral load and CD4 tests once every 6 months. Participants receive viral load and CD4 test results only by request, and adherence is generally not assessed. The intervention consisted of three sessions over the course of one month. Participants in both arms were assessed at four time points: baseline "pre-intervention", immediately post-intervention (IPI), 3-month follow-up, and 6-month follow-up.

3.2. Trial Randomization

3.3. Data Sources

3.2.1. Participant Surveys.

Participants completed surveys via Qualtrics at four timepoints -- baseline "pre-intervention", immediately post-intervention (1-month after baseline), three-month and six-months follow-up. Among the many topics assessed, surveys included sociodemographic items, the validated self-reported three-item adherence scale[1], and use of OST.

3.2.2. EMD Data.

HIV-positive participants were given an EMD that continuously monitored device openings. The EMD sends a signal to a secure website via cellular data networks whenever the device is opened as a proxy for ART adherence. If connection or SIM card problems prevent real-time monitoring, records and diagnostics are stored (up to 1 year) and uploaded to the server when a connection is re-established. The device can store a 30-day supply of medication.

3.2.3. Biological Measures.

Dried blood spot (DBS) samples were collected from HIV-positive participants at their regular AIDS Center visit to assess feasibility and acceptability of the biological collection process and measure biological levels of medication. A nurse collected DBS samples by venipuncture, using a pipette to spot blood directly onto Whatman 903 ProteinSaver cards. The cards will be air-dried, individually packaged in a plastic bag with desiccant, and transferred to a -20 to -80°C freezer for storage. Only samples of participants on tenofovir-based regimens were shipped to the US.

Hair samples. Participants self-collected hair samples at baseline and immediately post-intervention to measure ART adherence levels. To collect hair samples, participants cut a small thatch of hair (~50 strands of hair) close to the scalp from a bottom layer of their hair. Hair was placed in a piece of tinfoil and inside a Ziploc bag labeled with their study ID. Participants shipped samples to GHRCCA staff, who then batch shipped samples to the US for testing.

Viral load. Participants are tested for viral load levels during every patient visit at the AIDS Center, which occurs every six months. Viral load data were abstracted from the AIDS Center electronic medical record at the closest visit prior to the baseline assessment and at the closest visit to the six-month follow-up assessment.

3.4. Statistical Interim Analysis and Stopping Guidance

There are no interim analyses or interim stopping rules that must be considered for this statistical analysis plan (SAP).

4. STATISTICAL PRINCIPLES AND ANALYSIS

4.1 Confidence Intervals and P-values

All statistical computations will be performed by the PI (Dr. Davis), who is an epidemiologist, in consultation with quantitative data scientists. For descriptive summaries of study data, the following will be presented:

- Nominal/categorical measures will be summarized using frequencies and percentages;
- Interval or ratio scale measures will be summarized using means and standard deviations;
- Ordinal measures will be summarized depending on the number of levels. An ordinal measure with five levels or less will be summarized as a nominal measure. An ordinal measure with more than five levels will be summarized as an interval or ratio scale measure.

The balance or imbalance of baseline characteristics will be studied and reported, particularly for analyses comparing the two study arms.

Reported p-values will be based on two-sided tests at an $\alpha=0.05$ unless otherwise specified. When p-value correction is appropriate, Benjamini-Hochberg[2] False Discovery Rate adjustments will be used to address multiplicity and preserve Type I error rate.

Unless required otherwise by a journal, the following rules are standard:

- Test statistics will be reported to two decimal places.
- P-values will be reported to two significant figures. If less than 0.001, p-values will be reported as '<0.001'.
- No preliminary rounding will be performed; rounding will only occur after analysis. To round, consider digit to right of last significant digit: if < 5 round down, if ≥ 5 round up.

4.2. Dependent Variables

The dependent variables in this analysis are:

1. EMD measured ART adherence – Number of days EMD is opened from baseline to six-month follow-up (0 to 180 days) among participants in each arm.

2. Biological measures of ART adherence – Number of participants in each arm who are virally suppressed and adherent as measured through biological testing.

3. Self-reported ART adherence – Assessed by a validated three-item ART adherence scale. The three items are: 1) *Days taken* -- number of days the patient missed at least one dose of their medication (number of days, 0-30); 2) *Frequency* -- how often the patient took their medication in the way they were supposed to in the past 30 days (never/rarely/sometimes/usually/almost always/always); and 3) *Rating* – how good a job the patient did in taking their medication in the way they were supposed to in the past 30 days (very poor/poor/fair/good/very good/excellent). Responses were then linearly transformed into a scale of 0 to 100 (higher scores indicate greater adherence). The average score across the three items was calculated to represent overall adherence. Scale reliability among this sample was high (Cronbach's α= .90).

4. Uptake of OST – Number of participants who reported they were currently taking OST at the six-month follow-up.

4.3. Independent Variables

The key independent variables in this analysis are:

Study Arm – Randomized to the Intervention arm or the SOC arm.

Timing of Assessment – pre-intervention, immediately post-intervention, three-month follow-up, six-month follow-up.

4.4. Covariates

As appropriate in the analyses, multivariable modeling of changes in ART adherence will include adjustment for the following participant-level characteristics:

a. Age
b. Sex
c. Partner HIV status
d. Baseline levels of adherence

4.5. Statistical Analysis Methods

Descriptive statistics of key independent variables and covariates will be summarized within each arm using frequencies or means (standard deviations) as appropriate. Overall participation rates will be reported in each arm based on the proportion of baseline participants who completed the six-month follow-up assessment.

To test **hypothesis 1 (H1)**, we will conduct t-tests to assess for differences in ART adherence rates over six months (0 to 180 days) between study arms.

To test **hypothesis 2 (H2)**, we will conduct chi-square tests to assess for differences in biological measures of ART adherence between study arms. Adjustments for small sample sizes will be made as appropriate.

To test **hypothesis 3 (H3)**, we will conduct generalized linear mixed modeling to assess for change in self-reported ART adherence from baseline to the six-month follow-up period and compare between arms. Generalized linear mixed modeling will be used to account for the effect of response clustering within participants. Models will adjust for the covariates specified above (section 4.4) and include random effects for dyad and intercept.

The model testing H3 is summarized as Equation H3:

> ART adherence = Intercept + B_1(Arm) + B_2(Categorical variable corresponding to timepoint of adherence assessment; two degrees of freedom) + **B_3 (Arm * Timepoint of adherence assessment**; two degrees of freedom**)** + B_4(Baseline adherence level) + B_5(Partner HIV status) + B_6(Respondent Age) + B_7 (Respondent Sex) + Error
> + Random effects for the clustering within participant across timepoints and by dyad.

If the statistical test for the interaction between arm and timepoint is significant at the α=0.05 level, then we conclude that study arm significantly impacts the change in ART adherence. Within arm, adjusted estimates of changes in adherence will be presented along with 95% confidence intervals.

To test **hypothesis 4 (H4)**, we will conduct chi-square tests to assess for differences in uptake of OST between study arms. Adjustments for small sample sizes will be made as appropriate.

4.6. Missing Data

The analysis sample is restricted to participants with data for the outcomes and with complete demographic information. Missing data for outcomes or demographics will not be imputed.

5. REFERENCES

1. Wilson IB, Lee Y, Michaud J, Fowler FJ, Rogers WH. Validation of a New Three-Item Self-Report Measure for Medication Adherence. AIDS Behav 2016;20(11):2700–8.

2. Yoav Benjamini, YH. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society: Series B (Methodological) 1995;57(1):289–300.