Pre-exposure Prophylaxis Implementation in Central-Eastern European Countries

NCT05323123

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Contents

Statistical Analysis Plan (SAP)

1. Administrative Information

Title and Trial Registration

Statistical Analysis Plan for the "Prepare Romania" Trial: Promoting Pre-Exposure Prophylaxis (PrEP) Adherence Among Gay, Bisexual, and Other Men Who Have Sex with Men (GBMSM) in Romania.

Trial registration number: This trial is formally registered with the identifier NCT05323123 on ClinicalTrials.gov.

2. Introduction

2.1 Background and Rationale

HIV transmission among gay, bisexual, and other men who have sex with men (GBMSM) in Romania remains a critical public health issue. Romania has one of the highest rates of new HIV cases among GBMSM in Central and Eastern Europe, despite efforts to improve prevention services. A significant contributor to this trend is the lack of formal access to pre-exposure prophylaxis (PrEP) within the national healthcare system, limiting the availability of this essential HIV prevention method for GBMSM. Although PrEP has been shown to reduce the risk of HIV transmission by up to 96% with high adherence, GBMSM in Romania face numerous barriers to consistent use.

In response to these challenges, the Prepare Romania intervention was developed as a culturally adapted program that combines counseling with mobile health (mHealth) tools to enhance PrEP adherence and persistence. The intervention is tailored specifically for GBMSM living in Romania, integrating behavioral support with technology to increase accessibility, convenience, and acceptability among this population. The intervention is based on two evidence-based U.S.-developed PrEP adherence tools: the Sparking PrEP Awareness Research and Knowledge (SPARK) counseling protocol and the HealthMpowerment (HMP) app, adapted to meet the cultural and contextual needs of Romanian GBMSM. Previous pilot trials in Romania demonstrated both the feasibility and acceptability of this combined intervention among the target population.

2.2 Objectives

The primary aim of this trial is to evaluate the effectiveness of a culturally adapted intervention that combines in-person counseling with mobile health (mHealth) support to improve adherence to PrEP medication among GBMSM in Romania. This population faces

substantial barriers to HIV prevention, including stigma and limited access to PrEP. By hypothetically increasing adherence, the intervention tested in this study seeks to mitigate HIV transmission risks within this high-risk group, ultimately contributing to public health initiatives targeting HIV prevention.

3. Study Methods

3.1 Trial Design

The Prepare Romania trial is a two-arm, parallel-group randomized controlled trial (RCT) with a 1:1 allocation ratio, designed to evaluate the effectiveness of a culturally adapted intervention to improve adherence to PrEP among GBMSM in Romania. Participants are randomized to either the intervention or control arm. The intervention arm includes a combination of counseling and a culturally tailored mobile health app that supports PrEP adherence. Participants in the control arm receive standard PrEP education only. Randomization is stratified by city (Bucharest and Cluj-Napoca), and the study is unmasked, with both participants and staff aware of group assignments. Primary and secondary outcomes will be collected at baseline, 3 months, and 6 months post-randomization.

3.2 Randomization

Upon completing informed consent and screening, participants in the Prepare Romania trial are randomized to either the intervention arm (Prepare Romania) or the control arm (PrEP education-only). This randomization process is managed by the study coordinator using a computerized algorithm that ensures a 1:1 allocation ratio. The trial aims for 60 participants per city (Bucharest and Cluj-Napoca), but the randomization sequence accommodates up to 70 participants per city. This approach provides flexibility, allowing for up to 10 potential exclusions after the initial medical visit due to PrEP contraindications or personal choices to forgo PrEP initiation based on clinical assessments. The study's randomization plan does not involve additional stratification factors outside the city-level allocation.

3.3 Sample Size

The Prepare Romania trial's sample size was chosen to provide preliminary data on the hypothesized intervention outcomes, PrEP adherence and persistence among GBMSM in Romania, as well as to inform the design of a future effectiveness trial. A formal power calculation was not conducted due to the exploratory nature of this pilot study. Instead, we followed guidance from Whitehead et al., which provides recommendations for sample sizes in pilot studies.

The sample size of 60 participants per arm (120 total) was selected based on conservative effect size estimates drawn from previous studies evaluating PrEP adherence interventions. In a comparable RCT testing the SPARK intervention, protective PrEP levels (defined as adherence of ≥4 doses per week) were observed in 94% of the intervention arm and 85% of the control arm at the 3-month follow-up, with corresponding rates of 92% and 86% at 6 months. This difference in adherence rates translates to an estimated effect size of 6%, with a pooled standard deviation of approximately 0.32, yielding a standardized effect size of 0.19. Whitehead et al. suggest a minimum sample size of 25 participants per condition for pilot trials when the effect size falls between 0.1 and 0.3. To ensure adequate power, the Prepare Romania trial conservatively enrolls 60 participants per arm, exceeding this minimum and allowing for a robust assessment of intervention impact.

Each site (Bucharest and Cluj-Napoca) is tasked with recruiting 60 participants. With this sample size, the study expects retention at 6 months to exceed 25 participants per arm, even accounting for potential attrition. This estimate is supported by high retention rates (approximately 89%) reported in prior SPARK trials.

3.4 Timing of Outcome Assessments

Outcomes measured at baseline, 3 months, and 6 months post-randomization.

4. Statistical Principles

4.1 Confidence Intervals and P-Values

For the Prepare Romania trial, statistical analyses will be conducted with a two-sided significance level set at 0.05. This alpha level applies to all primary and secondary outcomes, ensuring that results are interpreted consistently across study endpoints. The 95% confidence intervals will be calculated for the primary and other key secondary outcomes.

4.2 Analysis Populations

The primary analysis for the Prepare Romania trial will follow the intention-to-treat (ITT) principle.

4.3 Baseline Patient Characteristics

Baseline characteristics will be summarized with descriptive statistics, including study site, age, sexual identity, outness, relationship status, high school location, ethnicity, education level, occupation, income, previous PrEP usage, and current PrEP status.

To assess baseline balance between the groups, we will calculate the Absolute Standardized Difference (ASD) for each characteristic.

6. Analysis

6.1 Outcome Definitions

Primary Outcome

The primary outcome of this study is PrEP adherence, which will be measured objectively using dried blood spot (DBS) testing to detect levels of tenofovir diphosphate (TFVdp). Adherence is defined as achieving a TFVdp concentration of ≥1000 fmol/punch, corresponding to a mean dosing frequency of at least four doses per week. This measure will be evaluated at 3- and 6-months post-randomization. The binary operationalization of this measure will serve as the primary outcome.

Secondary Outcomes

The secondary outcomes include continuous DBS PrEP adherence, self-reported PrEP adherence (binary and continuous), PrEP knowledge (binary: all questions answered correctly vs. not), PrEP motivation (ordinal categorical), PrEP attitudes and beliefs (continuous), PrEP barriers, PrEP facilitators (continuous), PrEP stigma (continuous), anxiety symptomology (continuous), depression symptomology (continuous), STI diagnoses in the last 6 months (binary: any vs. none), and number of condomless anal sex (CAS) acts with HIV-positive or unknown-status partners in the last 6 months (continuous). For all secondary outcomes, missing data will be recorded and addressed.

6.2 Analysis Methods

We will use Generalized Linear Mixed Models (GLMMs) to handle the various types of outcomes across primary and secondary measures, which allows for fixed effects (intervention, site, time, baseline outcome, the interaction between intervention and time baseline covariates) and random effects (including participant-level variability). The adjusted covariates include age, relationship status, and PrEP usage. All analyses will be conducted in R. The general form of the GLMM is:

$$g\left(\mu(Y_{ij})\right) = \beta_1 I(Time_{ij} = 3) + \beta_2 I(Time_{ij} = 3) \times Intervention + \beta_3 I(Time_{ij} = 6) + \beta_4 I(Time_{ij} = 6) \times Intervention + \boldsymbol{\beta}_5 Covariates_i + \mu_i + \epsilon_{ij}$$

Y_{ij} represents the outcome for participant i at time j,
$\mu(Y_{ij})$ is the expected value of Y_{ij} given the covariates,

$g(.)$ is the link function that relates the linear predictors to the expected value of outcome (e.g., logit link for binary outcomes, identity link for continuous outcomes),
β_1 is the effect of time at 3 month,
β_2 is the interaction effect between intervention and time at 3 month,
β_3 is the effect of time at 6 month,
β_4 is the interaction effect between intervention and time at 6 month,
$\boldsymbol{\beta_5}$ is the covariate effect,
μ_i is the random intercept for each participant, where $\mu_i \sim N(0, {\sigma_i}^2)$,
$I(.)$ is the indicator function.

Treatment effects from the model

3 month	6 month
β_2	β_4

Then the average treatment effect is

$$\frac{\beta_2 + \beta_4}{2}$$

Primary Outcome

The primary outcome, PrEP adherence obtained through DBS, is a binary variable and will be analyzed using a GLMM with a logit link function. The intervention effect will be represented by an adjusted odds ratio, and will be assessed across different follow-up points. This analysis will be implemented in R using the "glmer()" function, specifying a logit link and a random intercept for participants to account for within-subject correlations.

Secondary Outcomes

The secondary outcomes include continuous, binary, and categorical variables, each analyzed with an appropriate link function based on the outcome type. For continuous outcomes, such as depression and anxiety symptomology scores, an identity link function will be used, estimating the intervention effect as the difference in mean scores between groups. This model will be implemented using the "lmer()" function in R. Binary secondary outcomes, will use the same logit link function and implementation details as the primary outcome analysis.

Table 1. List of secondary outcomes.

Outcome Measure	Variable coding
DBS PrEP adherence	Continuous
Self-Report PrEP adherence	Binary and continuous
Depression symptomology	Continuous
Anxiety symptomology	Continuous
PrEP knowledge	Binary
PrEP motivation	Ordinal categorical, with 7 categories

PrEP stigma	Continuous
Positive attitudes/beliefs about PrEP	Continuous
Negative attitudes/beliefs about PrEP	Continuous
PrEP barriers	Continuous
PrEP facilitators	Continuous
STI diagnoses	Binary
CAS acts	Continuous

Each model will output the intervention effect at baseline, 3 months, and 6 months (with the exception of STI diagnoses and CAS acts, which are only measured at baseline and 6 months as well as DBS and self-report PrEP adherence, which are only measured at 3 months and 6 months), with 95% confidence intervals to provide estimates of precision.

6.3 Missing Data

Missing data are expected at follow-up time points (3 months and 6 months) in primary and secondary outcomes. Assuming that the missing data follow a Missing at Random (MAR) pattern, Multiple Imputation by Chained Equations (MICE)(Van Buuren and Groothuis-Oudshoorn 2011) will be used to handle missing values for the primary outcome and secondary outcome adjusting all baseline covariates. Imputations will be generated with 15 datasets using the "mice()" function from the "mice" package in R. The imputation methods will include predictive mean matching (pmm) for continuous outcomes, logistic regression (logreg) for binary outcomes, and polytomous regression (polyreg) for categorical outcomes. Each imputed dataset will be analyzed separately, and results will be combined using Rubin's Rules(Rubin 2004) to obtain pooled estimates and confidence intervals, accounting for the variability within and between imputations.

7. References

Rubin, Donald B. 2004. *Multiple Imputation for Nonresponse in Surveys*. Vol. 81. John Wiley & Sons.

Van Buuren, Stef, and Karin Groothuis-Oudshoorn. 2011. "Mice: Multivariate Imputation by Chained Equations in R." *Journal of Statistical Software* 45:1–67.