**Statistical Analysis Plan**
Version 1.5

# Evaluation of stepped PrEP adherence support for young South African women using a SMART design

**Short Title: *PrEP SMART***

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Drafted by: Jennifer Velloza, PhD MPH; Deborah Donnell, PhD; Allison Meisner, PhD


***Statistical Team***

*University of Washington, Seattle, USA*
Deborah Donnell, PhD
Jennifer Velloza, PhD MPH
Allison Meisner, PhD
Cole Grabow, MPH
Kathy Thomas, MS

*University of the Witwatersrand, Johannesburg, South Africa*
Richard Munthali, PhD

***Investigators***

Connie Celum, MD, MPH *University of Washington, Seattle, USA*
Sinead Delany-Moretlwe, MBChB, PhD, *University of the Witwatersrand, Johannesburg, South Africa*
Jared Baeten, MD, PhD, *University of Washington, Seattle, USA*
Sybil Hosek, PhD, *Stroger Hospital of Cook County, Chicago, USA*

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## Background and Rationale

The purpose of this study is to test a stepped model of scalable adherence support strategies in young South African women who initiate PrEP using a sequential multiple assignment randomized trial (SMART) design.

A SMART is a useful experimental design for the development of high-quality adaptive interventions. The SMART design utilized in this study will allow us to evaluate which of four embedded dynamic treatment strategies achieves highest PrEP adherence.

## Study Objectives and Hypotheses

**The primary objectives of this study are:**

- To evaluate the effect of the mHealth interventions, in addition to regular clinic visits, on PrEP adherence at the nine-month study visit.
- To evaluate the effect of the additional adherence support interventions, in addition to regular clinic visits and first-line mHealth interventions, on PrEP adherence at the nine-month study visit, separately for (1) non-responders to the first-line SMS intervention and (2) non-responders to the first-line WhatsApp intervention.
- To evaluate the four embedded dynamic treatment strategies in terms of PrEP adherence at the nine-month study visit to identify the best adherence support strategy.

**The secondary objectives of this study are:**

- To evaluate (1) the effect of the mHealth interventions on early PrEP adherence (month 2), (2) the effect of the mHealth interventions on long-term PrEP adherence (month 12), and (3) the effect of the additional adherence support interventions on long-term PrEP adherence (month 12) among (a) non-responders to the first-line SMS intervention and (b) non-responders to the first-line WhatsApp intervention.
- To identify correlates of PrEP adherence at the two-, nine- and twelve-month study visits, adjusting for study arm, such as sociodemographic factors, individual-level and partner-level characteristics, and risk practices.
- To assess the proportion of young women who discontinue PrEP, timing of discontinuation, and factors associated with PrEP discontinuation, adjusting for study arm.
- To characterize SMS response rates and the content and frequency of use of WhatsApp groups
- To qualitatively explore factors that influence women's decisions to use PrEP, adhere to PrEP, and their satisfaction with their assigned intervention(s) and preferences for another intervention option.

**The exploratory objectives of this study are:**

- To assess HIV incidence and the presence of detectable TFV-DP in PrEP users who acquire HIV infection during the study.
- To describe antiretroviral drug resistance among women who acquire HIV infection.

## Study Design

This is a single-site sequential multiple assignment randomized trial (SMART) to assess a stepped model of scalable adherence support strategies in young South African women who initiate PrEP.

Women will be consented to have 12 months of follow-up. PrEP will be offered as part of a comprehensive package of sexual and reproductive health care that includes: HIV testing, counseling, condoms, contraception, and testing for and treatment of STIs. Approximately 350 women will be consented and randomized (about 175 participants in each initial intervention arm). Women will receive their assigned interventions until month 9 (the primary endpoint) and will be exited from the SMS and WhatsApp messages at the month-9 visit. They will be followed for an additional three months to assess the intervention's sustained effect on PrEP adherence at month 12.

At enrollment women will be randomly assigned in a 1:1 ratio to receive both standard adherence support counseling with monthly clinic visits and one-way SMS reminder messages and *either* participation in WhatsApp groups *or* weekly two-way SMS communication.

At month 3, women who have TFV-DP <500 fmol/punch (based on a blood sample collected at month 2) or who miss PrEP refills at the month 1 or 2 visits will undergo a secondary 1:1 randomization to either continue monthly follow-up visits with problem-focused counseling until month 8 or attend quarterly visits with drug level feedback counseling at months 3 and 6. Women will also continue to receive the adherence support from their original randomization (i.e., WhatsApp groups or weekly two-way SMS).

Women who have TFV-DP ≥500 fmol/punch at month 3 (based on a blood sample collected at month 2) will continue to receive the adherence support in their original randomization (SOC adherence counseling, one-way SMS reminder messages, and either WhatsApp groups or weekly two-way SMS) with quarterly visits. **Table 1** depicts the four embedded dynamic treatment strategies and decision rules for determining non-adherence and re-randomizing women at their month 3 visit:

**Table 1. Embedded dynamic treatment strategies and corresponding decision rules**

| **Dynamic treatment strategy** | **Decision rule** |
|---|---|
| **Non-response definition:** TFV-DP levels <500 fmol/punch or missed refills | |
| First, offer monthly SOC counseling and WhatsApp group intervention; then add drug level feedback with quarterly visits for non-responders and switch responders to quarterly SOC visits and continued WhatsApp groups | First-stage intervention option = {SOC + WhatsApp}<br><br>IF evaluation = {non-response}<br><br>THEN second-stage intervention option = {DLFB +<br><br>first-stage intervention option + quarterly study visits} |

| | |
|---|---|
| | ELSE continue on first-stage intervention option + quarterly study visits |
| First, offer monthly SOC counseling and weekly two-way SMS intervention; then add drug level feedback with quarterly visits for non-responders and switch responders to quarterly SOC visits and continued weekly two-way SMS | First-stage intervention option = {SOC + SMS}<br>IF evaluation = {non-response}<br>THEN second-stage intervention option = {DLFB +<br>first-stage intervention option + quarterly study visits}<br>ELSE continue on first-stage intervention option + quarterly study visits |
| First, offer monthly SOC counseling and WhatsApp group intervention; then add monthly follow-up visits with problem-focused adherence counseling for non-responders and switch responders to quarterly SOC visits and continued WhatsApp groups | First-stage intervention option = {SOC + WhatsApp}<br>IF evaluation = {non-response}<br>THEN second-stage intervention option = {Continue monthly<br>visits and problem-focused counseling + first-stage intervention option}<br>ELSE continue on first-stage intervention option + quarterly visits |
| First, offer monthly SOC counseling and weekly two-way SMS intervention; then add monthly follow-up visits with problem-focused adherence counseling for non-responders and switch responders to quarterly SOC visits and continued weekly two-way SMS | First-stage intervention option = {SOC + SMS}<br>IF evaluation = {non-response}<br>THEN second-stage intervention option = {Continue monthly<br>visits and problem-focused counseling + first-stage intervention option}<br>ELSE continue on first-stage intervention option + quarterly visits |

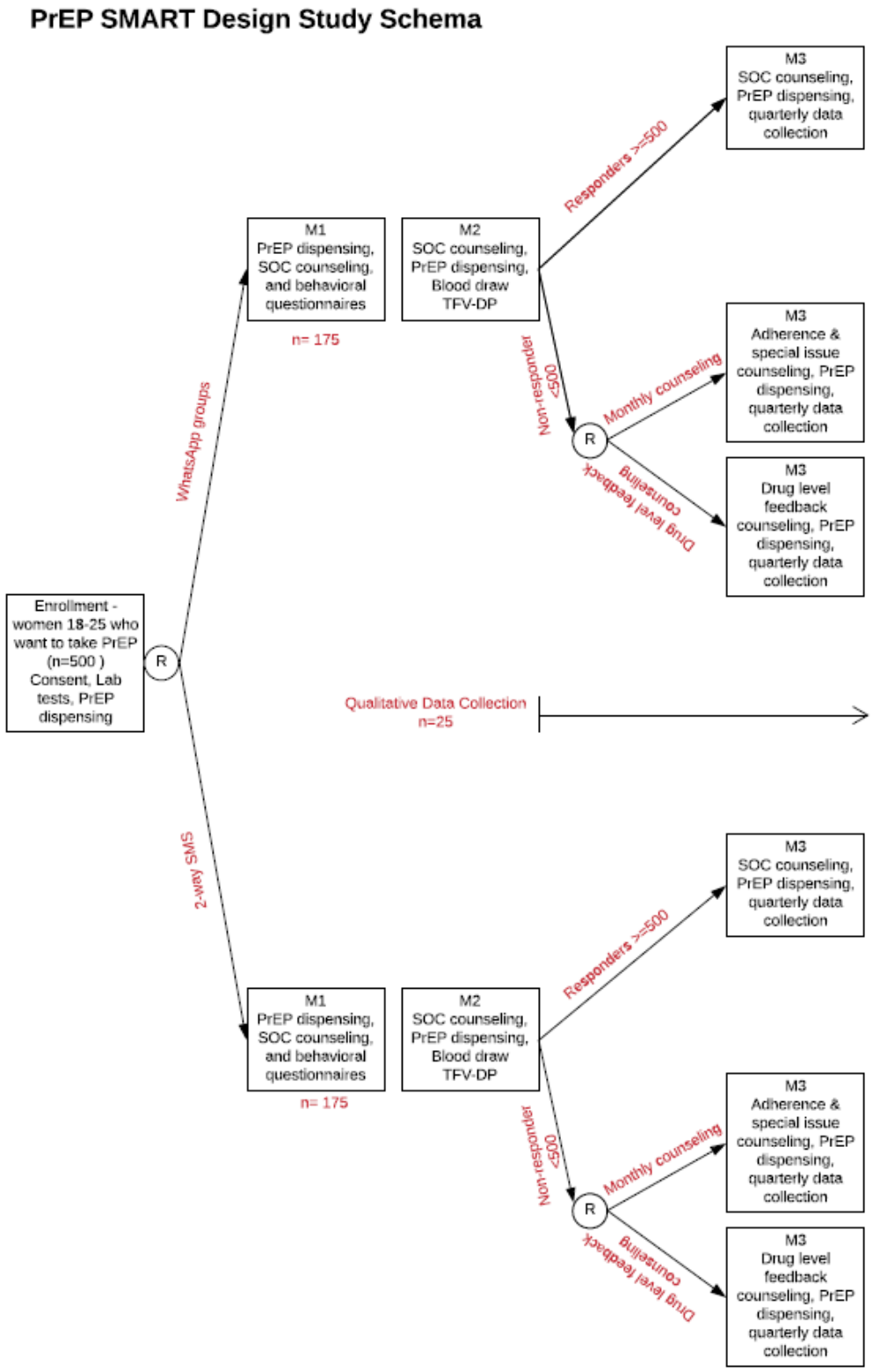
All women will receive periodic one-way SMS messages reminding them about upcoming clinic appointments during the follow-up period.

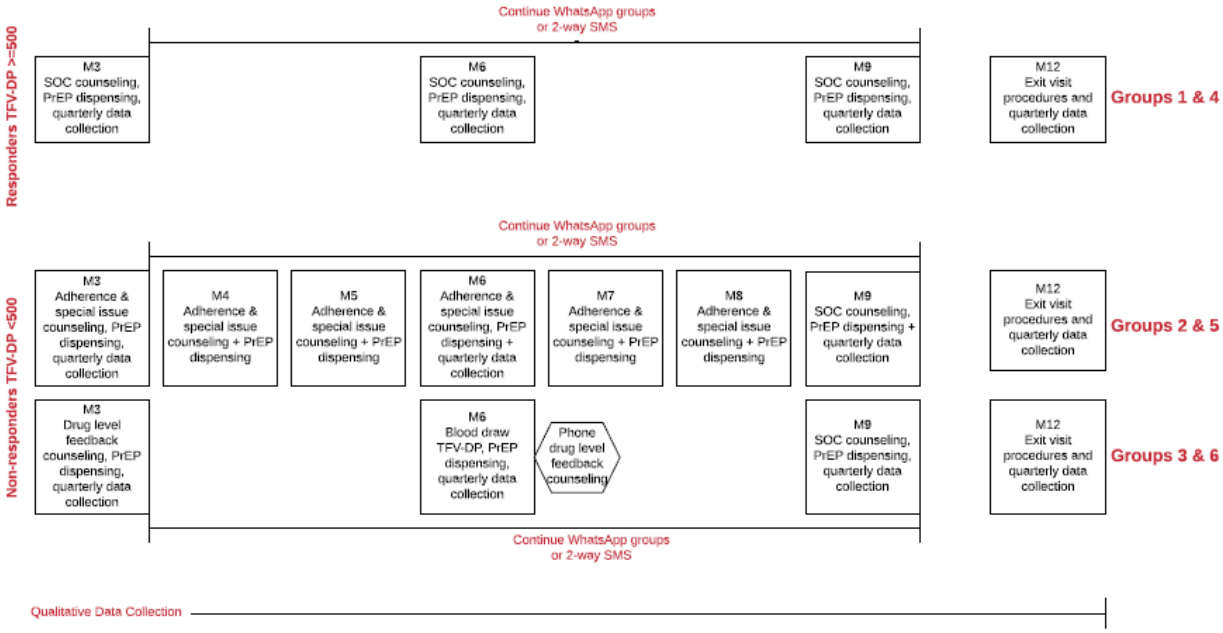
## Schema

| | |
|---|---|
| **Rationale:** | Sequential multiple assignment randomized trials (SMARTs) are useful experimental designs for the development of high-quality adaptive interventions. The SMART design utilized in this study will allow us to identify the best embedded dynamic treatment strategy to facilitate PrEP adherence support. |
| **Purpose:** | To test a stepped model of scalable adherence support strategies in young South African women who initiate PrEP, using a SMART design. |
| **Design:** | Eligible women who accept open-label daily oral PrEP will be enrolled and randomized to both SOC adherence support (brief counseling) and one-way SMS reminder messages and either WhatsApp groups or weekly two-way SMS messages. These mHealth interventions are aimed at increasing PrEP adherence during follow-up by providing peer support for PrEP adherence (WhatsApp groups), clinical support to manage side effects and address adherence issues (two-way SMS messages), and reminders about daily PrEP pill-taking (both WhatsApp groups and two-way SMS messages).<br><br>Follow-up visits will occur monthly for three months and, in both groups, tenofovir drug levels at month 2 will be used as an objective measure of adherence to determine whether they have achieved high adherence based on their initial randomization. Women with high adherence (i.e., TFV-DP ≥500 fmol/punch from DBS, 'responders') will continue with the adherence support to which they were initially randomized and will attend quarterly visits from months 3-12.<br><br>'Non-responders' will be identified based on TFV-DP <500 fmol/punch or missed drug refills at their month 1 or 2 study visits and will continue initial randomization (WhatsApp or weekly two-way SMS) plus be randomized to either more intensive adherence support: continued monthly visits from months 3-9 with problem-focused counseling modules delivered at months 3-8 *or* quarterly visits between months 3-9 with feedback about adherence based on drug levels at the month 3 and 6 study visits<br><br>The primary outcome for the combined intervention is the proportion in each first-line treatment arm (mHealth intervention) with high adherence measured by TFV-DP levels at 9 months.<br><br>We will also conduct qualitative data collection with a purposive sample of approximately 25 responders and non-responders after their 2-, 6-, and 12-month or exit study visits. |
| **Population:** | HIV-uninfected women ages 18-25 in Johannesburg, South Africa. |
| **Study Size:** | Approximately 350 women who accept PrEP will be consented and randomized. |

| | |
|---|---|
| **PrEP Regimen:** | All participants will be provided once daily oral emtricitabine 200 mg / tenofovir disoproxil fumarate 300 mg (FTC/TDF). |
| **Study Duration:** | Approximately 48 months, including submissions to Institutional Review Boards (IRBs) and the South African Health Products Regulatory Authority (SAHPRA, formerly the Medicines Control Council), recruitment, and 12 months of follow-up per participant. |
| **Primary Objective:** | To evaluate the effect of mHealth interventions (first-line intervention), in addition to regular clinic visits, on PrEP adherence, the effect of additional adherence support interventions (second-line intervention: monthly visits and special issue counseling or quarterly visits and drug-level feedback) among women who do not respond to (1) the first-line SMS intervention or (2) the first-line WhatsApp intervention (separately), and the best embedded dynamic treatment strategy of intensifying adherence support among young women who have low adherence after the first two months of use. |
| **Secondary Objectives:** | • To evaluate (1) the effect of the first-line interventions on early PrEP adherence (month 2), (2) the effect of the first-line interventions on long-term PrEP adherence (month 12), and (3) the effect of the second-line interventions on long-term PrEP adherence (month 12) among those who do not respond to the first-line interventions.<br>• To assess the correlates of PrEP adherence at the two-, nine- and twelve-month visits, after adjusting for study arm, including sociodemographic factors, individual-level and partner-level characteristics, and risk practices.<br>• To assess the proportion of young women who discontinue PrEP, timing of discontinuation, and factors associated with PrEP discontinuation.<br>• To characterize SMS response rates and the content and frequency of use of WhatsApp groups.<br>• To qualitatively explore factors that influence women's decisions to use PrEP and adhere to PrEP and their satisfaction/preferences with their assigned intervention(s). |
| **Exploratory Objectives:** | • To assess HIV incidence and the presence of detectable TFV-DP in PrEP users who acquire HIV infection during the study.<br>• To describe antiretroviral (ARV) drug resistance among women who acquire HIV infection. |
| **Study Site:** | Ward 21 CRS, Wits RHI, University of the Witwatersrand, in Johannesburg, South Africa. |

## Overview of Study Design




Responders TFV-DP >=500
Continue WhatsApp groups or 2-way SMS
M3 SOC counseling, PrEP dispensing, quarterly data collection
M6 SOC counseling, PrEP dispensing, quarterly data collection
M9 SOC counseling, PrEP dispensing, quarterly data collection
M12 Exit visit procedures and quarterly data collection
Groups 1 & 4
Non-responders TFV-DP <500
Continue WhatsApp groups or 2-way SMS
M3 Adherence & special issue counseling, PrEP dispensing, quarterly data collection
M4 Adherence & special issue counseling + PrEP dispensing
M5 Adherence & special issue counseling + PrEP dispensing
M6 Adherence & special issue counseling, PrEP dispensing + quarterly data collection
M7 Adherence & special issue counseling + PrEP dispensing
M8 Adherence & special issue counseling + PrEP dispensing
M9 SOC counseling, PrEP dispensing + quarterly data collection
M12 Exit visit procedures and quarterly data collection
Groups 2 & 5
M3 Drug level feedback counseling, PrEP dispensing, quarterly data collection
M6 Blood draw TFV-DP, PrEP dispensing, quarterly data collection
Phone drug level feedback counseling
M9 SOC counseling, PrEP dispensing, quarterly data collection
M12 Exit visit procedures and quarterly data collection
Groups 3 & 6
Continue WhatsApp groups or 2-way SMS
Qualitative Data Collection

## Sample size

We estimate that we will need to enroll about 350 AGYW to detect an approximately 12% difference in PrEP adherence between the two-way SMS and WhatsApp groups (with brief standard of care counseling) at the nine-month study visit.

## Data Analysis

**Primary Analysis 1: Evaluate the effect of the mHealth interventions, in addition to regular clinic visits, on PrEP adherence at the nine-month study visit.**

*Analysis population:* PrEP SMART participants enrolled and randomized to either the weekly two-way SMS arm or the WhatsApp groups.

- For an intent-to-treat analysis, we will include all randomized participants.
- For a modified intent-to-treat analysis, we will include only participants who attend at least one study visit between months 1-3.
- For a per protocol analysis, we will include all randomized participants who received full doses of the SMS and WhatsApp interventions as specified below.

*Outcome:* PrEP adherence at the nine-month study visit, defined as a binary outcome variable around TFV-DP levels of 700 fmol/punch

- We will also conduct secondary analyses with PrEP adherence measured as continuous TFV-DP levels and will provide descriptive summaries of PrEP adherence measured as a five-category variable: BLQ, BLQ-349 fmol/punch, 350-699 fmol/punch, 700-1249 fmol/punch, and ≥1250 fmol/punch.

*Predictor(s) of interest:* Randomized first-line intervention arm, either weekly two-way SMS or WhatsApp groups

*Measures of intervention use for per protocol analysis:*

- Completed SMS intervention: 1 = remained enrolled in the SMS intervention for the full follow-up period. 0 = opted out of the SMS intervention for at least 1 week of the follow-up period.
- Completed the WhatsApp intervention: 1 = remained enrolled in the WhatsApp intervention for the full follow-up period. 0 = opted out of the WhatsApp intervention for at least 1 week of the follow-up period.

*Statistical analyses:*

1) We will compute descriptive statistics to describe the full study population of all randomized individuals and to describe the study population in each of the six intervention groups.
2) We will compute descriptive statistics to describe the study population who were randomized and returned for at least one study visit between months 1-3 (the sample for the modified intent-to-treat analysis). We will also summarize reasons for discontinuing

PrEP or study participation among participants who were randomized but did not return to a study visit between months 1-3, as data are available.

3) Primary analysis: We will conduct an intent-to-treat analysis using modified Poisson regression to assess the effect of the first-line randomized intervention on 9-month adherence by comparing TFV-DP levels (</≥ 700 fmol/punch) across participants receiving two-way SMS versus participants enrolled in the WhatsApp intervention. This main effect can be interpreted as the difference between the two first-line intervention options. The effect of the intervention will be estimated as a relative risk (RR) from the regression model, with study arm assigned at first randomization as the only predictor and robust standard error estimates.

   Sensitivity analyses: If overall loss to follow-up in the full sample is >10% by month 9 or if differential loss to follow-up between the two arms is >10%, we will repeat the primary ITT analysis using inverse probability weights to adjust for the potential impact of missing data.

   Modified ITT analyses: We will conduct a modified ITT analysis using the same procedures described above (primary analysis) but restricting to participants who returned for at least one study visit between months 1 and 3.

   - Since this analysis breaks randomization, we will adjust for important prognostic variables (including demographics, sexual behavior factors, and HIV risk factors) to account for treatment imbalances.

   Per-protocol analyses: We will also conduct a per-protocol analysis using the same procedures described above but restricting to participants who received full doses of the SMS and WhatsApp interventions as described above in the *Measures of intervention use* section and adjusting for the same prognostic variables as in the modified ITT analysis.

   Subgroup analyses: We will perform subgroup analyses to obtain subgroup-specific estimates of the intervention. These estimates will be generated using the same model and analysis population as described in the primary analysis (above) but will also include subgroup indicator variable(s) and an interaction term between the subgroup indicator and study arm in the model. Subgroups will be defined as follows:

   - Age ≤20 years versus >20 years

4) Secondary analysis: We will assess the effect of the intervention on the continuous PrEP adherence outcome using linear regression models to estimate the difference in mean TFV-DP levels between those in the SMS group compared with those in the WhatsApp group at month 9. If less than 40% of the sample has drug undetectable drug levels, we will assign those with undetectable drug levels a numeric drug level equal to half the limit of detection. If more than 40% of the sample has undetectable drug levels, we will reassess the value of this analysis.

**Primary Analysis 2: Evaluate the effect of the additional adherence support interventions, in addition to regular clinic visits and first-line mHealth interventions, on PrEP adherence at the nine-month study visit, separately for (1) non-responders to the first-line SMS intervention and (2) non-responders to the first-line WhatsApp intervention.**

*Analysis population:* PrEP SMART participants who are re-randomized to either monthly problem-focused counseling or drug-level feedback counseling.

*Outcome:* PrEP adherence at the 9-month study visit, defined as a binary outcome variable around TFV-DP levels of 700 fmol/punch

- We will also conduct secondary analyses with PrEP adherence measured as continuous TFV-DP levels and will provide descriptive summaries of PrEP adherence measured as a five-category variable: BLQ, BLQ-349 fmol/punch, 350-699 fmol/punch, 700-1249 fmol/punch, and ≥1250 fmol/punch.

*Predictor(s) of interest:* Randomized arm, either monthly problem-focused counseling or drug-level feedback counseling with quarterly study visits.

*Statistical analyses:*

1) We will summarize reasons for re-randomization (e.g., missed visits, TFV-DP levels <500) and provide descriptive statistics for re-randomized participants, stratified by first-line treatment, in order to understand the characteristics of non-responders.
2) Primary analysis: We will use modified Poisson regression to assess the effect of the randomized second-line intervention on 9-month adherence by comparing TFV-DP levels across participants receiving monthly counseling versus participants receiving drug-level feedback, separately for (1) non-responders to the first-line SMS intervention and (2) non-responders to the first-line WhatsApp intervention. The effect of the randomized intervention will be estimated as a relative risk (RR) from the regression models, with study arm assigned at second randomization as the predictor of interest and robust standard error estimates.

   Sensitivity analysis: If overall loss to follow-up in the full sample is >10% by month 9 or if differential loss to follow-up between the two arms is >10%, we will repeat the primary analysis using inverse probability weights to adjust for the potential impact of censoring due to missing data.

   Subgroup analysis: Subgroup-specific estimates of the intervention will be generated using the same models and analysis population as described in the primary analysis (above) but will also include subgroup indicator variable(s) and an interaction term between the subgroup indicator and second-line study arm in the model. Subgroups will be defined as follows:
   - Age ≤20 years versus >20 years

3) Secondary analyses:
   - We will also assess the effect of the intervention on the continuous PrEP adherence outcome using linear regression models to assess the difference in mean TFV-DP levels between those in the monthly problem-focused counseling group and those in the drug-level feedback group at month 9. If less than 40% of the sample has drug undetectable drug levels, we will assign those with undetectable drug levels a numeric drug level equal to half the limit of detection. If more than 40% of the sample has undetectable drug levels, we will reassess the value of this analysis.

- We will also consider evaluating the effect of second-line treatment stratified by reason for non-response; however, if the number of participants available for these stratified analyses is insufficient, we will not pursue these analyses.

**Primary Analysis 3: Among the embedded dynamic treatment strategies, identify the optimal adherence support strategy in terms of PrEP adherence at the nine-month study visit, where additional adherence support is provided to those who have low adherence after the first two months of PrEP use.**

*Analysis population:* All PrEP SMART participants. For a secondary modified intent-to-treat analysis, we will include only participants who attend at least one study visit between months 1-3.

*Outcome:* PrEP adherence at the nine-month study visit, defined as a binary outcome variable around TFV-DP levels of 700 fmol/punch

*Predictor(s) of interest:* Embedded dynamic treatment strategies

*Statistical analyses:*

1) We will estimate the proportion of participants adherent to PrEP for each of the four embedded dynamic adherence support strategies (as shown in Table 1) and will compare the proportion adherent to PrEP across the four groups.
    a. We will pursue a weighted-and-replicated approach to account for non-response and re-randomization as well as overlap in dynamic treatment regimes (Kidwell et al., *J Appl Stat*, 2018). The weighted-and-replicated approach involves two steps:
        i. Weighting: In a SMART, for a given embedded dynamic treatment strategy, there is imbalance in the responders and non-responders who followed the strategy. In particular, assuming 1:1 randomization in each of the two phases of the SMART, the chance of a responder following the strategy is 0.5, while the chance of a non-responder following the strategy is 0.5*0.5 = 0.25. Thus, responders are over-represented, by design. However, all participants should be equally represented. To achieve this, we assign a weight of 1/0.5 = 2 to responders and 1/0.25 = 4 to non-responders. We must also use robust standard error estimates because the weights depend on responder status, which is unknown ahead of time.
        ii. Replicating: If we would like to compare two embedded dynamic treatment strategies that have the same first-line treatment, we have to account for the fact that one participant can be in two treatment strategies. Namely, in PrEP SMART, if a participant is a responder to one first-line treatment, they are automatically in two embedded treatment strategies. Thus, to model the data, we have to replicate this person's observation (i.e., make a copy of their row in the dataset). Non-responders are not replicated as they only follow one embedded dynamic treatment strategy. Note that this is not needed if we are evaluating embedded dynamic treatment strategies that begin with different first-line treatments.

The replicated data are then modeled using a weighted GEE with an independence working correlation matrix where the clusters are the individual subjects. The model can be written as:

$$\log(p) = \beta_0 + \beta_1 x_1 + \beta_2 x_{2NR} + \beta_3 x_1 x_{2NR}$$

where p is the probability of PrEP adherence, $x_1$ is the indicator of first-line treatment (0 for SMS, 1 for WhatsApp), and $x_{2NR}$ is the indicator of the second-line treatment among non-responders to the first-line treatment (0 for monthly counseling, 1 for drug-level feedback). The linear predictors for each of the four embedded dynamic treatment regimes are:

| **Embedded dynamic treatment regime** | **Linear predictor** |
|---|---|
| SMS, monthly counseling | $\beta_0$ |
| SMS, drug-level feedback | $\beta_0 + \beta_2$ |
| WhatsApp, monthly counseling | $\beta_0 + \beta_1$ |
| WhatsApp, drug-level feedback | $\beta_0 + \beta_1 + \beta_2 + \beta_3$ |

**Code**: This analysis will compare the four embedded DTS using a 'weighted and replicated' approach (see above for details on why this approach is needed). Here, 'responder' means those who responded to 1st-line treatment.

- Weighting:
  - Assign a weight of 2 to responders and 4 to non-responders
  - Use robust SEs
- Replicating:
  - Replicate the data for each participant who responded to 1st-line treatment (i.e., they appear in the data twice)
  - Use a (weighted) GEE: $\log(p) = \beta_0 + \beta_1 x_1 + \beta_2 x_{2NR} + \beta_3 x_1 x_{2NR}$
    - Independence working correlation matrix
    - Each participant is a cluster
    - Log link function (to estimate RRs)
    - Predictors:
      - Indicator of 1st-line treatment ($x_1$)
        - 0 = SMS, 1 = WhatsApp
      - Indicator of 2nd-line treatment for non-responders to 1st-line treatment ($x_{2NR}$)
        - 0 = monthly counseling, 1 = drug-level feedback
      - Interaction between 1st- and 2nd-line treatment
- SAS Code:
  - olddata = dataset
  - newdata = weighted and replicated data
  - R = indicator of response to 1st-line treatment
  - X1 = indicator of 1st-line treatment
  - X2 = indicator of 2nd-line treatment
  - y = indicator of response at 9 months
  - id = participant ID

```
data newdata; set olddata;
   if R=1 then do;
         ob=1; X2=0; weight=2; output;
```

```
            ob=2; X2=1; weight=2; output;
            end;
      else if R=0 then do;
            ob=1; weight=4; output;
            end;
   run;

   proc genmod data=newdata descending;
      class id;
      model y = X1 X2 X1*X2 / dist = poisson
                              link = log
                              ;
      scwgt weight;
      repeated subject=id / type=ind;
      estimate 'DTS1' int 1 X1 0 X2 0 X1*X2 0 / exp;
      estimate 'DTS2' int 1 X1 0 X2 1 X1*X2 0 / exp;
      estimate 'DTS3' int 1 X1 1 X2 0 X1*X2 0 / exp;
      estimate 'DTS4' int 1 X1 1 X2 1 X1*X2 1 / exp;
      contrast 'globalnull' X1 1,
                            X2 1,
                            X1*X2 1 / e;
   run;
```

- The 'estimate' statements will provide estimates of the probability of adherence (response) for each of the 4 embedded DTS, along with 95% CIs.
- The 'contrast' statement is a test of the global null hypothesis that the 4 embedded DTS are equal. The 'e' option will produce the contrast matrix, which should be checked to ensure the appropriate test is being done.

2) We will also include inverse probability weights to account for censoring if overall loss to follow-up in the full sample is >10% by month 9 or if differential loss to follow-up between the two arms is >10%.
3) Secondary analysis: We will conduct a modified intent-to-treat analysis using the same procedures described above (i.e., the weighted and replicated approach) but restricting to participants who returned for at least one study visit between months 1 and 3.
    - Since this analysis breaks randomization, we will adjust for important prognostic variables (including demographics, sexual behavior factors, and HIV risk factors) to account for treatment imbalances.

**Secondary Analysis 1: Evaluate (1) the effect of the mHealth interventions on early PrEP adherence (month 2), (2) the effect of the mHealth interventions on long-term PrEP adherence (month 12) and (3) the effect of the additional adherence support interventions (second-line interventions) on long-term PrEP adherence (month 12) separately for (a) non-responders to the first-line SMS intervention and (b) non-responders to the first-line WhatsApp intervention**

*Analysis population:*

- All individuals randomized to two-way SMS or WhatsApp groups who have drug levels available at month 2 and/or month 12.

- All individuals randomized to either monthly counseling or drug-level feedback who had drug levels available at month 12.

*Outcome:* PrEP adherence at the 2- and 12-month study visits, defined as a binary outcome variable around TFV-DP levels of 700 fmol/punch

- We will also conduct secondary analyses with PrEP adherence measured as continuous TFV-DP levels and will conduct descriptive summaries of PrEP adherence measured as a five-category variable: BLQ, BLQ-349 fmol/punch, 350-699 fmol/punch, 700-1249 fmol/punch, and ≥1250 fmol/punch.

*Predictor(s) of interest:* Randomized arm (SMS vs. WhatsApp groups; drug level feedback counseling vs. monthly counseling)

*Statistical analysis:* We will assess the short-term and long-term effects of the intervention on PrEP adherence using the same methods as described for the primary outcome (primary analyses 1 and 2). Shorter term efficacy will be estimated by comparing month 2 TFV-DP levels between primary randomization groups. Longer term efficacy will be estimated by comparing month 12 TFV-DP levels between primary randomization groups and also between secondary randomization groups, among non-responders to the first-line intervention.

**Secondary Analysis 2: Create models to estimate the probability of adherence based on sociodemographic factors, individual- and partner-level characteristics, risk practices, and study arm.**

*Analysis population:* All enrolled study participants

*Outcome:* PrEP adherence the 9-month study visit, defined as a binary outcome variable around TFV-DP levels of 700 fmol/punch

*Predictor(s) of interest:* Demographics (e.g., age, education), sexual behavior, sexually transmitted infections, perceived risk of HIV, HIV-related stigma, self-efficacy to use PrEP, relationship power dynamics, transactional sex, intimate partner violence, depression, traumatic stress symptoms, alcohol use, treatment arm.

*Statistical analyses:*

1) We will construct risk prediction tools to estimate the probability of adherence at 9 months based on the predictors listed above. We will fit Poisson regression models with all predictors of interest and binary 9-month adherence (TFV-DP >= 700 fmol/punch) as the outcome. We will construct two risk prediction tools:
    i. **Baseline tool:** this model will include all participants, and so will be applicable to the population of AGYW in South Africa. Here, the treatment arm variable will simply be an indicator of SMS/WhatsApp. All predictors will be measured at baseline.
    ii. **Non-responder tool:** this model will include those individuals who did not respond to first-line treatment, so it will be generalizable to this subgroup of the population. In this model, the treatment arm variable will be an indicator of DLFB/enhanced counseling. We will also include first-line

treatment. This model will use the most recent values of the predictors of interest (i.e., the most recent available at the time of rerandomization).

2) We will evaluate model performance by estimating the area under the receiver operating characteristic curve (AUC) and the calibration intercept and slope. These measures will be adjusted for resubstitution bias via the bootstrap (see Harrell, *Regression Modeling Strategies*).
3) There is very little missingness of predictors of interest at baseline. Thus, the first model ("baseline tool") will be based on a complete case analysis, and the second model ("non-responder tool") will be based on last observation carried forward for participants with baseline data available; those without baseline data will be excluded.

*Code for risk prediction model fitting and performance assessment*

```
library(rms)

## Simulate data with 200 observations, 3 predictors (x1, x2, x3), and
outcome y
set.seed(17)
x1 <- rnorm(200, 0, 1)
x2 <- rnorm(200, 1, 1)
x3 <- rnorm(200, -1, 1)
lp <- -2 + 0.5*x1 + 1*x2 - 0.5*x3
expit <- function(x) exp(x)/(1+exp(x))
y <- rbinom(200, 1, expit(lp))

fit <- lrm(y ~ x1 + x2 + x3, x=TRUE, y=TRUE) ## fit prediction model with 3
predictors (logistic regression model)
pentrace.fit <- pentrace(fit, seq(0, 100, by=0.5)) ## search for best
penalty
fit.penal <- update(fit, penalty = pentrace.fit$penalty) ## update model
with best penalty to get penalized model

set.seed(29)
validation_rslt <- validate(fit.penal, bw=F, B=1000, Dxy.method="somers2")
## validate penalized model
validation_rslt[3,5] ## calibration intercept
validation_rslt[4,5] ## calibration slope
validation_rslt[1,5]/2 + 0.5 ## AUC
```

**Secondary Analysis 3: Assess the degree of prevention-effective adherence and factors associated with prevention-effective adherence.**

*Analysis population:* All enrolled study participants

*Outcome:* Prevention-effective adherence

*Predictor(s) of interest:* Demographics (e.g., age, education), sexual behavior, sexually transmitted infections, perceived risk of HIV, HIV-related stigma, self-efficacy to use PrEP, relationship power dynamics, transactional sex, intimate partner violence, reported side effects, social harms, serious adverse events, depression, traumatic stress symptoms, alcohol use

*Statistical analyses: The planning for this analysis is in the preliminary stages. The SAP will be updated with more details when available.*

**Secondary Analysis 4: Characterize SMS response rates and the content and frequency of use of WhatsApp groups.**

*Analysis population:* All enrolled study participants in either the SMS or WhatsApp groups (will assess outcomes separately for each group)

*Outcome:* SMS response rates, WhatsApp group engagement

*Statistical analyses:*

1) We will compute descriptive statistics to summarize the engagement in SMS messaging and WhatsApp groups over time in the study, defined as follows:
    a. Level of engagement in SMS intervention: 1 = successful completion of an SMS contact in a given week, defined as either successful response of "yes" to an SMS message or a successful phone call attempt after either a "no" response or the first non-response after a prior response. 0 = an unsuccessful phone call attempt after a "no" or first non-response in a given week or participant removed themselves from the SMS messages.
    b. Level of engagement in WhatsApp groups: 1 = enrolled in a WhatsApp group for a given week; 0 = removed from a WhatsApp group in a given week.
2) We will also compute descriptive statistics to describe the total number of SMS and WhatsApp messages sent on average each week, the number of participants responding to the WhatsApp messages during a given week, and any differences in WhatsApp activity by group number (e.g., WhatsApp group 1 versus WhatsApp group 2).
3) We will conduct thematic content analysis to identify key themes across the different WhatsApp groups and time in the study. Potential themes include: PrEP pill-taking, PrEP reminder messaging, relationship dynamics, job resources, and sexual health.

**Exploratory Analysis 1: Assess HIV incidence and presence of detectable TFV-DP in PrEP users who acquire HIV infection during the study.**

*Analysis population:* All enrolled study participants

*Outcome:* HIV seroconversion following study enrollment, proportion of HIV seroconverters who had detectable TFV-DP levels at their last study visit prior to seroconversion

*Statistical analysis:* We will compute descriptive statistics to assess the number of seroconversions, HIV incidence over follow-up, and TFV-DP levels at the last visit before seroconversion and at seroconversion (separately) for those who seroconvert. We will report aggregate data and data by primary and secondary randomization arm (among non-responders to first-line intervention).

**Exploratory Analysis 2: Describe antiretroviral drug resistance among women who acquire HIV infection.**

*Analysis population:* All enrolled study participants

*Outcome:* Mutations in first sample after HIV seroconversion

*Statistical analysis:* We will compute descriptive statistics to assess the frequency of resistance mutations at first visit after seroconversion. We will report aggregate data and data by primary and secondary randomization arm.

**References**

Kidwell et al. “Design and analysis considerations for comparing dynamic treatment regimes with binary outcomes from sequential multiple assignment randomized trials.” *J Appl Stat*, 2018.

Oetting et al. “Statistical methodology for a SMART design in the development of adaptive treatment strategies.” In “Causality and Psychopathology: Finding the Determinants of Disorders and their Cures,” Shrout, Keyes, and Ornstein, eds. 2011.