Statistical Analysis Plan

Study Title: Optimizing viral load suppression in Kenyan children on antiretroviral therapy (Opt4Kids)

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Introduction: This statistical analysis plan (SAP) describes the analyses to be performed for Aims 1 and 2 of the Opt4Kids study. Decisions made in this plan were finalized prior to unblinding study results unless otherwise noted.

Study Background:
As many as 40% of the 1 million children living with HIV (CLHIV) receiving antiretroviral treatment (ART) in resource limited settings have not achieved viral suppression (VS). Kenya has a large burden of pediatric HIV with an estimated 105,000 CLHIV. Feasible, scalable, and cost-effective approaches to ensure VS in CLHIV are urgently needed. The goal of this study is to determine the feasibility and impact of point-of-care (POC) viral load (VL) and targeted drug resistance mutation (DRM) testing to improve VS in children on ART in Kenya**.**

Study Objectives:
Our leading objective is to determine the impact of POC VL by comparing the proportion of children on ART achieving VS 12 months after study enrollment (Aim 1a) and time to VS among those not suppressed or initiating ART (Aim 1b) between the study arms. We hypothesize that the proportion of children virally suppressed will be higher in the intervention vs. control arm.

Our secondary objectives are to determine the impact of targeted DRM testing and patterns of DRMs among children on ART without VS (Aim 2) and better understand how POC VL and targeted DRM may be scaled for programmatic use, via key informant interviews (Aim 3a) estimate programmatic costs and project incremental cost-effectiveness of the intervention (Aim 3b).

Study Design:
Opt4Kids is an open-label randomized, controlled study to pilot the use of POC VL testing and targeted DRM testing among children with HIV on ART age 1-14 years over a 12-month period in high-volume HIV treatment facilities in Kisumu, Kenya. At each facility, eligible children will be randomized 1:1 to either receive the intervention testing, consisting of quarterly POC VL testing and targeted DRM testing, or standard-of-care (SOC) testing based on the existing Kenyan national guidelines. For each child, we will follow the viral outcomes to 12 months after enrollment and compare VL suppression rates.

Study setting
The study is being conducted Kisumu County, western Kenya at low-resource, high-volume, high-HIV burden primary health care facilities. These government facilities are supported by the Ministry of Health (MOH), Kenya and HIV implementing partners funded by PEPFAR/CDC.

Comprehensive pediatric HIV care and treatment services are provided in accordance with the Kenyan MOH ART Guidelines. Children diagnosed with HIV are initiated on ART regardless of CD4 count per national guidelines. Routine VL monitoring is offered through a laboratory network connected to a national, centralized laboratory and is recommended for children after six months of continuous ART and every six months thereafter for those with VL <1000 copies/mL. Management of children with VL > 1000 copies/mL includes enhanced adherence counseling and repeat of VL testing in three months followed by possible switch of ART for those who are still not virologically suppressed. Only those children on 2nd or 3rd line ART who continue to have viremia after adherence optimization, undergo DRM testing at a national reference laboratory. This testing process requires

approval by the Kenyan MOH HIV ART treatment committee, who also guides the local provider on clinical management.

Study population
The study population was recruited from CLHIV ages 1-14 years newly initiating or already receiving ART at the study facilities. Infants <1 year of age were not included in the study as they frequently require more than 6 months of ART to suppress their initially high VL, and thus require specialized interpretation and management of VL results.

Eligibility criteria include:
- Children aged 1-14 years living with HIV (documented HIV positive or HIV VL)
- Already on ART or newly initiating ART

Randomization, allocation, and blinding
We randomized study participants 1:1 to the intervention vs. control arms using a blocked randomization scheme with varying block sizes, stratified by facility site and age groups (Group A: 1-9 or Group B: 10-14 years of age). Study staff ensured fidelity to the arm allocations by being the only ones able to order a POC VL. All investigators were blinded but participants, site coordinators, and the data team were not blinded to the randomization procedures.

Power calculations
Power for the study is based on the primary outcome of proportion of VS in children in the intervention vs. control arms at 12 months after enrollment for each child (Aim 1). We estimated requiring a sample size of 90% (630) of an estimated 700 children eligible to enroll to have 80% power to detect an effect size of a difference between arms of at least 11%. Accounting for 10% loss to follow-up over 12 months, this sample size is expected to provide total of 567 children (or 284 per arm) with outcomes for analysis. These calculations are based on Fisher's exact test, two-sided α=0.05, and initial VS rates of approximately 65% (estimated from historical facility data).

Table 5: Effect sizes and power calculations

VS rates, control (SOC) vs. intervention (POC) arms	Difference (effect size)	Power	Expected Total N for analysis	Expected N per arm for analysis
65% vs. 70%	5.0%	0.22	567	284
65% vs. 72.5%	7.5%	0.45	567	284
65% vs. 75%	10%	0.71	567	284
65% vs. 76%	**11%**	**0.80**	**567**	**284**
65% vs. 77.5%	12.5%	0.89	567	284
65% vs. 80%	15%	0.98	567	284
70% vs. 80%	10%	0.76	567	284
70% vs. 82.5%	12.5%	0.93	567	284
70% vs. 85%	15%	0.99	567	284

A power calculation was also performed for how precisely the prevalence of DRMs could be estimated. With 284 participants randomized to the intervention arm, we expected approx. 35% (100) to not achieve VS at their 1st POC VL test and undergo DRM testing, and we would be able to estimate prevalence of DRMs to within +/- 5% to 10% of the 95% CIs. For example, for a class of DRMs with prevalence of 80%, we anticipate generating exact 95% CIs of 71.3%-87.0%; a less common DRM at 10% will generate 95% CIs of 5.1%-17.1%.

Outcome Variables

Aim 1 primary outcome

Our study's primary outcome is the child's VS (defined as VL<1000 copies/mL) at 12 months after enrollment (defined as from a blood draw within 12 months +/- 16 weeks after the enrollment date). VL quantity is determined using results from the study's POC HIV VL GeneXpert test result which was planned for both randomization groups at the 12-month time point. Where a participant has no POC result available at 12 months but does have a standard laboratory VL available at 12 months, the standard laboratory VL is used. A month is considered to be 28 days.

Aim 1 VL sensitivity analysis outcomes

VS outcomes for sensitivity analyses of our primary comparison are the same as the primary VS outcome but use lower VL cutoffs of suppression (<40 copies/mL, which was the lower limit of detection for both the study POC and some standard lab-based VL assays, and <400 copies/mL, which is the operational VS threshold at which clinical management for potential non-VS changes based on updated Kenyan and WHO guidance).

Aim 1 Secondary outcomes

Secondary study outcomes for Aim 1 include process outcomes used to understand implementation of POC VL testing; these are:

- Uptake, defined as whether the study POC VL test was performed at the study visits (intervention group only)
- Turn-around time for the VL testing results at each study visit (intervention group, and if available, SOC group)
- Retention in care (both groups)
- ART switches (both groups)

Aim 2 outcomes

The study DRM testing outcomes include:

- Frequency of DRM test being performed, among tests at which child is not virally suppressed (test result of VL≥1000 copies/ml)
- Frequency and proportion of DRM tests showing any major DRM: major mutations within each class of HIV drugs, e.g., NRTIs, NNRTIs, and PIs.
- Among children tested for DRM,
    - frequency recommended to change regimens
    - among those recommended to change, frequency who actually did so by month 12.

Exposure Variables

Consistent with principles of intent-to-treat analysis (ITT), the primary exposure in this trial is randomly assigned intervention group. Children are analyzed in the group to which they were randomly assigned regardless of post-randomization events, such as the caregiver or child refusing the intervention, or a child being allocated in practice to the incorrect arm.

General statistical considerations

Binary variables will be described with frequency and proportion (as a percentage); continuous outcomes will be described with median (IQR), with IQR represented as (Q1, Q3). Statistical testing will be at the 0.05 significance level (α=0.05).

Missing Data
Analyses will be complete case analysis, meaning we will not impute VL outcomes for participants who do not contribute an observed VL measurement at 12 months.

Statistical Analyses
Enrolled population:
We will provide descriptive statistics for study population by randomization group for baseline demographics, disease, treatment, caregiver and household factor and facility characteristics in order to understand how well randomization worked to generate balanced groups. Baseline variables to be described are:

1) Demographics: age, sex
2) HIV disease factors: WHO clinical stage, VS 0-2 years prior to enrollment
3) ART treatment factors: age at ART initiation, years on ART, type of ART regimen
4) Caregiver factors: relation to child, marital status, educational attainment, parental HIV status, parental treatment status (ART), caregiver VS status, educational attainment
5) Household socioeconomic status (SES) factors: level of food insecurity; household commodities of electricity, radio, television, phone, quality of floor, quality of roof, number of rooms, quality of cooking fuel
6) Facility characteristics: type of hospital, rural vs urban, size of facility and size of HIV program in facility, as measured by monthly outpatient visits

Retention to study visits and intervention process outcomes:
We will provide descriptive statistics for study processes by randomization group for each study visit, where study visits are as assigned by study staff.

1) Retention to study visits (defined as attending a study visit among those expected to attend, where those who died or transferred to another clinic were not expected. Both in-person visits and phone visits at which questionnaires were obtained were considered attending the visit due to interruptions of regular clinic in-person visits due to COVID restrictions).
2) Uptake of POC testing (defined as whether POC test was performed at the study visit, among those attending the study visit).
3) Turn-around time for the VL testing results at each study visit (intervention group, and if available, SOC group), This is calculated as days from blood draw to when result was communicated to (a) participant; (b) provider. Median (IQR) days is presented, as well as frequency (%) reported within 24 hours, as would be expected to happen for POC tests.

VLs over time:
While not our primary outcome, interim descriptive statistics of VLs over time are provided for VS at baseline and quarterly thereafter. For participants in the POC group, POC test results are used. For participants in the SOC group, SOC test results are used. Testing over time is shown descriptively using testing intervals defined by days since enrollment: Enrollment = 0-2 years prior to enrollment; 3 months = 1 day to 3 months + 6 weeks after enrollment; 6 months = 6 months +/- 6 weeks; 9 months = 9 months +/- 6 weeks. This could differ from study visits as assigned by visit staff, for example if a 6 visit was attended over 6 weeks late it could fall in the 9-month interval. If more than one test had been performed in an interval, the test closest to the target date is used.

Study Aim 1. Effect of the intervention:
All analyses of the effect of the intervention are ITT, using randomized group assignment as the exposure variable as described in the Exposure Variables section (p3).

Aim 1a: We hypothesize that the proportion with VS one year after enrollment will be higher among children randomized to POC VL testing compared to those in the control group. Our primary analysis will compare the proportion of children with VS 12 months after enrollment as defined by our primary outcome in the intervention vs. control groups, estimating the RR for the intervention effect using a modified Poisson regression model with robust standard errors, adjusting for facility and age group strata (1-9 or 10-14 years). We provide supporting descriptive statistics of the primary outcome by randomized group. Sensitivity analyses using lower cutoffs for VS follow the same methods as the primary analysis except outcomes are as specified for sensitivity analyses of the primary outcome; they present an *a priori* sensitivity analysis comparing VS at 12 months defined as <40 copies/mL. A *post hoc* sensitivity analysis defines VS at 12 months as <400 copies/mL.

Subgroup analyses for the effect of the intervention will examine potential effect modification by age group (1-9 years old or 10-14 years old) using the same model as the primary analysis and including an interaction term between age group and intervention group in the model. While the study is not powered to test the intervention effect within age subgroups, particularly in the subgroup of 10–14 year-olds, this analysis will provide data for the intervention effect within each, as well as for any difference in effect between the younger and older children. Gender, time on ART (< 2 years, 2-5 years, or >5 years), VS status of the caregiver, and whether the caregiver is the biological parent are also *a priori* subgroup variables considered for effect modification, in each case by adding the main effect of the subgroup variable and the interaction term between subgroup variable and randomization group.

Aim 1b; we hypothesize that among children newly initiating ART or initially unsuppressed, the proportion with VS one year after enrollment will be higher among children randomized to POC VL testing compared to those in the control group. The analysis among children newly initiating ART or initially unsuppressed will follow the same methods as the primary analysis to compare the proportion of children with VS 12 months after enrollment as defined by our primary outcome except that VS will be defined as <400 copies/mL and the analysis will be limited to the population of children meeting our definition of newly initiating ART or initially unsuppressed. Children newly initiating ART are defined as those initiating ART within 30 days of study enrollment. Children initially unsuppressed are those with VS ≥400 copies/mL. for the POC group, at the initial POC VL test. Because in SOC only a subset were tested at enrollment, we used first VL test up to and including 6 months to identify SOC children who were initially unsuppressed (i.e., from enrollment testing interval to 6 month testing interval).

Subgroup analyses for the effect of the intervention among children newly initiating ART or initially unsuppressed are conducted for subgroups defined by age group, and for subgroups defined by children newly initiating ART vs. those already on ART but initially unsuppressed. For each, the effect of the intervention within subgroups is estimated by including the appropriate main effect and interaction term in the model.

Other associations with VS
Additional analyses including multivariate modified Poisson regression models will estimate associations between VS and all potentially related individual-level factors (such as age, sex, duration on ART, prior VS patterns (e.g., previously virally suppressed vs. not), and

family demographics), as well as facility factors (such as patient volume, staff volume, urban/rural location, and tier of facility), in order to explore predictors of VS in this context. We will also do a secondary analysis separating the outlined outcomes for children on 1st vs. 2nd line therapy.

Validation of POC HIV-1 VL tests

We intend to validate POC HIV-1 VL testing against current in-country gold standard VL testing by generating concordance/discordance rates and Cohen's kappa to determine assay agreement for VS using <1000 copies/mL. We will also conduct sensitivity analyses to determine concordance rates using lower thresholds of VL cut offs for VS (down to <40 copies/mL, the lower limit of quantification for the POC VL testing).

Study Aim 2 (DRM testing)

We hypothesize that we will find high levels of antiretroviral DRMs in this population, and both individual factors, such as prior exposure to antiretrovirals, and facility-level factors, such as facility type, will predict the presence of DRMs. We will describe the proportion of episodes of viremia (VL ≥1000 copies/mL) that were tested for DRMs by randomization group. Among those tests, we will also describe the proportion of samples that have any DRMs by HIV drug classes, e.g. NRTIs, NNRTIs, and PIs. We will report the proportion of samples with each type of mutation detected by drug class, and further group these mutations into major and minor ones. For example, we will examine major mutations in M184V/I and K65R for NRTIs, K103N, Y181C, G190A, and V106M for NNRTIs, and V82A, I76V, 184V, L47A, L90M, M46I, and D30N for PIs. We will report the frequency of drug regimen changes being recommended as a result of the DRM testing result, and, among those recommended to change, the proportion who actually do change prior to the month 12 visit.

We will use multivariate logistic regression models to identify risk factors associated with major and any DRMs. We will also explore how sociodemographic, behavioral, clinical, and facility factors may be contributing to the DRM patterns we observe.

ART regimens over time

The distribution of ART regimens at enrollment and each post-enrollment visit will be described by randomization group.

Study Aim 3

Study Aim 3 does not involve statistical summaries or analysis and is not covered in this plan.

Changes to the analysis plan:

Study Aim 1b: For this analysis among children initiating ART or not virally suppressed at enrollment, we originally planned to compare time to VS using a Cox regression model by randomization group. Although VS was expected to be assessed on different schedules for the intervention arm (3-monthly) versus control arm (6-monthly unless unsuppressed and then 3-monthly until suppressed), because both arms are to test quarterly in those not suppressed, we expected the assessment plan to be similar in intervention vs control groups for those initially unsuppressed in spite of the overall difference in testing frequency.

A number of adjustments were made to this analysis. All were made prior to unblinding this analysis unless otherwise specified. First, we noted that post-enrollment POC testing in the

POC group was not consistently performed quarterly due to COVID interruptions either delaying or preventing in-person visits, especially at study months 6 and 9. Also, for SOC children, the routine testing schedule did not necessarily align with study enrollment, resulting in SOC VLs performed at enrollment only for a subset of SOC children, and subsequently, SOC was not necessarily performed quarterly. Because in neither arm was time to VS measured consistently, we changed from comparing time to VS by estimating a hazard ratio from Cox regression, to comparing VS at 12 months by estimating a relative risk from a modified Poisson regression so that our outcome is measured in the same manner in both groups. Second, because in SOC only a subset were tested at enrollment, we used first VL test up to and including 6 months to identify SOC children who were initially unsuppressed. Third, we defined the cutoff for being initially unsuppressed and for achieving VS after enrollment to be based on 40 copies/mL to allow us to explore the question of value of our intervention in those not clearly initially suppressed. After unblinding, we discovered that enrollment VL data in those with both POC and SOC tests showed that POC tests missed a noticeable number of those with VL>40 by SOC, but not in those with VL>400 copies/mL; to reduce any bias from differences in the tests we changed our plan to use a cutoff of 400 copies/mL both for identifying initially unsuppressed children and for the VS outcome at 12 months for this analysis.

Study Aim 2: To estimate the effect of providing timely DRM results on VS for intervention participants undergoing DRM, separately from the effect of POC alone, we planned a secondary analysis with outcome of time to VS using a Cox model with primary predictor of intervention vs. control arm. To distinguish the effect of DRM results from that of POC VL testing, we planned to add a time-varying covariate which indicates, for each visit with a VL test, whether the clinician was notified of a positive DRM result since the child's prior VL test. If implemented as expected, all children without VS will be provided DRM testing and providers notified of the results. This model would allow us to divide the estimated effect of the intervention between the effect of POC VL testing alone (on those who do not have DRM), and the effect of POC VL testing plus DRM testing for those who do undergo DRM testing. In practice, however, almost no participants in the SOC group were given DRM testing while nearly all those ever unsuppressed in the POC group were; DRM testing was essentially part of the intervention and could not be disentangled from POC testing. We instead describe the rate of DRM testing by randomization group, the rate of major DRMs found, and what clinical recommendations followed from DRM testing and whether implemented by the end of the study.