STATISTICAL ANALYSIS PLAN FOR HVTN PHARMACOKINETICS AND FUNCTIONAL ACTIVITIES

Protocol HVTN 128 (v1.0)

A phase 1 clinical trial to evaluate the safety and pharmacokinetics of VRC-HIVMAB075-00-AB (VRC07-523LS) in the sera and mucosae of healthy, HIV-1–uninfected adult participants

Date finalized for signature: 19 February 2025
Effective Date: Date of last signature
SAP version: 4.0

Statistical Analysis Plan for Pharmacokinetics and Functional Activities

Protocol: HVTN 128 (v1.0)

Document will become effective on date of last signature.

Author	
Legal Name	Shiyu Chen
Job Title	Statistical Research Associate III
Signature & Date	

Approval	
Legal Name	Ollivier Hyrien
Job Title	Professor
Signature & Date	

SAP Modification History

The version history of, and modifications to, this statistical analysis plan are described below.

SAP Version	Modification
1.0	Initial
2.0	Adding ADA
3.0	Adding PK-SMC Assay
4.0	Modifying PK-SMC Assay

Table of Contents

1 OVERVIEW

This document describes the Statistical Analysis Plan (SAP) for the analysis of pharmacokinetics data from HVTN 128. The plan will be reviewed and updated prior to additional immunogenicity analyses and before the final safety analysis, with all major revisions of the plan archived.

2 PROTOCOL SUMMARY

2.1 Title

A phase 1 clinical trial to evaluate the safety and pharmacokinetics of VRC-HIVMAB075-00-AB (VRC07-523LS) in the sera and mucosae of healthy, HIV-1–uninfected adult participants

2.2 Design

Multicenter, randomized, unblinded trial

2.3 Study products

- VRC07-523LS: VRC-HIVMAB075-00-AB (VRC07-523LS) is a human monoclonal antibody (mAb) targeted to the HIV-1 CD4 binding site. It was developed by the VRC/NIAID/NIH and manufactured under current Good Manufacturing Practice regulations at the VRC Pilot Plant operated under contract by the Vaccine Clinical Materials Program, Leidos Biomedical Research, Inc., Frederick, MD. VRC07-523LS will be supplied as 10 mL glass vials with a 6.25 ± 0.1 mL fill volume and 3 mL glass vials with a 2.25 mL ± 0.1 mL fill volume, at a concentration of 100 ± 10 mg/mL.

2.4 Study population

24 healthy, HIV-1–uninfected volunteers aged 18 to 50 years; 24 study product recipients

2.5 Study plans and schema table

Group	N*	Route	VRC07-523LS Dose	Product administration Schedule		
				D0	D112	D224
				W0	W16	W32
1	12	Intravenous (IV) infusion	10 mg/kg	X	X	X
2	12	Intravenous (IV) infusion	30 mg/kg	X	X	X
Total	24					

IV = intravenous infusion

N* = number of participants receiving at least one product infusion

2.6 Duration per participant

12 months of scheduled clinic visits followed by biopsy safety contact 2 weeks after the last scheduled clinic visit

2.7 Estimated total study duration

17 months (includes 5 months for enrollment, and 12 months of scheduled visits)

2.8 Study Sites

HVTN Clinical Research Sites (HVTN CRSs) to be specified in the Site Announcement Memo.

3 OBJECTIVES AND ENDPOINTS

3.1 Primary objectives and endpoints

Primary objectives 1:

- To evaluate the safety and tolerability of VRC07-523LS administered at 10 mg/kg IV infusion every 4 months
- To evaluate the safety and tolerability of VRC07-523LS administered at 30 mg/kg IV infusion every 4 months

Primary endpoint 1:

- To determine whether multiple infusions of VRC07-523LS reach and maintain detectable levels in the mucosa

Primary objective 2:

- To determine whether multiple infusions of VRC07-523LS reach and maintain detectable levels in the mucosa
- To correlate levels of VRC07-523LS in serum and the mucosa

Primary endpoints 2:

- Levels of VRC07-523LS in genital and rectal secretions, as well as cervical, vaginal, and rectal tissues at the collection timepoints
- Levels of VRC07-523LS in serum out to Week 48, 16 weeks after the last product administration

3.2 Secondary objectives and endpoints

Secondary objective 1:

- To compare dose regimens of VRC07-523LS delivery for their ability to sustain mucosal mAb levels at serial timepoints post repeat administration

Secondary endpoint 1:

- Levels of VRC07-523LS in genital and rectal secretions and tissue at specified timepoints

Secondary objective 2:

- To determine whether ADA can be detected in serum

Secondary endpoint 2:

- Serum concentration of ADA in each group measured at multiple timepoints from baseline through the final study visit

3.3 Exploratory objectives

Exploratory objective 1:

To assess the acceptability and tolerability of repeat mucosal sampling, including secretions and biopsies, for application in future studies

Exploratory objective 2:

To develop predictive mucosal population pharmacokinetic models of VRC07-523LS administered IV

Exploratory objective 3:

To conduct analyses related to furthering the understanding of HIV, monoclonal antibodies, immunology, vaccines, and clinical trial conduct

4 COHORT DEFINITION

Participants will enroll in the study at the first biopsy collection. Only participants who remain in the trial after completion of the first biopsy visit will be randomized. Since enrollment is concurrent with the first biopsy sample collection, all enrolled participants will provide some data regarding the tolerability of mucosal sample collections, regardless of if they are randomized.

Since randomization followed with receiving the first study product administration, all randomized participants will provide some data regarding the safety of the study product. Participants who are randomized but do not receive the study product are released from randomization, so the randomized cohort will consist only of participants who received study product and contribute some data regarding the safety of the study product.

All safety data from randomized participants will be analyzed according to the initial randomization assignment regardless of how many infusions they received. The analysis is a modified intent-to-treat analysis in that individuals who are enrolled but not randomized do not contribute immunogenicity data about the study product and hence are excluded.

5 RANDOMIZATION

Participants will enroll in the study at the first biopsy collection. Only participants who remain in the trial after the completion of the first biopsy visit will be randomized.

Accrual will continue until 24 participants have received first product administration.

The randomization sequence will be obtained by computer-generated random numbers and provided to each HVTN CRS through the SDMC via a Web-based randomization system.

Groups 1 and 2 will be randomized simultaneously.

The randomization will be stratified by assigned sex at birth and done in blocks to ensure balance across both groups.

6 BLINDING

Participants and site staff will be unblinded as to participant treatment group assignments. VRC07-523LS concentration and ADA assessments will be performed in a blinded fashion.

7 STATISTICAL ANALYSIS

This section describes the final study analyses, unblinded as to treatment arm assignment. All analyses pertaining to safety and drug levels objectives of the study will be conducted with an intent-to-treat analysis that includes all randomized individuals per their randomization allocation. Additional analyses will be performed that account for the actual infusions and dose levels that each participant received. Analyses for primary endpoints will be performed using SAS and R. Additional software may be used to perform non-compartmental PK and population PK analyses (eg, Monolix). All other descriptive and inferential statistical analyses will be performed using SAS, or R statistical software.

No formal multiple comparison adjustments will be employed for multiple primary or secondary endpoints. Unless otherwise noted, all statistical tests will be 2-sided and will be considered statistically significant if $p < 0.05$.

8 PHARMACOKINETICS TABLES AND FIGURES, BY ASSAY

8.1 Serum Concentration and Pharmacokinetics Analysis

VRC07-523LS levels will be measured in serum collected at the designated timepoints and in female and/or male assigned sex at birth.

The method for detection of VRC07-523LS utilizes Carboxylated magnetic beads (Magplex, Luminex) are covalently coupled to NeutrAvidin and are then subsequently bound to a biotinylated goat anti-mouse IgG Fc antibody. This is then followed by the binding of the 5C9 anti-idiotype monoclonal. Through this process, 5C9 is oriented to freely bind to the paratope of the VRC07- 523LS. The sample is then added to the coated beads and incubated for 120-125 minutes in a plate shaker. At the end of the incubation, plates are washed using BAMA wash buffer. Bound VRC07-523LS is detected using a goat anti-human IgG Fc, multi-species serum protein (SP), conjugated to phycoerythrin (PE). Microspheres are read on a Bioplex 200

instrument to measure Fluorescence Intensity (FI). Mean Fluorescence Intensity (MFI) values are used to quantify VRC07-523LS that has been bound to 5C9 anti-idiotype antibody in solution VRC07-523LS IgG standard curves are run on each plate to quantify VRC07-523LS IgG in human serum and mucosal test samples. Standard curves are fit to a 5-PL logistic regression to convert MFI of samples to VRC07-523LS IgG mAb concentration. Duplicate wells containing HIV-1 seronegative normal human reference serum and negative control beads are also run in each assay to ensure specificity of binding.

All samples will be shown in the plot, including concentrations below the lower limit of quantification (LLoQ). Samples with concentrations above the LLoQ at a 1:100 dilution will be further tested at various dilution factors to obtain MFI's in the linear range of the standard curve, and the in-well concentration closest to the EC50 of the 5PL standard curve will be reported.

Several criteria will be used to determine if data from an assay are acceptable and can be statistically analyzed. The standard curve EC50 values and MFI values will be tracked against historical data using Levey-Jennings charts and points with an MFI > 100 must have a %CV <20% between replicates. Any sample without at least 2 observed concentrations in agreement with each other, or with baseline MFI > 1000 will be repeated to obtain an accurate measurement.

The primary analysis will be restricted to participants who received all scheduled administrations as per protocol. This analysis will exclusively use data from study participants who received the study product at the intended dose level, during the specified scheduled visit window, and at the scheduled visit, as specified in the protocol, by the time serum samples were collected. Other statistical analysis (e.g., population PK) may be conducted on data from randomized participants according to the initial randomization assignment regardless of how many administrations they received.

Serum specimens are collected before the first study product administration (visit 2, day 0), 2 weeks after the first study product administration (visit 4, day 14), at the second study product administration (visit 5, day 112), 2 weeks after the second study product administration (visits 6, day 126), at the third study product administration (visit 7, day 224), 2 and 16 weeks after the third study product administration (visit 8 and visit 9, respectively).

Statistical analysis of drug level data will be performed on VRC07-523LS concentrations or normalized concentrations in the serum. The descriptive statistics of serum and mucosal mAb concentration will include their geometric mean and associated 95% confidence intervals based on a *t*-distribution, as well as their median and Interquartile Range (IQR). These summary statistics will be tabulated by timepoint and treatment group. Spaghetti plots will be used to display participant-level concentrations as a function of the study day, with the treatment group median and IQR overlaid on top of the participant-level dots, by timepoint, treatment group. Serum concentrations may also be presented as box/scatter plots, by timepoint or study visit, treatment group, and tissue type. Observations will be colored to indicate the treatment group and assigned sex at birth.

Serum concentration data that appear unreliable, from specimens collected outside of the visit window, or from HIV-infected participants post-infection may be excluded. In these analyses, serum concentrations below the lower limit of quantification (LLoQ) will be replaced by half the LLoQ. Concentrations below the LLoQ will be set to 0 mcg/mL.

8.1.1 List of Tables

- Table 1, Counts of concentrations > LLoQ

- Table2, Descriptive statistics (i.e., sample size (N), geometric mean, median, min, max and IQR) of participant-level mAbs concentrations by treatment groups, visits, among the per protocol cohort, including values below the LLoQ.
- Comparison of dose-normalized concentrations between groups within time points

8.1.2 List of Graphs

- Figure 1: Boxplots of the VRC07-523LS concentrations by visit day and treatment groups. Within each treatment group panel, boxplots of VRC07-523LS concentrations will be displayed side by side against the visit day.
- Figure 2: Spaghetti plots of VRC07-523LS concentrations by treatment group over visit day at the individual- and at group-level (median and geometric mean). VRC07-523LS concentrations from each treatment group will be overlaid in the same panel with different colors. Individual-level concentrations will be displayed in grey lines and group-level in solid colored lines.
- Figure 3: Spaghetti plots and boxplots of does-normalized VRC07-523LS concentrations by treatment group over visit day.

8.2 Anti-Drug Antibodies (ADA)

To address Secondary Objective 2 of the study, anti-drug antibody (ADA) detection and characterization assays will be run to evaluate whether infusion of VRC07-523LS may induce an antibody response that target the study product and/or whether some subjects exhibit anti-drug reactivity prior to infusion, for each desired participant specimen of the study protocol.

Antidrug antibodies (ADA) are most typically detected and characterized using a tiered testing strategy. In Tier I, a sensitive binding assay is used to determine if samples may have ADA present. In Tier II, the response is confirmed, typically by establishing the specificity of the response by competition with free drug. In Tier III, the response is characterized, typically with a neutralization reduction assay and/or a titering assay. For Tiers I and II as well as the titering assay, “bridging” assay formats are amongst the most common.

A bridging assay to detect ADA against a biologic drug product begins with covalently conjugating drug product with either biotin or the Sulfo-Tag label. Biotinylated and Sulfo-Tagged mAb are then combined and mixed with serum that may contain ADA. When an ADA response is present, complexes comprised of the ADA and one or both types of labeled mAb species can form. In some cases, because antibodies are multivalent, the ADA will act as a bridge between the biotinylated and Sulfo-Tagged drug in a ternary complex. Following an incubation to allow complex formation, this mixture is added to a proprietary streptavidin-functionalized plate, washed, and the presence of ADA responses, as indicated by the presence of ternary or “bridged” complexes, is detected. The Meso Scale Diagnostics (MSD™) instrument passes an electrical current through the plate, exciting any Sulfo-Tag within proximity of the plate surface, and resulting in electrochemiluminescence, which is expressed in relative light units (RLU). During assay qualification, a positivity cut point is established and utilized to determine whether a sample is ADA Screening Assay positive based on its RLU signal intensity. While only complexes containing both biotinylated and Sulfo-Tagged drug will result in signal, additional complexes are likely to form that are nonconductive to signal measurement (e.g. biotin:biotin or Sulfo-Tag: Sulfo-Tag bridged products). Formation of these nonproductive complexes has been taken into consideration during the development phase, and efforts have been made to maximize sensitivity by reducing the likelihood of formation of these nonproductive complexes. The criteria for establishing positivity cut points in the Tier I assay are designed to minimize the risk of false negatives. As such, a certain proportion of ADA-negative subjects will be classified as positive in

this screening assay but can later be investigated with Tier II confirmatory and, in some cases, Tier III characterization assays. The assays are all performed in 96well plates for high throughput capacity. The screening assay has been qualified, and the specificity and titering assays have been deemed fit for purpose.

All designated serum samples will be assayed at a 1:12 dilution of serum for Tiers I and II. In Tier III, titers will be defined as lowest fold dilution from a starting dilution of 1:12 at which the test sample remains above the assay cut point.

Screening assay

i. If the average value of a test sample exceeds the assay cut point, that sample is classified as Tier I positive.

ii. %CVs are calculated by the Discovery Workbench Software. If any control exceeds an average CV of 20%, an investigation of the run will be required. If any sample exceeds a CV of 20%, that sample will need to be rerun.

Confirmation assay

i. Tier 1 positive samples are retested in the presence of free drug. If free drug reduces signal by more than the specificity threshold, these samples are considered Tier II confirmed.

Titering assay

i. Test samples that are Tier II confirmed will be titered using the screening assay across a dilution series. The lowest fold dilution at which the test sample remains above the assay cut point is considered its titer.

The following evaluation criteria will be employed for screening, confirmatory and tittering assays:

i. %CVs must be below 20% for sample replicates

ii. Positive controls must exceed values established during qualification

ADAs will be categorized as treatment induced, boosted, or independent based on the longitudinal profile observed, with boosted responses defined by a 3-fold increase in titer from baseline. Any sample that was either Tier I or Tier II negative at baseline, then Tier II positive at a later timepoint is considered as induced ADA. Only samples that were ADA positive at baseline have their titers compared to follow up timepoints to determine if a response is boosted or independent.

The statistical analysis tabulates frequencies of serum samples in which ADA were detected, along with their 95% confidence intervals, by treatment group, timepoint, and ADA assay tier. The analysis will also report summary statistics (e.g., mean, median, range, as appropriate) of ADA levels among samples with detectable ADA levels.

If ADA is detected in more than one sample, the percentages of participants with detectable ADA levels, along with their 95% confidence intervals, will also be plotted as a function of time by treatment group.

8.2.1 List of Tables

- Response rate and corresponding 95% confidence intervals using the Wilson score method by tier, visit and treatment arm.

- List of participants with Tier II confirmed positive responses.

8.2.2 List of Graphs

- Line plots of ADA Tier II confirmed response by treatment arm.
- Line plots of ADA Tier III titering responses for participants with Tier II confirmatory response.

8.3 PK-Single Molecule Counting (PK-SMC)

SMCxPro for VRC07-523LS concentrations in mucosal tissues and secretions

Pharmacokinetic - Single Molecule Counting (PK-SMC) assay will be used to measure passively infused VRC07-523LS broadly neutralizing monoclonal antibody in HVTN 128 participant mucosa (secretions and biopsies) using 5C9 anti-idiotype antibody (anti-ID).

This assay will address the testing necessary to satisfy applicable primary, secondary, and exploratory objectives & endpoints as described in the HVTN 128 Protocol.

i. Primary Objectives 2:

- To determine whether multiple infusions of VRC07-523LS reach and maintain detectable levels in the mucosa
- To correlate levels of VRC07-523LS in serum and the mucosa

ii. Primary Endpoints 2:

- Levels of VRC07-523LS in genital and rectal secretions, as well as cervical, vaginal, and rectal tissues at the collection timepoints
- Levels of VRC07-523LS in serum out to Week 48, 16 weeks after the last product administration

iii. Secondary Objective 1:

- To compare dose regimens of VRC07-523LS delivery for their ability to sustain mucosal mAb levels at serial timepoints post repeat administration

iv. Secondary Endpoint 1:

- Levels of VRC07-523LS in genital and rectal secretions and tissue at specified timepoints

v. Exploratory Objective 2:

- To develop predictive mucosal population pharmacokinetic models of VRC07-523LS administered IV

The SMCxPro assay is a highly sensitive sandwich-based immunoassay that uses a magnetic bead system akin to flow cytometry to detect very low concentrations (theoretically down to single molecules) of biomarkers such as monoclonal antibodies. It is the successor to the Singulex instrument system and operates on similar principles, but the instrument software and thus expected output format are significantly different from Singulex. In HVTN studies, this assay is typically used to assess the mucosal concentrations of administered monoclonal antibodies, as part of PK (pharmacokinetic) analyses. The ultra-sensitive capabilities of the SMCxPro instrument enables a high degree of sensitivity that allows for the analysis of proteins down to femtogram/mL levels. VRC07-523LS levels are normalized relative to total protein and/or total IgG concentrations. Hemoglobin measurements are used to perform quality control of mucosal secretions.

Using the proprietary SMC Capture Antibody Labeling Kit, anti-idiotype (anti-ID) antibody is labeled and then coated onto magnetic microparticle beads. Anti-human Fc secondary detection antibody of the appropriate isotype (ex: anti-human IgG1 Fc) is labeled with a fluorophore using the SMC Detection Antibody Labeling Kit. Labeled and coated microparticle beads are mixed separately with samples, standards, and controls and incubated to allow antibody capture. Captured monoclonal antibodies are detected using the labeled secondary detection antibody. This forms a sandwich complex with the target analyte between the magnetic bead anti-ID antibody and the labeled secondary detection antibody, comparable to a sandwich ELISA.

The magnetic properties of the microparticle beads are used to hold the beads in place for washing steps. After several rounds of washing to remove excess unbound reagents, the complexes are broken apart by the additional of an acidic elution buffer and the released detection molecules, which are now proportional in quantity to the analyte, are quantified by fluorescence detection using the SMCxPro instrument. The method is derived from the Singulex three-curve weighting system, but without the ability to export the separate curves and their weights for each assay point.

Study samples and controls are run in triplicate. Where possible, all of a participant's samples of a given specimen type are included in the same assay batch, in order to minimize batch effects on PK trajectories. Standard curves containing known concentrations of the target analyte in human mucosal test samples will be run on each plate to establish quantification criteria. These standard curves are fit to a 5-PL logistic regression to convert response readouts of samples to a derived mAb concentration. Triplicate wells containing HIV-seronegative human reference serum and negative control beads are also run in each assay batch as a negative control to ensure specificity of binding. Sample data is released only where both preset criteria are met: 1) Five consecutive standard curve points recover 70-130% of expected concentration. 2) Three consecutive spiked positive controls recover 70-130% of the expected concentration. Linear ranges vary by specific analyte and are available with standard curve data for each dataset. Results are reported in pg/mL.

PK-Binding Antibody Multiplex Assay (PK-BAMA) for VRC07-523LS concentration in serum

Pharmacokinetic-Binding Antibody Multiplex Assay (PK-BAMA) will be used to measure levels of passively infused VRC07-523LS broadly neutralizing monoclonal antibody in HVTN 128 participant sera using 5C9 anti-idiotype antibody (anti-ID).

One of the primary objectives of this study is to determine VRC07-523LS drug levels using the anti-idiotype PK-BAMA in serum collected at the designated timepoints.

Carboxylated magnetic beads (Magplex, Luminex) are covalently coupled to NeutrAvidin and are then subsequently bound to a biotinylated goat anti-mouse IgG Fc antibody. This is then followed by binding of the 5C9 anti-idiotype monoclonal. Through this process, 5C9 will be oriented to freely bind to the paratope of the VRC07-523LS. Sample is then added to the coated beads and incubated for 120-125 minutes in a plate shaker. At the end of the incubation, plates are washed using BAMA wash buffer. Bound VRC07-523LS are detected using a goat anti-human IgG Fc, multi-species serum protein (SP), conjugated to phycoerythrin (PE). Microspheres are read on a Bioplex 200 instrument to measure Fluorescence Intensity (FI). Mean Fluorescence Intensity (MFI) values are used to quantify VRC07-523LS that has been bound to 5C9 anti-idiotype antibody in solution.

VRC07-523LS IgG standard curves are run on each plate to quantify VRC07-523LS IgG in human mucosal test samples. Standard curves are fit to a 5-PL logistic regression to convert response readouts of samples to VRC07-523LS IgG mAb concentration. Duplicate wells containing HIV-1 seronegative normal human reference serum and negative control beads are also run in each assay to ensure specificity of binding.

ELISA for Hemoglobin (Hb) and protein concentration

The purposes of the hemoglobin (Hb) quantitation assays are to measure blood contamination in mucosal secretions. Oricol devices permit the collection of rectal secretions from the rectum, and vaginal secretions will be collected via Softcup collection. Integral to the detection of VRC07-523LS in secretions is the need to demonstrate that collections are free of contaminating blood, where the antibody is found at high concentrations. This study aims to provide early feedback to the sites regarding the levels of Hb in rectal secretions from multiple clinicians, to ensure the sample is optimal for the measurements of the primary endpoint.

Preliminary Results are determined using the ELISA Plate Reader (Molecular Devices) SpectraMax i3X absorbance (Hemoglobin) and fluorescence (protein concentration). Assays are assessed for QC using the standards ran on each plate.

The Quant-iT™ Protein Assay Kit (Invitrogen™) is used to measure the total protein in samples provided by study participants. The kit provides concentrated assay reagent, dilution buffer, and pre-diluted BSA standards. Simply dilute the reagent, load it into the wells of a microplate, add 1-20 μL of sample, mix, then measure the fluorescence. The assay is highly selective for protein and exhibits very little protein-to-protein variation. The assay is performed at room temperature, and the signal is stable for 3 hours. Common contaminants, such as salts, solvents, or DNA—but not detergents—are well tolerated in the assay.

Bio-Plex Pro™ Human Isotyping Assay for IgG concentration

The Bio-Plex Pro™ Human Isotyping Assay (Bio-Rad Laboratories, Inc.) is used to measure IgG in mucosal samples provided by study participants. The kit provides magnetic beads, detection antibodies, standards, controls, and reagents for detecting human IgG1, IgG2, IgG3, IgG4, IgA, and IgM. Samples are diluted in the provided kit buffer and mixed with the multiplex magnetic bead panel in a 96-well plate. Plates are washed with the kit-provided wash buffer, and secondary detection antibodies are added per the package insert instructions. Plates are read on a BioPlex 200 instrument to measure Fluorescence Intensity (FI). Mean Fluorescence Intensity (MFI) values are used to quantify isotype concentrations. Sample isotype concentrations are derived from the kit-supplied standard curve.

Specimens

A total of 24 participants and 504 secretion (semen, cervicovaginal, and rectal) samples (168 of each specimen type) will be tested at 7 timepoints (visits 2, 4, 5, 6, 7, 8 and 9) from Groups 1 and 2. A total of 24 participants and 168 serum samples will be also tested at 7 timepoints (visits 2, 4, 5, 6, 7, 8 and 9) from Groups 1 and 2. A total of 24 participants and 288 biopsy (colorectal, cervical and vaginal) samples (96 of each specimen type) will be tested at 4 timepoints (visits 2, 4, 8 and 9) from Groups 1 and 2.

Summary of sample numbers and specimen types proposed for testing is summarized in the table below:

No. of Participants	Specimen Type		Timepoint (visit)	Total no. of samples
24	Secretion	Semen	2,4,5,6,7,8,9	168
		Cervicovaginal	2,4,5,6,7,8,9	168
		Rectal	2,4,5,6,7,8,9	168

24	Biopsy	Colorectal	2,4,8,9	96
		Cervical	2,4,8,9	96
		Vaginal	2,4,8,9	96

Statistical endpoints

To account for heterogeneity in size of biopsies and absolute concentration (e.g., rectal secretions flushed with media during collection) of the samples, IgG- and protein-normalizations for mucosal samples are performed by dividing VRC07-523LS levels (pg/mL) by the total IgG (ng/mL) and protein concentrations (mg/mL), respectively, as following:

- VRC07-523-LS concentration normalized by total IgG (pg VRC07-523LS/ng total IgG):
  - Units: VRC07-523LS units are pg/mL, and total antibody units are ng/mL. Truncate as specified in the Truncation Rule section below.
  - Antibody normalized concentration = truncated observed concentration / truncated total antibody
- VRC07-523-LS concentration normalized by total protein (pg VRC07-523LS/ng total protein):
  - Units: VRC07-523LS units are pg/mL, and total protein units are mg/mL (milligrams per milliliter). To convert mg to ng, first truncate as specified in the truncation rule section below, then multiply total protein by $1x10^6$ (1,000,000).
  - Protein normalized concentration = truncated observed concentration / (10^6 x truncated total protein)

IgG-normalizations for serum samples are performed by dividing VRC07-523LS levels (µg/mL) by the average reference values for total IgG concentrations in serum in the USA (1165 mg/dL), as following:

- VRC07-523-LS concentration in serum normalized by total IgG (pg VRC07-523LS/ng total IgG):
  - Units: VRC07-523LS units are µg/mL, and total antibody units are mg/dL. Truncate as specified in the Truncation Rule section below, then divided total antibody by 100.
  - Antibody normalized concentration = truncated observed concentration / (10^{-2} x U.S. reference total antibody)

Truncation rules are applied for SMCxPro, serum, total IgG, and total protein data as following:

- SMCxPro truncation: For results under the smaller value of limit of detection (LLoD) and sample type specific limit of quantification (LLoQ), they are truncated at lower limit of quantification (LLoX) / 2 where LLoX is minimum(LLoQ, LLoD)
- serum truncation: Baseline serum concentrations below the lower limit of quantification (LLoQ = 45.7 ng/ml = 0.0457μg/ml) are replaced with LLoQ/2. No post-baseline concentrations are below the LLoQ.

- Total IgG truncation: Total IgG (total IgG = Hu IgG1 + Hu IgG2 + Hu IgG3 + Hu IgG4). Any missing subclass will be treated as zero for the purpose of calculating total IgG. If IgG1, IgG2, IgG3, and IgG4 subclasses are all missing for a sample, the sample will be treated as not collected/no sample recovered, and total IgG will be set to NA. Total IgG concentration will be compared to 48 ng/mL; if it is less than 48 ng/mL, the sample will be flagged in the analysis dataset and excluded from the analysis without truncation.
- Total protein truncation: Compared total protein concentration to 0.167 mg/mL, which will be the last curve point and considered as the LLoQ. If concentration < 0.167 mg/mL, then it will be truncated as 0.5 * 0.167 mg/mL

Body-weight adjustment will be performed by dividing the IgG- and protein-normalized VRC07-523LS levels by the body weight at baseline. Dose-amount adjustment will be performed by dividing the IgG- and protein-normalized VRC07-523LS levels by the body weight at baseline multiplied the dose amount of the products (T1: 10 mg/kg VRC07-523LS, T2: 30 mg/kg VRC07-523LS). The % penetration into different mucosal compartments will be calculated as the protein-normalized VRC07-523LS level in each of the mucosal specimen type divided by that in serum, multiplied by 100.

Pre-treatment participants and all secretion samples deemed hemoglobin-contaminated or unreliable (1. Total IgG concentration < 48 ng/mL, or 2. Total protein concentration < 0.167 mg/mL, with total IgG concentration also < 48 ng/mL) are excluded from the analysis.

Statistical methods

Tabular summaries of the distributions of body-weight-adjusted, IgG- and protein-normalized VRC07-523LS levels, and % penetration of protein-normalized VRC07-523LS is presented by specimen type, treatment arm, sex at birth and visit. Body weight comparisons between treatment arms (T1 vs. T2) and between sexes at birth (Female vs. Male) are presented using the Wilcoxon Rank Sum Test. A table of partial Spearman's rank correlation coefficients of specimen comparison and p-values, adjusted for treatment group (T1 vs. T2) and sex at birth (males vs. females), is presented by visit. P-values are adjusted for multiple comparisons by controlling the false discovery rate (FDR) using the Benjamini-Hocherg procedure [9] and the q-value [10]. Results are significant if the adjusted p-value or q-value $\leq$ 0.05.

Comparisons of dose-amount-adjusted, IgG-normalized VRC07-523LS concentrations between visits (v2 vs. v5, v2 vs. v7, v2 vs. v9, v4 vs. v6, v4 vs. v8, v6 vs. v8) are made using the Wilcoxon Signed-Rank test, and comparisons between sexes at birth at visits 4 and 5 are made using the Wilcoxon Rank Sum test. Additionally, comparisons of body-weight-adjusted, IgG-normalized VRC07-523LS concentrations between treatment arms at visits 4 and 5 are made using the Wilcoxon Rank Sum test. Comparisons are made for the % penetration of protein-normalized VRC07-523LS between visits, and specimen types using Wilcoxon Signed-Rank test; and between treatment arms using Wilcoxon Rank Sum test; and between sex at birth, controlling for treatment arms using Van Elteren Test. Comparisons are conducted between levels normalized using actual total protein concentrations and those normalized using the U.S. reference protein concentration (70 g/L) with treatment arms (T1 and T2) pooled using the Wilcoxon Signed Rank Test. Linear mixed-effect regression analyses are performed modeling natural log-transformed concentrations as a function of time since most recent infusion, sex at birth, and treatment group. Linear regressions are performed modeling the difference between natural log-transformed concentrations between visits adjusting for the difference between number of days since last infusion at compared visits, as well as sex at birth and treatment group. P-values are adjusted for multiple comparisons by controlling the false discovery rate (FDR) using the Benjamini-Hocherg procedure [9]

and the q-value [10]. Results are significant if the adjusted p-value or q-value ≤ 0.05. Comparisons are conduct for groups with at least 3 data points for paired test and at least 5 for unpaired test.

The distributions of body-weight-adjusted IgG- and protein-normalized VRC07-523LS levels, and total IgG over time are displayed graphically by specimen type, assigned sex at birth, and treatment arm. Data points for each participant are connected by a gray solid line. Additionally, the distributions of body-weight-adjusted IgG- and protein-normalized VRC07-523LS levels over time are displayed graphically by treatment arm, with all specimen types plotted together. Data points for each participant are connected by a gray dashed line, while median levels at each timepoint for each specimen type are connected by a colored solid line. The distributions of body-weight-adjusted IgG-normalized VRC07-523LS levels over actual study days since enrollment are displayed graphically by specimen type, assigned sex at birth, and treatment arm. Data points for each participant are connected by the colored dashed line.

Correlations of dose-amount-adjusted, IgG-normalized VRC07-523LS concentrations across specimen types by visits are reported in two ways: (1) sex at birth plotted together but distinguished by shapes, and treatment arms plotted together but distinguished by colors (2) separately for female and male participants, and treatment arms plotted together but distinguished by colors. Correlations of IgG-normalized VRC07-523LS concentrations across specimen types by visits are reported in two ways: (1) separately for treatment arms, and sex at birth plotted together but distinguished by shapes (2) separately for treatment arms, and sex at birth. Correlation of IgG levels and protein levels across specimen types by treatment arms and visits are reported as well. Spearman's rank correlation coefficients and their associated p-values are calculated. Comparisons only done when there are at least 5 data points in each group.

8.3.1 List of Tables

- Comparisons of body weight at baseline (T1: VRC07-523LS 10 mg/kg vs T2: VRC07-523LS 30 mg/kg) using Wilcoxon Rank Sum Test, by sex at birth.
- Comparisons of body weight at baseline (Female vs Male) using Wilcoxon Rank Sum Test, by arm.
- Summary statistics (i.e., N, min, 25%ile, mean, median, 75%ile, max, geometric mean, standard deviation) of body-weight-adjusted IgG-normalized VRC07-523LS concentration for each specimen type, by specimen type, treatment arm, assigned sex at birth and visit.
- Summary statistics (i.e., N, min, 25%ile, mean, median, 75%ile, max, geometric mean, standard deviation) of body-weight-adjusted protein-normalized VRC07-523LS concentration for each specimen type, by specimen type, treatment arm, assigned sex at birth and visit.
- For secretions, dose normalized IgG-normalized VRC07-523LS concentrations comparisons between visits 4, 6, and 8 (Cmax after a different number of infusions) using Wilcoxon signed-rank test, by specimen type. P-values will be adjusted for multiple comparisons by controlling the false discovery rate (FDR) using the Benjamini-Hochberg (BH) procedure and the q-value. The unadjusted p-values, BH-adjusted p-values, and q-values will be reported.
- For secretions, dose normalized IgG-normalized VRC07-523LS concentrations comparisons between visits 2, 5, 7, and 9 (trough after a different number of infusions) using Wilcoxon signed-rank test, by specimen type. P-values will be adjusted for multiple comparisons by controlling the false discovery rate (FDR) using the Benjamini-Hochberg (BH) procedure and the q-value. The unadjusted p-values, BH-adjusted p-values, and q-values will be reported.
- For mucosal tissues, comparisons between visits 4 vs 8 dose normalized IgG-normalized VRC07-523LS concentrations (Cmax after a different number of infusions) using Wilcoxon

signed-rank test, by specimen type. P-values will be adjusted for multiple comparisons by controlling the false discovery rate (FDR) using the Benjamini-Hochberg (BH) procedure and the q-value. The unadjusted p-values, BH-adjusted p-values, and q-values will be reported.

- For mucosal tissues, comparisons between visits 2 vs 9 dose normalized IgG-normalized VRC07-523LS concentrations (trough after a different number of infusions) using Wilcoxon signed-rank test, by specimen type. P-values will be adjusted for multiple comparisons by controlling the false discovery rate (FDR) using the Benjamini-Hochberg (BH) procedure and the q-value. The unadjusted p-values, BH-adjusted p-values, and q-values will be reported.
- For secretions, body-weight-adjusted IgG-normalized VRC07-523LS concentrations comparisons between treatment groups at visits 4 and 5 using Wilcoxon signed-rank test, by specimen type with sex at birth combined. P-values will be adjusted for multiple comparisons by controlling the false discovery rate (FDR) using the Benjamini-Hochberg (BH) procedure and the q-value. The unadjusted p-values, BH-adjusted p-values, and q-values will be reported.
- For mucosal tissues, body-weight-adjusted IgG-normalized VRC07-523LS concentrations comparisons between treatment groups at visit 4 using Wilcoxon signed-rank test, by specimen type with sex at birth combined. P-values will be adjusted for multiple comparisons by controlling the false discovery rate (FDR) using the Benjamini-Hochberg (BH) procedure and the q-value. The unadjusted p-values, BH-adjusted p-values, and q-values will be reported.
- Comparisons for body-weight-adjusted IgG-normalized VRC07-523LS concentration at peak timepoints (T1 vs T2) using Wilcoxon rank sum test, by specimen type and for (1) both assigned sexes at birth combined for rectal biopsies and rectal secretions and (2) each assigned sex at birth separately for other specimen types. P-values will be adjusted for multiple comparisons by controlling the false discovery rate (FDR) using the Benjamini-Hochberg (BH) procedure and the q-value. The unadjusted p-values, BH-adjusted p-values, and q-values will be reported.
- For rectal secretions, dose-amount-adjusted IgG-normalized VRC07-523LS concentrations comparisons at visit 4 and 5 among different sexes, using Wilcoxon rank sum test, by specimen type. P-values will be adjusted for multiple comparisons by controlling the false discovery rate (FDR) using the Benjamini-Hochberg (BH) procedure and the q-value. The unadjusted p-values, BH-adjusted p-values, and q-values will be reported.
- For rectal biopsies, dose-amount-adjusted IgG-normalized VRC07-523LS concentrations comparisons at visit 4 among different sexes, using Wilcoxon rank sum test, by specimen type. P-values will be adjusted for multiple comparisons by controlling the false discovery rate (FDR) using the Benjamini-Hochberg (BH) procedure and the q-value. The unadjusted p-values, BH-adjusted p-values, and q-values will be reported.
- Specimen comparison using partial Spearman's rank correlation, adjusted for treatment arms (T1 and T2) and sex assigned at birth, stratified by visit. P-values will be adjusted for multiple comparisons by controlling the false discovery rate (FDR) using the Benjamini-Hochberg (BH) procedure and the q-value. The unadjusted p-values, BH-adjusted p-values, and q-values will be reported.
- Summary of the percentage of penetration for protein-normalized VRC07-523LS concentration, by specimen type, treatment arm, assigned sex at birth and visit.
- For secretions, comparisons between visits 4, 6, and 8 penetration percentages (protein-normalized VRC07-523LS of mucosal divided by serum at Cmax after a different number of infusions) using Wilcoxon signed-rank test, by specimen types with treatment groups combined. P-values will be adjusted for multiple comparisons by controlling the false

discovery rate (FDR) using the Benjamini-Hochberg (BH) procedure and the q-value. The unadjusted p-values, BH-adjusted p-values, and q-values will be reported.

- For secretions, comparisons between visits 2, 5, 7, and 9 penetration percentages (protein-normalized VRC07-523LS of mucosal divided by serum at trough after a different number of infusions after a different number of infusions) using Wilcoxon signed-rank test, by specimen types with treatment groups combined. P-values will be adjusted for multiple comparisons by controlling the false discovery rate (FDR) using the Benjamini-Hochberg (BH) procedure and the q-value. The unadjusted p-values, BH-adjusted p-values, and q-values will be reported.
- For mucosal tissues, comparisons between visits 4 vs 8 penetration percentages (protein-normalized VRC07-523LS of mucosal divided by serum at Cmax after a different number of infusions) using Wilcoxon signed-rank test, by treatment group and spec type. P-values will be adjusted for multiple comparisons by controlling the false discovery rate (FDR) using the Benjamini-Hochberg (BH) procedure and the q-value. The unadjusted p-values, BH-adjusted p-values, and q-values will be reported.
- For mucosal tissues, comparisons between visits 2 vs 9 penetration percentages (protein-normalized VRC07-523LS of mucosal divided by serum at trough after a different number of infusions) using Wilcoxon signed-rank test, by treatment group and spec type. P-values will be adjusted for multiple comparisons by controlling the false discovery rate (FDR) using the Benjamini-Hochberg (BH) procedure and the q-value. The unadjusted p-values, BH-adjusted p-values, and q-values will be reported.
- For secretions, comparison of penetration percentages (protein-normalized VRC07-523LS of mucosal divided by serum) between treatment groups, using Wilcoxon rank sum test, at visits 4 and 5. P-values will be adjusted for multiple comparisons by controlling the false discovery rate (FDR) using the Benjamini-Hochberg (BH) procedure and the q-value. The unadjusted p-values, BH-adjusted p-values, and q-values will be reported.
- For mucosal tissues, comparison of penetration percentages (protein-normalized VRC07-523LS of mucosal divided by serum) between treatment groups, using Wilcoxon rank sum test, at visit 4. P-values will be adjusted for multiple comparisons by controlling the false discovery rate (FDR) using the Benjamini-Hochberg (BH) procedure and the q-value. The unadjusted p-values, BH-adjusted p-values, and q-values will be reported.
- For rectal secretions, comparison of penetration percentages (protein-normalized VRC07-523LS of mucosal divided by serum) at visits 4 and 5, among different sexes, controlling for treatment arms, using Van Elteren Test. P-values will be adjusted for multiple comparisons by controlling the false discovery rate (FDR) using the Benjamini-Hochberg (BH) procedure and the q-value. The unadjusted p-values, BH-adjusted p-values, and q-values will be reported.
- For rectal biopsies, comparison of penetration percentages (protein-normalized VRC07-523LS of mucosal divided by serum) at visit 4, among different sexes, controlling for treatment arms, using Van Elteren Test. P-values will be adjusted for multiple comparisons by controlling the false discovery rate (FDR) using the Benjamini-Hochberg (BH) procedure and the q-value. The unadjusted p-values, BH-adjusted p-values, and q-values will be reported.
- Among females, comparison of penetration percentages (protein-normalized VRC07-523LS of mucosal divided by serum) at visits 4 and 5 for rectal biopsies vs cervical biopsies, rectal biopsies vs vaginal biopsies, cervical biopsies vs vaginal biopsies, and rectal secretions vs cervicovaginal secretions, using Wilcoxon signed-rank test, with treatment arms combined, by visit. P-values will be adjusted for multiple comparisons by controlling the false discovery rate (FDR) using the Benjamini-Hochberg (BH) procedure and the q-value. The unadjusted p-values, BH-adjusted p-values, and q-values will be reported.

- Among males, comparison of penetration percentages (protein-normalized VRC07-523LS of mucosal divided by serum) at visit 4 for rectal secretions vs semen, using Wilcoxon signed-rank test, with treatment arms combined, by visit. P-values will be adjusted for multiple comparisons by controlling the false discovery rate (FDR) using the Benjamini-Hochberg (BH) procedure and the q-value. The unadjusted p-values, BH-adjusted p-values, and q-values will be reported.
- Comparisons for dose-amount-adjusted protein-normalized VRC07523LS levels in serum between actual total protein levels and U.S. reference protein levels (70 g/L) with T1+T2 pooled using Wilcoxon signed rank test, by specimen type and visit. P-values will be adjusted for multiple comparisons by controlling the false discovery rate (FDR) using the Benjamini-Hochberg (BH) procedure and the q-value. The unadjusted p-values, BH-adjusted p-values, and q-values will be reported.
- Analysis of the impact of time since the most recent infusion, sex at birth, and treatment on log-transformed, body-weight-adjusted IgG-normalized VRC07523LS levels across specimen types using a linear mixed-effects model. P-values will be adjusted for multiple comparisons by controlling the false discovery rate (FDR) using the Benjamini-Hochberg (BH) procedure and the q-value. The unadjusted p-values, BH-adjusted p-values, and q-values will be reported.
- Analysis of the impact of time difference between visits since the most recent infusion, sex at birth, and treatment on log-transformed, body-weight-adjusted IgG-normalized VRC07523LS levels across specimen types using a linear regression model. P-values will be adjusted for multiple comparisons by controlling the false discovery rate (FDR) using the Benjamini-Hochberg (BH) procedure and the q-value. The unadjusted p-values, BH-adjusted p-values, and q-values will be reported.

8.3.2 List of Graphs

- Lineplots of body-weight-adjusted IgG-normalized VRC07-523LS levels for each specimen type, by specimen type, assigned sex at birth and treatment arm (“visit week” as x-axis variable. Note, the spacing between two consecutive visit weeks indicate the actual number of weeks between them.) (all treatment arms plotted together but distinguished using different colors).
- Lineplots of body-weight-adjusted protein-normalized VRC07-523LS levels for each specimen type, by specimen type, assigned sex at birth and treatment arm (“visit week” as x-axis variable. Note, the spacing between two consecutive visit weeks indicate the actual number of weeks between them.) (all treatment arms plotted together but distinguished using different colors).
- Lineplots of body-weight-adjusted IgG-normalized VRC07-523LS levels for each specimen type, with sex at birth combined, by treatment arm (“visit week” as x-axis variable. Note, the spacing between two consecutive visit weeks indicate the actual number of weeks between them.) (all specimen types plotted together, separated for treatment arm).
- Lineplots of body-weight-adjusted protein-normalized VRC07-523LS levels for each specimen type, with sex at birth combined, by treatment arm (“visit week” as x-axis variable. Note, the spacing between two consecutive visit weeks indicate the actual number of weeks between them.) (all specimen types plotted together, separated by treatment arm).
- Lineplots of body-weight-adjusted IgG-normalized VRC07-523LS levels for each specimen type, by specimen type, assigned sex at birth and treatment arm (Actual number of days since enrollment as x-axis variable.) (all treatment arms plotted together but distinguished using different colors).

- Scatterplots of correlation as following:
  - Correlation analysis in rectal biopsy, cervical biopsy, vaginal biopsy, rectal secretions, cervicovaginal secretions, and semen, with sex at birth combined and treatment arms combined, by visit, for IgG-normalized and dose-amount-adjusted VRC07-523LS concentration (with Spearman's rank correlation coefficient for raw values and associated p-value category included. The data points included in the visualization will use different symbols reflecting assigned sex at birth, different colors will be used for different treatment arm. The correlation coefficients will base on all data included in the plot).
  - Correlation analysis in rectal biopsy, cervical biopsy, vaginal biopsy, rectal secretions, and cervicovaginal secretions for females with treatment arms combined, by visit, for IgG-normalized and dose-amount-adjusted VRC07-523LS concentration (with Spearman's rank correlation coefficient for raw values and associated p-value category included, different colors will be used for different treatment arm).
  - Correlation analysis in rectal biopsy, semen, and rectal secretions for males, with treatment arms combined, by visit, for IgG-normalized and dose-amount-adjusted VRC07-523LS concentration (with Spearman's rank correlation coefficient for raw values and associated p-value category included, different colors will be used for different treatment arm).
  - Correlation analysis in different specimen types with sex at birth combined, by treatment arm and visit, for IgG-normalized VRC07-523LS concentration (with Spearman's rank correlation coefficient for raw values and associated p-value category included. The data points included in the visualization will different symbols reflecting assigned sex at birth. The correlation coefficients will base on all data included in the plot).
  - Correlation analysis in rectal biopsy, cervical biopsy, vaginal biopsy, rectal secretions, and cervicovaginal secretions for females, by treatment arm and visit, for IgG-normalized VRC07-523LS concentration (with Spearman's rank correlation coefficient for raw values and associated p-value category included, different colors will be used for different treatment arm).
  - Correlation analysis in rectal biopsy, semen, and rectal secretions for males, by treatment arm and visit, for IgG-normalized VRC07-523LS concentration (with Spearman's rank correlation coefficient for raw values and associated p-value category included, different colors will be used for different treatment arm).
  - Correlation analysis of total IgG levels and total protein levels in rectal biopsy, semen, and rectal secretions, by treatment arm, assigned sex at birth, and visit (with Spearman's rank correlation coefficient for raw values and associated p-value category included); treatment groups may also be pooled together to construct correlation matrices.

9 REFERENCES

1. Agresti A, Coull BA. Approximate is better than "exact" for interval estimation of binomial proportions. Am Stat. 1998;52(2):119-26.
2. Lydersen S, Fagerland MW, Laake P. Recommended tests for association in 2 x 2 tables. Stat Med. 2009;28(7):1159-75.
3. Hudgens MG. Estimating cumulative probabilities from incomplete longitudinal binary responses with application to HIV vaccine trials. Stat Med. 2003;22(3):463-79.

4. Brookes ST, Whitely E, Egger M, Smith GD, Mulheran PA, Peters TJ. Subgroup analyses in randomized trials: risks of subgroup-specific analyses; power and sample size for the interaction test. J Clin Epidemiol. 2004;57(3):229-36.
5. Lachenbruch PA. Comparisons of two-part models with competitors. Stat Med. 2001;20(8):1215-34.
6. Hu Z, Proschan M. Two-part test of vaccine effect. Stat Med. 2015;34(11):1904-11.
7. Hughes JP. Mixed effects models with censored data with application to HIV RNA levels. Biometrics. 1999;55(2):625-9.
8. Rotnitzky A, Robins J. Analysis of semi-parametric regression models with non-ignorable non-response. Stat Med. 1997;16(1-3):81-102.
9. Benjamini, Yoav, and Yosef Hochberg. 1995. “Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing.” Journal of the Royal Statistical Society: Series B (Methodological) 57.
10. Storey, John D. 2003. “The Positive False Discovery Rate: A Bayesian Interpretation and the q-Value.” The Annals of Statistics 31.