nature portfolio

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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☒	☐	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☐	☒	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Clinical data were collected through Case Report Forms (CRFs) that are part of an electronic data capture (EDC) system. Laboratory data were collected at respective research labs: The IgG ELISA, hemoglobin ELISA and QuantiT assay data were collected on a Spectramax i3X Microplate Reader (Molecular Devices) using Softmax Pro 6.5.1 software. The Singlulex assay measurements of specific mAb concentration were collected on a Singulex Erenna instrument (Singulex/EMD Milllipore) using Sgx link TM Ver 1.4.56.39608 software. VRC01 and VRC01LS ELISA data was collected using Softmax Pro version 7.0.2. For ADA data, the MSD panel and platform were used. The images were acquired using a TissueFAXS system (TissueGnostics, Vienna, Austria, software v7.0.6245.134). See more details in Methods.
Data analysis	Data analysis was performed using SAS (version 9.4), nCal package (Version 2021.11-30), R (Version 4.0.4), and custom code which is provided as a Figshare link with the manuscript.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The data underlying the findings of this manuscript will be publicly available at the public-facing HVTN website. All individual participant data have been de-identified.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	We reported data from 14 participants assigned female sex at birth (AFAB) and 12 participants assigned male sex at birth (AMAB); all self-identified as cis-gender. Different mucosal specimens relevant to HIV transmission were sampled in participants AMAB (rectal and seminal) and participants AFAB (cervical and vaginal) and findings were reported based on sex assignment at birth.
Reporting on race, ethnicity, or other socially relevant groupings	We reported on Black/African American or other racial identities, given that VRC01 was isolated from an African American. We include age, weight, vital signs, safety labs, inflammatory markers, intestinal permeability and contraceptive use. We also include a behavioral risk score which assesses participant behavioral vulnerability to HIV. We also include tests sexually transmitted infections to help assess vulnerability to HIV and suitability for the mucosal sampling procedures. We adjusted for several pre-defined variables to ensure comparability between the groups.
Population characteristics	Sample population characteristics are included in Figure 1.
Recruitment	Clinical sites recruited healthy, HIV-uninfected (seronegative) adults who comprehended the purpose of the study and provided written informed consent. Volunteers were recruited and screened; those determined to be eligible, based on the inclusion and exclusion criteria, were enrolled in the study. Final eligibility determination depened on results of laboratory tests, medical history, physical examinations, and answers to self-administered and/or interview questions. Investigators were instructed that they should always use good clinical judgment in considering a volunteer's overall fitness for trial participation. Some volunteers may not be appropriate for enrollment even if they meet all inclusion/exclusion criteria. Medical, psychiatric, occupational, or other conditions may make evaluation of safety and/or pharmacokinetics difficult, and some volunteers may be poor candidates for retention. Determination of eligibility, taking into account all inclusion and exclusion criteria, had to be made within 56 days prior to enrollment unless otherwise noted in Sections 7.1 and 7.2 of the study protocol. For all exclusion and inclusion criteria, including those specific for mucosal sampling unique to this study, can be found in the HVTN 116 protocol (included as supplemental information)
Ethics oversight	The Institutional Review Boards at Case Western Reserve University Hospitals (825319), University of Pennsylvania (06-16-12), Vaccine Research Center (IR8023) and Fred Hutchinson Cancer Center (IR8447) approved all studies and procedures.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	The planned sample size provided reasonable precision to address the primary objectives of the study. For additional details refer to the study protocol.
Data exclusions	Data exclusions are detailed in the methods sections and in figure legends.
Replication	No replication was performed. Each assay was standardized included batch controls.
Randomization	The randomization sequence was computer-generated from random numbers in blocks of 2 stratified on sex-assigned-at-birth. Randomization

numbers were provided to each HVTN clinical research site pharmacist through the Statistical Data Monitoring Center (SDMC) to determine treatment.

Blinding

The study was open label, but laboratory staff conducting the assays were blinded to treatment assignment and time post-infusion.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
[ ]	[x] Antibodies
[x]	[ ] Eukaryotic cell lines
[x]	[ ] Palaeontology and archaeology
[x]	[ ] Animals and other organisms
[ ]	[x] Clinical data
[x]	[ ] Dual use research of concern
[x]	[ ] Plants

Methods

n/a	Involved in the study
[x]	[ ] ChIP-seq
[x]	[ ] Flow cytometry
[x]	[ ] MRI-based neuroimaging

Antibodies

Antibodies used

VRC01 (VRC-HIVMAB060-00-AB) and VRC01LS (VRC-HIVMAB080-00-AB) infusion products were supplied by Dale and Betty Bumpers Vaccine Research Center (VRC), NIAID, NIH, DHHS (Bethesda, Maryland, USA).
5C9 (mouse IgG2a anti-VRC01 mAb) was provided by John Mascola at the VRC.

Commerical antibodies:
- Horseradish peroxidase (HRP)-labelled goat anti-human IgG1(Invitrogen Cat # A-10648, Lot #. 2031436)
- Mouse anti-human IgG1 antibody (Invitrogen cat# MH1015, Clone HP6070, lot# 838051A2,TA264094 and TJ275311)
- Mouse IgG2a isotype control (R&D Systems cat# MAB003, clone 20102 ; Lot# MV0917051, MV0918031, MV0919011 and MV092001)
- PowerVision anti-mouse IgG HRP (Leica Cat# DPVM-110HRP; lot# 6053888, 6058018 and 6064298)

Validation

The use of 5C9 to measure VRC01 was described previously (Ledgerwood, J.E., et al. Safety, pharmacokinetics and neutralization of the broadly neutralizing HIV-1 human monoclonal antibody VRC01 in healthy adults. Clin Exp Immunol 182, 289-301 (2015) and referenced in the manuscript. Lot QC at the VRC included routinely doing binding to VRC01 by ELISA as an assay to validate its activity and for lot bridging. The protein was also run on a SDS-PAGE gel (non-reducing and reducing) to confirm that it has appropriate heavy and light chain bands (reducing) and that there is a predominant ~150 KDa band with very low levels of other bands in the non-reducing gel. At the Fred Hutch, SDS-PAGE and IHC staining with batch control of tissues incubated ex vivo with VRC01 and VRC01LS were performed to QC the 5C9 lots used for IHC and bridge lots.

Mouse anti-human IgG1 antibody (Invitrogen cat# MH1015) was conjugated to Alexa647 using the Singulex detection labeling kit (Millipore cat# 03-0076-02) according to the manufacturer's instructions. Each lot of labeled antibody was tested in bridging experiments using the assay standards.

Mouse IgG2a isotype control (R&D Systems cat# MAB003) lots were tested in bridging experiments using the assay positive and negative controls.

PowerVision anti-mouse IgG HRP consistency between lots was monitored using the run to run positive and negative controls.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	Clinicaltrials.gov NCT02797171
Study protocol	https://classic.clinicaltrials.gov/ProvidedDocs/71/NCT02797171/Prot_000.pdf
Data collection	Clinical data collection commenced at HVTN sites in Philadelphia and Cleveland approximately between the time of site activation (June 5, 2017, Philadelphia; March 6, 2017 Cleveland) and first enrollment (Aug 3, 2017, Philadelphia; May 18, 2017, Cleveland). Clinical data was collected through to the last participant visit in October 2018.
Outcomes	The primary clinical objectives of the study were to evaluate the safety and tolerability of VRC01/VRC01LS mAb administered through IV infusion. Safety endpoints were local and systemic reactogenicity, laboratory measures of safety, and adverse events (AEs). The primary laboratory objectives were to evaluate the PK of VRC01 in serum versus each mucosal compartment. The endpoints included unnormalized VRC01 levels measured in serum and protein-normalized and IgG-normalized VRC01 levels measured in serum and each mucosal compartment using 5C9 anti-idiotypic antibody for VRC01 in a custom Singulex assay (see manuscript for details).

Plants

Seed stocks	*Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*
Novel plant genotypes	*Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*
Authentication	*Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.*