A Clinical Trial to Evaluate the Impact of Broadly Neutralizing Antibodies VRC01LS and 10-1074 on Maintenance of HIV Suppression in a Cohort of Early-Treated Children in Botswana
(Dual bNAb Treatment in Children)

Phase I/II

A Collaboration of:

The Harvard School of Public Health AIDS Initiative

The Brigham and Women's Hospital

The Ragon Institute at Massachusetts General Hospital

The Ministry of Health of Botswana

DAIDS-ES 38551

NCT03707977

This file contains the current Dual bNAb Treatment in Children protocol, comprising the following documents, presented in reverse chronological order:

Clarification Memo #3, dated July 19, 2021
Letter of Amendment #3, dated May 19, 2020
Clarification Memo #2, dated April 9, 2020
Letter of Amendment #2, dated January 2, 2020
Letter of Amendment #1, dated October 4, 2019
Clarification Memo #1, dated April 1, 2019
Protocol version 2.0, dated December 20, 2018

CLARIFICATION MEMO #3

DATE: July 19, 2021

TO: Tatelo Co-Principal Investigators, CRS Leaders, and CRS Coordinators

FROM: Tatelo Protocol Team

SUBJECT: Clarification Memo #3 to the Tatelo Protocol (DAIDS ES-38551), Version 2.0 dated December 20, 2018 entitled "A Clinical Trial to Evaluate the Impact of Broadly Neutralizing Antibodies VRC01LS and 10-1074 on Maintenance of HIV Suppression in a Cohort of Early-Treated Children in Botswana"

This clarification memo does not result in a change in the protocol informed consent document. The Division of AIDS does not require you to forward it to your Institutional Review Board (IRB)/Ethics Committee (EC); however, as always you must follow your IRB/EC policies and procedures. If IRB/EC review of clarification memos is required at your site, please submit this document for review.

Each site should file a copy of this clarification memo with the protocol for reference.

The protocol clarifications contained in this memo will be included in the next version of the Tatelo (DAIDS-ES 38551) protocol if it is amended at a future date.

The following clarifications to the Tatelo protocol (DAIDS-ES 38551), Version 2.0, dated December 20, 2018 have been made:

1. Analysis of HIV-1-specific neutralizing antibody responses will be completed in collaboration with Monogram Biosciences, Inc. instead of Beth Israel Deaconess Medical Center. Specifically, specimens for these analyses already described in the IRB-approved protocol will be shipped from the Kuritzkes laboratory at Brigham and Women's Hospital in Boston, MA to Monogram Biosciences in South San Francisco, California for protocol-indicated testing. There are no changes required to the processing or shipping procedures in Botswana, or to the Botswana-Harvard Partnership Laboratory Processing Chart.

A Clinical Trial to Evaluate the Impact of Broadly Neutralizing Antibodies VRC01LS and 10-1074 on Maintenance of HIV Suppression in a Cohort of Early-Treated Children in Botswana (Dual bNAb Treatment in Children)

DAIDS-ES 38551

Protocol Version 2.0, Dated December 20, 2018
Letter of Amendment #3, dated May 19, 2020

PROTOCOL SIGNATURE PAGE

I will conduct the study in accordance with the provisions of this protocol and all applicable protocol-related documents. I agree to conduct this study in compliance with United States (US) Health and Human Service regulations (45 CFR 46); applicable U.S. Food and Drug Administration regulations; standards of the International Conference on Harmonization Guideline for Good Clinical Practice (E6); Institutional Review Board/Ethics Committee determinations; all applicable in-country, state, and local laws and regulations; and other applicable requirements (e.g., US National Institutes of Health, Division of AIDS) and institutional policies.

Signature of Investigator of Record:

_____

Date signed: ____/____/____ (dd/mm/yy)

Name of Investigator of Record (printed):

_____

Date: May 19, 2020

RE: LETTER OF AMENDMENT #3 FOR DUAL BNAB TREATMENT IN CHILDREN Version 2.0, dated December 20, 2018

"A Clinical Trial to Evaluate the Impact of Broadly Neutralizing Antibodies VRC01LS and 10-1074 on Maintenance of HIV Suppression in a Cohort of Early-Treated Children in Botswana"

DAIDS-ES #38551

TO: Study Coordinators at Sites Participating in Dual BNAb Treatment in Children

FROM: Roger Shapiro, MD, MPH (Corresponding Principal Investigator)

The following information impacts the Dual BNAb Treatment in Children Study (DAIDS-ES#38551) and must be forwarded to your Institutional Review Board (IRB)/Ethics Committee (EC) as soon as possible for their information and review. This must be approved by your IRB/EC before implementation.

The following information may also impact the sample informed consent. Your IRB/EC will be responsible for determining the process of informing subjects of the contents of this letter of amendment.

Upon receiving final IRB/EC and any other applicable Regulatory Entity (RE) approval(s) for this LoA, sites should implement the LoA immediately. Sites are still required to submit a LoA registration packet to the DAIDS Protocol Registration Office (PRO) at the Regulatory Support Center (RSC). Sites will receive a registration notification for the LoA once the DAIDS PRO verifies that all the required LoA registration documents have been received and are complete. A LoA registration notification from the DAIDS PRO is not required prior to implementing the LoA. A copy of the LoA registration notification along with this letter and any IRB/EC correspondence should be retained in the site's regulatory files.

The purpose of this LOA is to 1) provide operational flexibility for conducting study visits and procedures in the context of circulating severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the associated coronavirus disease (COVID-19) pandemic; 2) correct the frequency of safety and accrual reporting to the IRBs. Safety and accrual reports are submitted to IRBs per their reporting criteria and requirements. This study also uses the SAE Reporting Category for Expedited Adverse Event reporting to DAIDS; EAE reports are also submitted to IRBs at the time of reporting to DAIDS; 3) to incorporate relevant revisions described in Clarification Memos #1 dated April 1, 2019 and #2 dated April 9, 2020); and 4) to incorporate relevant revisions made to the Investigator's Brochure for VRC01LS version 4.0 dated December 27, 2019.

These changes will affect the following sections of the protocol. Additions to text are shown in **bold**. Deletions are shown in ~~strikethrough~~.

1. Study Team Roster: The contact information for Rachel Bowman has been updated to include an email address.

Laboratory Data Manager
Rachel Bowman, PhD
Frontier Science and Technology Research Foundation, Inc.
4033 Maple Road
Amherst, NY 14226
Phone: (716) 834-0900 x7375
Email: bowman@fstrf.org

2. Section 1.7. Study Intervention: The following subsections have been revised to read:

Step 1 (first 6 participants)

All Step 1-3 participants must be from the EIT Study. After approval, Step 1 will begin for a maximum of 6 participants, all of whom will have participated in the PK Step (3 from each PK Step group), and all of whom will have been >12 weeks from last bNAb dosing. ART is continued throughout Step 1, and all participants receive both 10-1074 and VRC01LS. Following a recommendation from the study team and SMC to increase the maintenance dosing based on the PK Step, a VRC01LS loading dose of 30 mg/kg will be given at the start of Step 1, followed by 15mg/kg dosing at each 4-weekly visit, and 10-1074 dosing will be at 30mg/kg at each 4-weekly visit. At Week 0, Week 4, and Week 8 doses of Step 1, PK testing for both bNAbs will occur prior to infusion, and at Weeks 0 and 4 only at end of infusion, 1 hour post-infusion, 1 day post-infusion, and 1 week post-infusion. Post-infusion PK blood draws will occur at the end of and 1-hour after the completion of both infusions, if both are administered on the same day. HIV-1 RNA will be checked every 4 weeks in Step 1. The first 6 participants in Step 1 will continue in this Step, receiving both oral ART and dual bNAb infusions, until the second study team/SMC review occurs. This review of safety and PK data will occur within 8-10 weeks of the 6th participant's Week 8 PK sampling **(or as soon as is feasible in the event of Covid-19 related delays)**.

In the event that disruptions due to Covid-19 affect the Step 1 timeline, the first 6 participants in Step 1 will temporarily discontinue bNAbs and remain in Step 1, receiving at a minimum oral ART and safety monitoring every 12 weeks. These first 6 participants in Step 1 will resume dual bNAb infusions when the protocol team deems it is safe and feasible to do so. BNAb infusions are planned to occur throughout Step 1, but may resume later in the extended Step 1 schedule in the event of missed or discontinued infusions. If a child has missed more than 2 dual bNAb infusions, then dosing will be given as at the start of Step 1 upon resuming bNAbs: a VRC01LS loading dose of 30mg/kg and a 10-1074 dose of 30mg/kg will be given at the first resumed infusion visit. Maintenance dosing will follow as per protocol with VRC01LS given at 15mg/kg and 10-1074 given at 30mg/kg at each 4-weekly visit. BNAbs will be resumed at the next scheduled bNAb administration visit on the extended Step 1 schedule (i.e., at Week 12, 16, 20, 24, 28, 32, 36, etc.) and participants may enter Step 2 (if open) on

the day of the 3rd resumed bNAb infusion visit or later (8 weeks or later after resuming monthly bNAb infusions).

SMC Review of Safety and PK Data from Step 1 (first 6 participants)

Safety, PK, and viral suppression data for the first 6 participants from Step 1 will be reviewed within 8-10 weeks **(or as soon as is feasible in the event of Covid-19 related delays)** by the study team, sponsor, and SMC. *(No changes to the remainder of this subsection.)*

Step 2 (all participants)

Following a successful Step 1 review, Step 2 may begin. Step 2 will begin after a participant has spent at least 8 weeks in Step 1 **(and has completed at least 2 consecutive dual bNAb infusion visits with the third or later dual bNAb infusion scheduled for the Step 2 entry visit)**. *(No changes to the remainder of this paragraph.)*

3. Section 4. Study Design: The following subsections have been revised to read:

All Step 1-3 participants must be from the EIT Study. After approval, Step 1 will begin for a maximum of 6 participants, all of whom will have participated in the PK Step (3 from each PK Step group), and all of whom will have been >12 weeks from last bNAb dosing. ART is continued throughout Step 1, and all participants receive both 10-1074 and VRC01LS. Following a recommendation from the study team and the SMC to increase the maintenance dosing based on the PK Step, a VRC01LS loading dose of 30 mg/kg will be given at the start of Step 1, followed by 15mg/kg dosing at each 4-weekly visit, and 10-1074 dosing will be at 30mg/kg at each 4-weekly visit. At Week 0, Week 4, and Week 8 doses of Step 1, PK testing for both bNAbs will occur prior to infusion, and at Weeks 0 and 4 only at end of infusion, 1 hour post-infusion, 1 day post-infusion, and 1 week post-infusion. HIV-1 RNA will be checked every 4 weeks in Step 1. The first 6 participants in Step 1 will continue in this Step, receiving both oral ART and dual bNAb infusions, until the second study team/SMC review occurs. This review of safety and PK data will occur within 8-10 weeks **(or as soon as is feasible in the event of Covid-19 related delays)** of the 6th participant's Week 8 PK sampling.

In the event that disruptions due to Covid-19 affect the Step 1 timeline, the first 6 participants in Step 1 will temporarily discontinue bNAbs and remain in Step 1, receiving at a minimum oral ART and safety monitoring every 12 weeks. These first 6 participants in Step 1 will resume dual bNAb infusions when the protocol team deems it is safe and feasible to do so. BNAb infusions are planned to occur throughout Step 1, but may resume later in the extended Step 1 schedule in the event of missed or discontinued infusions. If a child has missed more than 2 dual bNAb infusions, then dosing will be given as at the start of Step 1 upon resuming bNAbs: a VRC01LS loading dose of 30mg/kg and a 10-1074 dose of 30mg/kg will be given at the first resumed infusion visit. Maintenance dosing will follow as per protocol with VRC01LS given at 15mg/kg and 10-1074 given at 30mg/kg at each 4-weekly visit. BNAbs will be resumed at the next scheduled bNAb administration visit on the extended Step 1 schedule (i.e., at Week 12, 16, 20, 24, 28, 32, 36, etc.) and participants may enter Step 2 (if open) on the day of the 3rd resumed bNAb infusion visit or later (8 weeks or later after resuming monthly bNAb infusions).

SMC Review of Safety and PK Data from Step 1 (first 6 participants)

Safety, PK, and viral suppression data for the first 6 Step 1 participants will be reviewed within 8-10 weeks **(or as soon as is feasible in the event of Covid-19 related delays)** by the study team, sponsor, and SMC. *(No changes to the remainder of this paragraph.)*

Step 2 (all participants)

Following a successful Step 1 review, Step 2 may begin. Step 2 will begin after a participant has spent at least 8 weeks in Step 1 **(and has completed at least 2 consecutive dual bNAb infusion visits with the third or later dual bNAb infusion scheduled for the Step 2 entry visit)**. *(No changes to the remainder of this subsection.)*

4. Section 6.2. 10-1074 Description, Supply, and Storage. The second sentence in the second paragraph has been revised to read:

10-1074 is supplied as single-use 20 mg/mL solution for IV injection in a 5ml **or 30ml** volume of buffered solution composed of sodium phosphate, potassium phosphate, potassium chloride, sodium chloride and polysorbate 80 with a pH of 7.0.

5. Section 6.3. Schedule of Intervention and Dosing. New text has been added after the second paragraph:

In the event that a) the PK testing for the SMC review cannot be completed on schedule (due to Covid-19 related disruptions), and there is not a clear path forward to completing the review without a delay of less than 6-8 weeks, or b) the study leadership determines that it is necessary to avoid regular in-person clinic visits for a period of time due to a Covid-19 outbreak, the study leadership team may choose to pause the bNAb infusions for the first 6 children in Step 1, while allowing children to remain in Step 1 and on ART. If such a pause in bNAb administration occurs, the study team may consider conducting visits in the extended schedule for the first 6 participants (Week 10 and beyond) by remote contact and prioritizing in-person clinic visits every 12 weeks for physical assessments and all laboratories normally collected at the Week 0 visit of Step 1 (with the exception of ADA testing and proviral genome sequencing). These laboratory tests are indicated in brackets [X] in Table 4 at Visits 20 and 32. Provision of antiretroviral medications will occur at the 12-week visit intervals, and also as required between these visits (preferably by home drop-off). Remote contact should also be performed whenever it becomes necessary to further reduce in-person clinic visits for staff or participant safety. Any missed procedures, including bNAb infusions will be recorded.

Once the Step 1 bNAb administration pause is ended by the study leadership team (when PK testing is imminent or completed and the study team has determined that it is feasible and safe to conduct infusion visits at the clinics), the 6 participants in Step 1 may resume dual bNAbs. If a child has missed more than 2 infusion visits, then dosing will be given as at the start of Step 1 upon resuming bNAbs: a VRC01LS loading dose of 30mg/kg and a 10-1074 dose of 30mg/kg will be given at the first resumed infusion visit. Maintenance dosing will follow as per protocol with VRC01LS given at 15mg/kg and 10-1074 given at 30mg/kg at each 4-weekly visit. BNAbs will be resumed at the next

scheduled bNAb administration visit on the extended Step 1 schedule (i.e., at Week 12, 16, 20, 24, 28, 32, 36, etc.) and participants may enter Step 2 (if open) on the day of the 3rd resumed bNAb infusion visit or later (8 weeks or later after resuming monthly bNAb infusions).

Additional enrollments **to Step 1** will not occur until approval is granted. If the PK data are in range and no dosing changes for the bNAbs are required, the dosing used in Step 1 will continue to be used for the remainder of the study unless new data emerges requiring additional review.

After approval is granted from this second review, the first 6 Step 1 participants will enter Step 2 and discontinue ART, provided they have been in Step 1 for at least 8 weeks**; have completed at least 2 consecutive dual bNAb infusion visits with the third or later dual bNAb infusion scheduled for the Step 2 entry visit;** and have remained with viral suppression <40 copies/mL (Table 5). Additional participants will also enroll in Step 1 at this time (both those who previously participated in the PK Step, and new participants). These participants may also proceed to Step 2 and discontinue ART after 8 weeks in Step 1, if they remain with viral suppression <40 copies/mL. A participant who experiences a confirmed viral rebound ≥40 copies/mL in Step 1 will discontinue bNAbs, complete visit procedures equivalent to those outlined for Step 1 Week 8, and enter Step 3. *(No changes to the remainder of this subsection.)*

6. Section 6.3.1. VRC01LS Dose Calculation Instructions. The first paragraph has been revised to read:

VRC01LS will be dosed at 30mg/kg at the first infusion visit, followed by 10mg/kg maintenance at subsequent infusion visits in the PK Step. Following a recommendation to increase the maintenance dosing of VRC01LS based on review of data from the PK Step, VRC01LS will be dosed at 30mg/kg at the first Step 1 infusion visit, followed by 15mg/kg maintenance at subsequent Step 1 infusion visits. **If a child misses more than 2 doses in Step 1, then VRC01LS will be reinitiated as a loading dose of 30mg/kg IV at the first resumed infusion visit followed by 15mg/kg IV every 4 weeks.**

7. Section 6.4.1. Preparation of VRC01LS IV Solution. This section has been revised to read:

To prepare VRC01LS IV infusion, the pharmacist will calculate the weight-based dose as in section 6.3.1 and remove the total number of vials needed as well as a 100 mL IV bag of Sodium Chloride for Injection USP, 0.9% from storage. **Do not use Dextrose 5% Water or other fluids to prepare VRC01LS IV infusions.** The pharmacist, using aseptic technique, will add the appropriate amount of VRC01LS to the 100 mL IV bag of Sodium Chloride for Injection USP, 0.9%. Typically 50–100mL of additional volume may be added to a 100mL bag of normal saline. Each pharmacist should test the capacity of the brand of saline bags that will be used at the site to confirm the capacity to add additional volume. The pharmacist will label the infusion bag including a Beyond Use Date and time.

VRC01LS is a highly concentrated protein solution and may develop white, opaque to translucent particles after thawing. ~~When particles are observed, they may disappear after a few hours at room temperature or storage at 2°C to 8°C.~~

The following instructions apply to thawing VRC01LS vialed product:
1. Thaw vials for a minimum of 1 hour at controlled room temperature (maximum 27°C) after removing from freezer.

2. Keep the material at controlled room temperature (maximum 27°C) during the entire preparation period, up to the maximum storage times described in the Section 6.1.

3. Prior to preparation for administration, vials should be swirled for 30 seconds with sufficient force to resuspend any visible particles, yet avoiding foaming. DO NOT SHAKE THE VIALS.

- **a) Visually inspect the vials; if 10 or fewer visible particles are present, the vial may be used for product preparation.**
- **b)** If **more than 10** particles are observed, return the vials to 2°C to 8°C storage. If the particles redissolve **or if 10 or fewer visible particles are present** within the maximum storage times described in Section 6.1, the vials may be used for product preparation.
- **c)** ~~If particles continue to be observed, do not use the vialed product for IV administration.~~ Refrigerated product must be equilibrated at controlled room temperature (maximum 27°C) for a minimum of 30 minutes before preparation and must be used within 8 hours of any subsequent return to room temperature.

4. If the thawed material is not administered within 24 hours of thaw, follow the storage information provided in Section 6.1.

The following instructions apply to VRC01LS Prepared Product (IV Bag and Syringe):
After product preparation in IV bags, the prepared VRC01LS may be stored at 2°C to 8°C up to 24 hours or at room temperature (maximum **27**~~30~~°C) for a maximum of **4**~~8~~ hours total including the infusion time. Product may not be stored in direct sunlight. If stored at 2°C to 8°C, prepared product must be equilibrated at room temperature (maximum **27**~~30~~°C) for a minimum of 30 minutes prior to product administration.

8. Section 6.6. Concomitant Medications: ART Management. A new subsection on "ART Drug Supply" has been added to the end of this section as follows:

ART Drug Supply

To avoid gaps in antiretroviral drug supply in the event of missed or cancelled study visits, study staff may dispense up to three months' supply of study drug, ensuring safety monitoring and addressing potential needs for dose modification due to weight gain. Further considerations are as follows:

- **Participants who have been dispensed an extra supply of antiretroviral medication should still come for their scheduled visits, if possible.**
- **If a participant is expected to grow into a higher weight band requiring a dose increase soon, sites should ensure accurate contact information with the family; if an in-person visit is not possible, study drug dose modifications may be implemented with dosing instructions provided to the participant's caregiver by telephone based on weight reported by the**

caregiver. Any such instruction should be source documented in the participant's study chart.

- **In the event of a public health emergency, such as Covid-19, requiring reduced in-person contact, where feasible, sites are encouraged to implement antiretroviral drug delivery options involving outdoor pick-up or drop-off. In such cases, sites are encouraged to provide ART adherence assessment, counseling, and support remotely (e.g., by telephone).**

9. Section 7.1. Clinical Evaluations and Procedures. If a pause in bNAb administration occurs for the first 6 participants in Step 1, the study team may consider conducting visits in the extended schedule for the first 6 participants (Week 10 and beyond) by remote contact and prioritized procedures for in-person clinic visits every 12 weeks for physical assessments and all laboratories normally collected at the Week 0 visit of Step 1 (with the exception of ADA testing and proviral genome sequencing). Table 4 has been revised to indicate these laboratory tests in brackets [X] at Visits 20 and 32 with updated blood volumes and explanatory footnotes.

Table 1: Follow-up and laboratory testing for Step 1 (all participants)

	Step 1: ART + dual bNAbs							Possible additional visits for first 6 participants in Step 1$											
Study week	0	0+ 1&	1	4	4+ 1&	5	8$	10*	12*	14*	16*	18*	20*	22*	24*	26*	28*	30*	32*
VRC01LS	X			X			X		X		X		X		X		X		X
10-1074	X			X			X		X		X		X		X		X		X
Exam	X		X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Baseline Characteristics/ Demographics/ SES (if required)	X																		
Adherence Assessment	X		X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Acceptability Assessment	X						X												
HIV RNA≠	X∞			X			X		X		X		X		X		X		X
CBC^	X		X			X		X		X		X	**[X]**	X		X		X	**[X]**
CD4/CD8	X							X		X		X	**[X]**	X		X		X	**[X]**
Chem / LFTs^	X		X#			X#		X#		X#		X#	**[X#]**	X#		X#		X#	**[X#]**
Stored Plasma	X			X			X		X		X		X		X		X		X
Stored PBMC	X			X			X		X		X		X		X		X		X
HIV-1 Qualitative DNA	X			X			X		X		X		X		X		X		X
Proviral Genome Sequencing**	X						X												

HIV-1 gag DNA Analysis by ddPCR[%]	X						X				X		**[X]**		X				X
Immune Profiling	(X)			(X)			X				X		(X)		X				X
ELISA	X						X				X		**[X]**		X				X
PK Testing[&]	X[&]	X[&]	X[&]	X[&]	X[&]	X[&]	X		X		X		X		X		X		X
ADA Testing[≠]	X																		
Blood Volume (mL)	12	2	4	9	2	4	11	4	8	4	9	4	8**[-12]**	4	9	4	8	4	9**[-12]**

SES, socioeconomic status
Laboratory test time-points are optional when marked in parentheses: (X).
[≠] If a participant has a confirmed viral load result ≥40 copies/mL during Step 1, he/she will discontinue bNAbs, complete visit procedures equivalent to those outlined for Step 1 Week 8 and enter Step 3.
[∞] May be drawn up to 4 weeks prior to Week 0 visit.
[&] For first 6 participants, PK draws will occur pre-infusion at Week 0, and also for both bNAbs at end of infusion at Week 0, 1 hour post-infusion at Week 0, Week 0 + 1 day after infusion, Week 1, Week 4 pre-infusion, at end of infusion at Week 4, 1 hour post-infusion at Week 4, Week 4 + 1 day after infusion, and Week 5. All subsequent PK testing will be pre-dose trough testing only. Other participants will not have PK on Day 0 or Week 1 or Week 5, but will have pre-dose trough testing at the visits noted beginning at 4 weeks.
[$] Step 2 testing schedule for "Step 2 Week 0" supersedes Step 1 schedule on the day of entry into Step 2.
* Additional Step 1 visits may be required only for the first 6 participants while awaiting SMC review of safety and PK data. In the unlikely event that a participant reaches the Week 32 Step 1 visit prior to the conclusion/recommendation of the SMC review **or the opening of Step 2**, Week 30 and Week 32 visit procedures will be repeated alternately every two weeks, if feasible, until a decision on Step 2 is reached.
** In the event of plasma rebound ≥400 copies/mL, viral sequencing from plasma samples will also be considered.
[%] CD4 T cell subsets will be considered if sufficient PBMC volume available.
^ CBC includes: hemoglobin (g/dl), Hematocrit (%), RBC (million/mm^3), MCV (microns), WBC (10^3/ cu mm), differential WBC, absolute neutrophil count (ANC), Platelets (10^3/cu mm). Chemistry includes: electrolytes, BUN, creatinine, glucose, albumin. LFTs include ALT, AST, total and direct bilirubin. Any draw that includes electrolytes should be performed with a butterfly needle when possible, to reduce the chances of hemolysis; specimens considered hemolyzed should be repeated at the next regularly scheduled visit. If clinical concern for hyperkalemia exists, hemolyzed specimens should be repeated as soon as possible.
[#] LFTs only
[≠]ADA testing will not be done on the first 6 participants in Step 1 who had this testing during the PK Step.
[X] indicate added laboratory tests to be done in the event that in-person clinic visits are reduced to 12-weekly on the extended Step 1 schedule for the first 6 participants.

(No changes to the remainder of this subsection.)

10. Section 7.7.1. Evaluations in the Setting of Maintained Viral Suppression. The following text has been added to the end of the subsection on Step 1:

<u>Step 1 Week 8-32 Visits (if remaining in Step 1 with reduced in-person visits due to Covid-19 related disruptions). Visits may be completed in full or in part, off-site, or by</u>

phone, text message, email, or other electronic means. Any missed procedures will be documented.

- **Perform physical exam**
- **Adherence Assessment**
- **Acceptability Assessment (at Week 8)**
- **Continue ART while remaining in Step 1**
- **VRC01LS and 10-1074 administration (scheduled for Weeks 8, 12, 16, 20, 24, 28, 32) must be completed at the study clinics and will therefore be completed only when local circumstances allow for in-person to be conducted safely. Missed infusions will be documented. If a child misses more than 2 doses of dual bNAbs in Step 1, then VRC01LS will be reinitiated as a loading dose of 30mg/kg IV at the first resumed infusion visit followed by 15mg/kg IV every 4 weeks.**
- **Samples obtained for: HIV-1 RNA monthly (at Weeks 8, 12, 16, 20, 24, 28, 32); hematology, CD4/CD8, and LFTs (at Weeks 10, 14, 18, 22, 26, and 30); stored PBMC/plasma monthly (at Weeks 8, 12, 16, 20, 24, 28, and 32), qualitative HIV DNA PCR monthly (at Weeks 8, 12, 16, 20, 24, 28, and 32), whole genome sequencing (Week 8), quantitative HIV DNA PCR from stored PBMC every 8 weeks (at Weeks 8, 16, 24, and 32), immune profiling (at Weeks 8, 16, 20 (optional), 24, and 32), ELISA (at Weeks 8, 16, 24, and 32), PK testing (at Weeks 8, 12, 16, 20, 24, 28, and 32). Missed evaluations will be documented.**

If limiting in person clinic visits to 12-weekly, add CBC, CD4/CD8, LFT to Week 20 and Week 32 evaluations. Add quantitative HIV DNA PCR from stored PBMC and ELISA to Week 20 evaluations.

11. Section 7.9. Timing **and method** of evaluations. The title of this subsection has been revised as shown. The second paragraph under Scheduled evaluations has been revised to read:

Visits will occur at the weeks indicated whenever possible. A visit may be counted as completed if attendance is either early (if performed no sooner than halfway between the previous visit target date and the target date for the upcoming scheduled visit) or late (no later than halfway between the target date for the scheduled visit and the target date for the next scheduled visit). Thus, a child is always eligible for a scheduled visit when seen in the clinic. However, the clinic staff may use their discretion to either perform visits as "unscheduled" if far from the target date and a reliable participant, or to count a visit as "scheduled" and perform all required tasks (see "Ill visits" below). **Unless paused due to Covid-19 related disruptions, i**nfusion visits that are not completed within 14 days of the scheduled visit will trigger review by the protocol team and, if in Step 2, weekly HIV RNA testing will occur.

Administration of bNAbs must be completed at the study clinics, and will therefore be completed only when local circumstances allow for in-person clinic visits to be conducted safely.

Sites may conduct study visits, in full or in part, off-site if permitted by applicable government, health authority, and institutional polices. If an off-site visit is planned, site staff should communicate with the parent/legal guardian to determine in advance where and when such visits will take place, with adequate protections for safety, privacy, and confidentiality. Staff will make arrangements for adequate biohazard containment, and specimen and data chain of custody.

Clinic visits for data collection and procedures other than bNAb administration (scheduled and ad hoc) may be conducted by phone, text message, email, or other electronic means.

If adverse events requiring further evaluation or management are identified during a remote contact, staff conducting the remote visit should arrange for appropriate clinical management, in consultation with the IoR or designee as needed.

The paragraph under Premature discontinuation of study treatment has been revised to read:

Premature discontinuation of study treatment
If the single bNAb needs to be discontinued during the PK Step the child will go off Step after completing the visit procedures equivalent to Week 12 of the PK Step. If the participant is permanently discontinuing study treatment in the PK Step he/she will go off-study. In general during participation in Step 1 or Step 2, if a child needs to discontinue study treatment for any reason, he/she will complete final visit evaluations for the Step and enroll to Step 3. **However, if the study leadership chooses to temporarily discontinue study treatment for the first 6 children in Step 1 as a result of the Covid-19 outbreak, these participants may resume dual bNAbs in Step 1 on the extended schedule when it is deemed feasible and safe to resume in-person clinic operations.**

New text on "Documentation" has been added to the end of this subsection as follows:

Documentation
Documentation should be entered in participant study charts in real-time should any of the following occur:

- **Missed visits**
- **Out-of-window visits**
- **Off-site visits (document the location of the visit)**
- **Incomplete or partial visits (document which procedures were performed and which were not)**
- **Remote contacts performed in lieu of in-person visits (document method used to complete the contact and which procedures were performed)**
- **Any other participant contacts**
- **Use of alternate laboratories or alternate laboratory assays**
- **Alternate provision of study drug**
- **Dose modifications implemented remotely**

12. Section 13.8.1. Quarterly Reporting and SMC Triggers for Safety or Stopping Criteria. The first sentence in the first paragraph has been revised to read: Monthly safety and accrual monitoring by the protocol team will occur throughout the study. Reports will be provided to the study sponsor ~~and IRBs~~ quarterly.

The above information will be incorporated into the next protocol version as necessary if the protocol is amended.

The following changes affect the informed consent form for Steps 1-3. Additions to text are shown in **bold**. Deletions are shown in ~~strikethrough~~.

Section 4. “What do I have to do if I am in this study?”

Study visits (third bullet)

- You will receive transportation money and compensation for your time at each visit. If you request it **(or if it is necessary for public health reasons during a Covid-19 outbreak)**, it may be possible to have some study visits at your home, **or at a different location than the study clinic,** but not when the antibody is given. **We will talk to you in advance about any change in location. If necessary, some study visits may also be done by phone, text, or email, whichever you prefer.** The study team may check in with you about your child by telephone between visits.

First 8 Weeks from the Start of Dual Antibody Therapy (third bullet, subsection on “Additional procedures for the first 6 children enrolled”)

- *Additional procedures for the first 6 children enrolled:*
    - There will be additional blood drawn at the first two infusions visits (before the infusion, at the end of the infusions, and one hour later). There will be an extra visit and blood draw the day after the first 2 infusions. At the Week 1 and Week 5 visits a small amount of additional blood will be drawn to check antibody levels.
    - There will also be about 4 additional visits (possibly up to 12) while we check the results of the tests done so far. We will check for safety and dosing of the antibodies when given together **during this time**. These extra 4-12 visits will occur on a regular schedule, every 2 weeks~~, with infusions once a month~~ until we have enough information to continue with the next step of the study.
    - **Your child may receive infusions once a month during this time. If there are delays caused by Covid-19 (for example, if the lab tests cannot be done on time, or if it is necessary to avoid lengthy clinic visits, we may temporarily stop the infusions until we can get back on the regular schedule. If that happens, we will still ask you to come to the clinic at least once every 12 weeks for a physical exam, blood tests, and antiretroviral medication refills. Tests are the same as at other study visits, and include FBC and chemistry, CD4, viral load, tests to determine antibody levels, and stored blood for specialized tests to study your child’s ability to fight the virus. Between 1.5 to 2.5 teaspoons of blood (8-12mL) will be drawn at these clinic visits every 12 weeks. The other visits (every two weeks) can be done by phone if necessary. If your child’s infusions were temporarily stopped: when we restart giving antibody infusions, your child will be given the antibody infusions two times, 4 weeks apart, before your child can move to or begin the next part of the study.**

CLARIFICATION MEMO #2

DATE: April 9, 2020

TO: Tatelo Co-Principal Investigators, CRS Leaders, and CRS Coordinators

FROM: Tatelo (Dual bNAb Treatment in Children) Protocol Team

SUBJECT: Clarification Memorandum #2 to the Tatelo Protocol (DAIDS ES-38551), Version 2.0 dated December 20, 2018 entitled "A Clinical Trial to Evaluate the Impact of Broadly Neutralizing Antibodies VRC01LS and 10-1074 on Maintenance of HIV Suppression in a Cohort of Early-Treated Children in Botswana"

This clarification memorandum (CM) does not result in a change in the protocol informed consent document. The Division of AIDS does not require you to forward it to your Institutional Review Board (IRB)/Ethics Committee (EC); however, as always you must follow your IRB/EC policies and procedures. If IRB/EC review of clarification memos is required at your site, please submit this document for review.

Each site should file a copy of this clarification memo with the protocol for reference.The protocol clarifications contained in this memo will be included in the next version of the Tatelo (DAIDS-ES 38551) protocol if it is amended at a future date.

This CM is being issued to safeguard the health and well-being of Tatelo study participants in the context of circulating severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the associated coronavirus disease (COVID-19) pandemic. The purpose of this CM is to provide operational flexibility for conducting study visits and procedures. Implementation of this CM is expected to be time-limited in relation to the COVID-19 pandemic. In consultation with the study Sponsor, the Tatelo Protocol Team will determine when, in the future, the guidance provided in this CM is no longer applicable. When such a determination is made, study sites will be formally notified and instructed to inform their IRBs/ECs.

Prioritization of Study Visit Procedures

- Administration of bNAbs must be completed at the study clinics, and will therefore be completed only when local circumstances allow for in-person clinic visits to be conducted safely.
- Sites may conduct study visits, in full or in part, off-site if permitted by applicable government, health authority, and institutional polices. The protocol currently states that home visits may be performed for procedures other than bNAb administration. If an off-site visit is planned, site staff should communicate with the parent/legal guardian to determine in advance where and when such visits will take place, with adequate protections for safety, privacy, and confidentiality. Staff will make arrangements for adequate biohazard containment, and specimen and data chain of custody.

- Clinic visits for data collection and procedures other than bNAb administration (scheduled and ad hoc) may be conducted by phone, text message, email, or other electronic means.
- If adverse events requiring further evaluation or management are identified during a remote contact, staff conducting the contact should arrange for appropriate clinical management, in consultation with the IoR or designee as needed.
- Six participants have been enrolled in Step 1. The protocol currently requires that safety, PK, and viral suppression data for these first 6 participants be reviewed by the Safety Monitoring Committee (SMC) within 8-10 weeks of the 6th participant completing the Step 1 Week 8 visit. Per the current protocol, the first 6 participants in Step 1 are to continue in Step 1 with bNAb infusions every 4 weeks until the SMC review occurs and approval to move forward to Step 2 is obtained. No additional children are to be enrolled to Step 1 until this SMC review occurs and approval to proceed is granted. In the event that the PK testing for the SMC review cannot be completed on schedule (due to Covid-19 related disruptions), and if there is not a clear path forward to completing the review without a delay of less than 6-8 weeks, the study leadership team may choose to pause the bNAb infusions for these 6 children, while allowing children to remain in Step 1 and on ART. If such a pause occurs, the study team may consider conducting visits in the extended schedule for the first 6 participants (Week 10 and beyond) by remote contact and prioritizing in-person clinic visits every 12 weeks for physical assessments and all laboratories normally collected at the Week 0 visit of Step 1 (with the exception of ADA testing). Provision of antiretroviral medications will occur at the 12-week visit intervals, and also as required between these visits (preferably by home drop-off). Remote contact should also be performed as described above whenever it becomes necessary to further reduce in-person clinic visits for staff or participant safety. Once the Step 1 bNAb administration pause is ended by the study leadership team (when PK testing is imminent or completed and the study team has determined that it is feasible and safe to conduct infusion visits at the clinics), the 6 participants in Step 1 may resume dual bNAbs. If a child has missed more than 2 infusion visits, then dosing will be given as at the start of Step 1 upon resuming bNAbs: a VRC01LS loading dose of 30mg/kg and a 10-1074 dose of 30mg/kg will be given at the first resumed infusion visit. Maintenance dosing will follow as per protocol with VRC01LS given at 15mg/kg and 10-1074 given at 30mg/kg at each 4-weekly visit. BNAbs will be resumed at the next scheduled bNAb administration visit on the extended Step 1 schedule (i.e., at Week 12, 16, 20, 24, 28, 32, 36, etc.) and participants may enter Step 2 (if open) on the day of the 3rd resumed bNAb infusion visit or later (8 weeks or later after resuming monthly bNAb infusions).

Antiretroviral Drug Supply

- Tatelo participants may obtain antiretroviral medication refills at scheduled bNAb administration visits at the clinic (monthly). However, to avoid gaps in drug supply in the event of missed or cancelled visits, sites may dispense up to three months' supply of study drug, ensuring safety monitoring and addressing potential needs for dose modification due to weight gain. Further considerations are as follows:
    - Participants who have been dispensed an extra supply of antiretroviral medication should still come for their scheduled visits, if possible.
    - If a participant is expected to grow into a higher weight band requiring a dose increase soon, sites should ensure accurate contact information with the family; if

an in-person visit is not possible, study drug dose modifications may be implemented with dosing instructions provided to the participant's caregiver by telephone based on weight reported by the caregiver. Any such instruction should be source documented in the participant's study chart.

- Where feasible, sites are encouraged to implement antiretroviral drug delivery options involving outdoor pick-up or drop-off.
- Sites are encouraged to provide adherence assessment, counseling, and support remotely (e.g., by telephone).

Documentation

- Site-specific contingency plans, and the implementation thereof, should be documented in essential document files for Tatelo.
- Documentation should be entered in participant study charts in real-time should any of the following occur:
  - Missed visits
  - Out-of-window visits
  - Off-site visits (document the location of the visit)
  - Incomplete or partial visits (document which procedures were performed and which were not)
  - Remote contacts performed in lieu of in-person visits (document method used to complete the contact and which procedures were performed)
  - Any other participant contacts
  - Use of alternate laboratories or alternate laboratory assays
  - Alternate provision of study drug
  - Dose modifications implemented remotely

A Clinical Trial to Evaluate the Impact of Broadly Neutralizing Antibodies VRC01LS and 10-1074 on Maintenance of HIV Suppression in a Cohort of Early-Treated Children in Botswana (Dual bNAb Treatment in Children)

DAIDS-ES 38551

Protocol Version 2.0, Dated December 20, 2018
Letter of Amendment #2, dated January 2, 2020

PROTOCOL SIGNATURE PAGE

I will conduct the study in accordance with the provisions of this protocol and all applicable protocol-related documents. I agree to conduct this study in compliance with United States (US) Health and Human Service regulations (45 CFR 46); applicable U.S. Food and Drug Administration regulations; standards of the International Conference on Harmonization Guideline for Good Clinical Practice (E6); Institutional Review Board/Ethics Committee determinations; all applicable in-country, state, and local laws and regulations; and other applicable requirements (e.g., US National Institutes of Health, Division of AIDS) and institutional policies.

Signature of Investigator of Record:

_____

Date signed: ____/____/____ (dd/mm/yy)

Name of Investigator of Record (printed):

_____

Date: January 2, 2020

RE: LETTER OF AMENDMENT #2 FOR DUAL BNAB TREATMENT IN CHILDREN Version 2.0, dated December 20, 2018

"A Clinical Trial to Evaluate the Impact of Broadly Neutralizing Antibodies VRC01LS and 10-1074 on Maintenance of HIV Suppression in a Cohort of Early-Treated Children in Botswana"

DAIDS-ES #38551

TO: Study Coordinators at Sites Participating in Dual BNAb Treatment in Children

FROM: Roger Shapiro, MD, MPH (Corresponding Principal Investigator)

The following information impacts the Dual BNAb Treatment in Children Study (DAIDS-ES#38551) and must be forwarded to your Institutional Review Board (IRB)/Ethics Committee (EC) as soon as possible for their information and review. This must be approved by your IRB/EC before implementation.

The following information may also impact the sample informed consent. Your IRB/EC will be responsible for determining the process of informing subjects of the contents of this letter of amendment.

Upon receiving final IRB/EC and any other applicable Regulatory Entity (RE) approval(s) for this LoA, sites should implement the LoA immediately. Sites are still required to submit a LoA registration packet to the DAIDS Protocol Registration Office (PRO) at the Regulatory Support Center (RSC). Sites will receive a registration notification for the LoA once the DAIDS PRO verifies that all the required LoA registration documents have been received and are complete. A LoA registration notification from the DAIDS PRO is not required prior to implementing the LoA. A copy of the LoA registration notification along with this letter and any IRB/EC correspondence should be retained in the site's regulatory files.

The purpose of this LOA is to 1) incorporate recommendations of the study team and Safety Monitoring Committee (SMC) following review of data from the PK Step of this trial, specifically a) to increase the maintenance dose of VRC01LS from 10mg/kg to 15 mg/kg every 4 weeks in Step 1 and for the remainder of the study if targets are met, and to provide data from the PK Step supporting this recommendation, b) to maintain the same dose of 10-1074 at 30mg/kg every 4 weeks and to provide data from the PK Step supporting this recommendation, and c) to clarify that the first 6 participants in Step 1 will be selected without consideration of individual results from the PK Step; 2) to extend the time between the first 6 participants completion of Step 1 and the Safety Monitoring Committee (SMC) review of Step 1 data from 8 weeks to 8-10 weeks to allow for adequate time to review; and 3) to update the in-use storage conditions for the prepared

10-1074 in IV bags following the release of the revised Investigator's Brochure version 6.0, dated September 1, 2019.

These changes will affect the following sections of the protocol. Additions to text are shown in **bold**. Deletions are shown in ~~strikethrough~~.

1. Section 1.7. Study Intervention: The following subsections have been revised to read:

Step 1 (first 6 participants)

All Step 1-3 participants must be from the EIT Study. After approval, Step 1 will begin for a maximum of 6 participants, all of whom will have participated in the PK Step (3 from each PK Step group), and all of whom will have been >12 weeks from last bNAb dosing. ART is continued throughout Step 1, and all participants receive both 10-1074 and VRC01LS. ~~If~~ **Following a recommendation from the study team and SMC to increase the maintenance** dosing ~~recommendations do not change~~ based on the PK Step, a VRC01LS loading dose of 30 mg/kg will be given at the start of Step 1, followed by 1**5**~~0~~mg/kg dosing at each 4-weekly visit, and 10-1074 dosing will be at 30mg/kg at each 4-weekly visit. At Week 0, Week 4, and Week 8 doses of Step 1, PK testing for both bNAbs will occur prior to infusion, and at Weeks 0 and 4 only at end of infusion, 1 hour post-infusion, 1 day post-infusion, and 1 week post-infusion. Post-infusion PK blood draws will occur at the end of and 1-hour after the completion of both infusions, if both are administered on the same day. HIV-1 RNA will be checked every 4 weeks in Step 1. The first 6 participants in Step 1 will continue in this Step, receiving both oral ART and dual bNAb infusions, until the second study team/SMC review occurs. This review of safety and PK data will occur within 8**-10** weeks of the 6th participant's Week 8 PK sampling.

SMC Review of Safety and PK Data from Step 1 (first 6 participants)

Safety, PK, and viral suppression data for the first 6 ~~Step 1~~ participants **from Step 1** will be reviewed within 8**-10** weeks by the study team, sponsor, and SMC. All PK data will be assessed by the study team based on pre-specified PK trough targets which are the same as those used in the PK Step **for 10-1074 (7.5 mcg/mL) at Day 56, and revised for VRC01LS based on data from the PK Step (160 mcg/mL at Day 56)**. The review will confirm that PK targets are met **for the updated VRC01LS dosing and** during dual bNAb administration. *(No changes to the remainder of this paragraph.)*

Step 2 (all participants)

Following a successful Step 1 review, Step 2 may begin. Step 2 will begin after a participant has spent at least 8 weeks in Step 1. For the first 6 participants, Step 2 will begin at a scheduled bNAb dosing visit (i.e. 4 weeks from last Step 1 bNAb dosing), and for all subsequent participants it will begin at the 8 Week visit date for Step 1 (which becomes the Week 0 Step 2 visit). Participants with ongoing viral suppression throughout Step 1 will undergo withdrawal of ART and will continue maintenance 10-1074 (30mg/kg) and VRC01LS (1**5**~~0~~mg/kg) treatment for up to 24 weeks in Step 2. HIV-1 RNA will be checked at all visits in Step 2, which occur weekly from Weeks 1-4 in Step 2, and every 2 weeks thereafter. *(No changes to the remainder of this paragraph.)*

2. Section 2.1.8.1. The subheading title is revised as follows: **Initial** PK Considerations for VRC01LS **in the PK Step**.

The second paragraph has been revised to read:

These PK and safety data for VRC01, coupled with results from adult studies of VRC01LS (VRC 606), allow for dosing estimation for children in the current study. In addition, VRC01LS dosing considerations have been described for children in IMPAACT P1112 [43]. VRC01LS differs by 2 amino acids from VRC01, and is expected to have a similar peak level with a longer half-life and improved concentration at mucosal surfaces. VRC01LS population PK parameters ~~can be~~**were** obtained from a PK analysis of study VRC606 **in healthy volunteers** [45] to generate a typical profile for a two-year-old given VRC01LS 30mg/kg loading dose IV followed by two maintenance doses of 10mg/kg (Figure 2).

The first sentence of the third paragraph has been revised to read:

Following administration of the 30mg/kg loading dose, the C_{max} and troughs **were**~~are~~ not expected to accumulate much with repeat 10 mg/kg dosing, with the expected steady-state trough concentrations with 10mg/kg IV greater than 400 µg/mL. *(No changes to the remainder of this paragraph.)*

3. Section 2.1.8.1.1. Pharmacokinetic Targets for VRC01LS: A new subsection heading **2.1.8.1.1.1 VRC01LS target for the PK Step:** has been added to the first paragraph to clarify this subsection pertains to the PK Step only. *(No changes to the remainder of this paragraph.)*

4. A new subsection **2.1.8.1.1.2. Updated PK Considerations for VRC01LS in Step 1:** has been added as follows:

2.1.8.1.1.2. Updated PK Considerations for VRC01LS in Step 1: Following the PK Step completion and PK data review, updated considerations for VRC01LS dosing in Step 1 are as below:

Participants in the VRC01LS arm received 30 mg/kg on Day 1, then 10mg/kg on Day 28 and 10mg/kg on Day 56 of the PK Step. The median (range) Cmax after the first dose of 30 mg/kg was 710.7 mcg/mL (559.2—798.8 mcg/mL). The median Cmax value fell to 429.2 mcg/mL and 453.5 mcg/mL after the second and third doses of 10 mg/kg. The median pre-dose (28 days post previous dose) concentrations were 223.3 mcg/mL (157.9-253.3 mcg/mL), 180.7 mcg/mL (164.9-227.1 mcg/mL) and 156.9 mcg/mL (125.8-201.4 mcg/mL) at Days 28, 56 and 84, respectively. Four of the six (67%) participants had VRC01LS concentrations above 200 mcg/mL at Day 28 while one had a concentration >200 mcg/mL at Days 56 or 84. However, all VRC01LS participants maintained VRC01LS concentrations above 125 mcg/mL throughout the study. The median AUC following the first dose (AUC_{0-28}) and across all three doses (AUC_{0-84}) were 7,983 mcg*d/mL (6,878-9,850 mcg*d/mL) and 22,247 mcg*d/mL (18,491-22,552 mcg*d/mL), respectively. All participants exhibited average concentrations (AUC divided by the collection duration) that exceeded 200 mcg/mL through Days 28 and 84 (Weeks 4 and 12) (Table 1).

Table 1. Median VRC01LS Concentrations for 6 Participants in the PK Step

$Cmax_{Dose1}$ (mcg/mL)	$Cmax_{Dose2}$ (mcg/mL)	$Cmax_{Dose3}$ (mcg/mL)	$Pre\text{-}Dose_{C28D}$ (mcg/mL)	$Pre\text{-}Dose_{C56D}$ (mcg/mL)	$Pre\text{-}Dose_{C84D}$ (mcg/mL)	$AUC_{0\text{-}84D}$ (mcg*d/mL)
710.7	429.2	453.5	223.3	180.7	156.9	22,247

In summary, the VRC01LS troughs were somewhat lower than expected, and lower than the protocol-specified target trough concentration of 200 mcg/mL at 84 days. However, the peaks were adequate and there were no outlier values below the pre-specified value of 115 mcg/mL, and the average concentrations exceeded 200 mcg/mL through Days 28 and 84.

Following SMC review of these data on December 12, 2019, a recommendation was made to increase the maintenance dose of VRC01LS from 10mg/kg to 15 mg/kg to better match the adult values (Figure 4; Figure 5). Therefore, in Step 1, VRC01LS will be initiated as a loading dose of 30mg/kg IV followed by 15mg/kg IV every 4 weeks.

The VRC01LS concentration 28 days after the second dose (one loading dose of 30mg/kg and one maintenance dose of 15mg/kg), called the Concentration at Day 56 (C56D), will be used to assess adequacy of the dosing regimen. Specifically, if the median C56D of VRC01LS is above 160 mcg/mL (~80% of the original PK Step threshold of 200 mcg/mL accounting for 10-20% accumulation expected with continued dosing) then VRC01LS dosing will be deemed adequate from a PK perspective. If the PK data are above 160 mcg/mL and no dosing changes for VRC01LS are required, the dosing used in Step 1 will continue to be used for the remainder of the study unless new data emerges requiring additional review.

Figure 4. VRC01LS Concentrations in Children in the PK Step compared to Adult Values (Adult simulation data from VRC606)

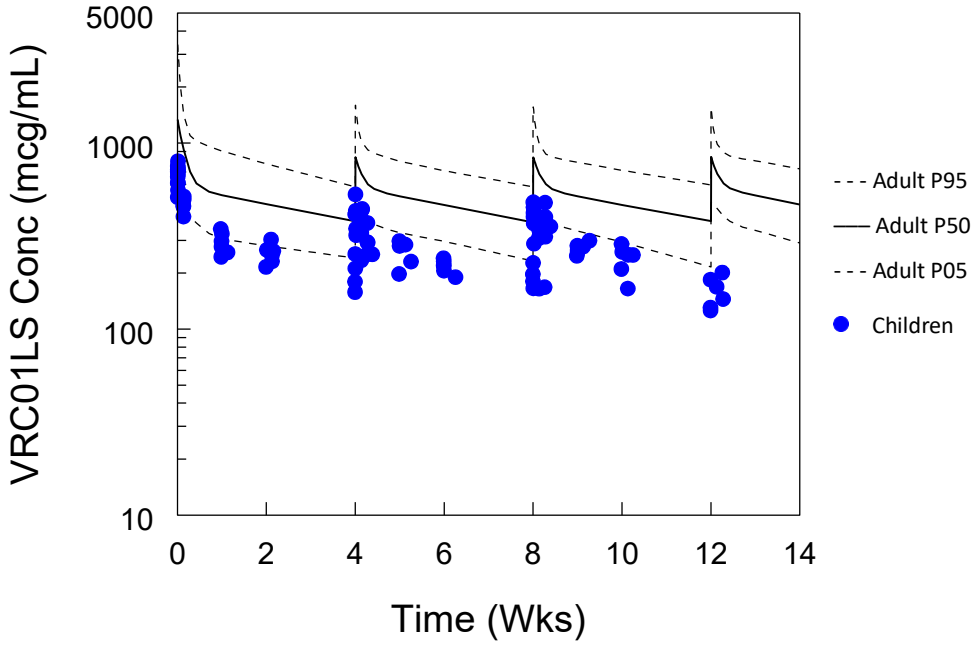
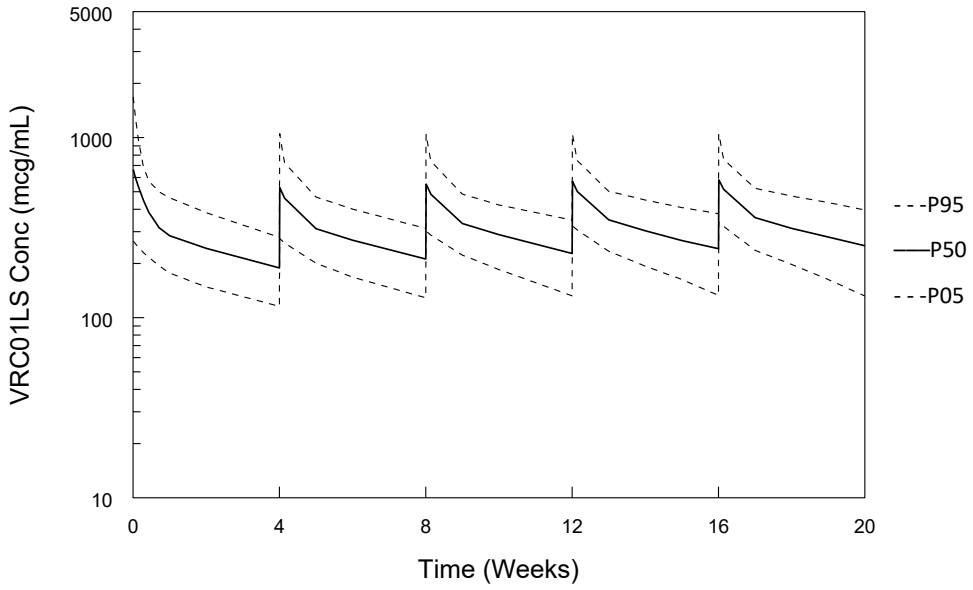


Figure 5. Predicted VRC01LS Concentrations using Updated Maintenance Dose of 15mg/kg every 4 weeks



5. Section 2.1.8.2 PK Considerations for 10-1074: A new subsection heading **Initial Dosing Considerations for the PK Step for 10-1074:** has been added to the first paragraph to clarify

this subsection pertains to the PK Step only. *(No changes to the remainder of this subsection.)*

6. Section 2.1.8.2.1. Pharmacokinetic Targets for VRC01LS: A new subsection heading **2.1.8.2.1.1. 10-1074 target for the PK Step:** has been added to the first paragraph to clarify this subsection pertains to the PK Step only. *(No changes to the remainder of this paragraph.)*

7. A new subsection **2.1.8.2.1.2. Updated PK Considerations for 10-1074 in Step 1:** has been added as follows:

Following the PK Step completion and PK data review by the SMC, updated considerations for 10-1074 dosing in Step 1 are as below:

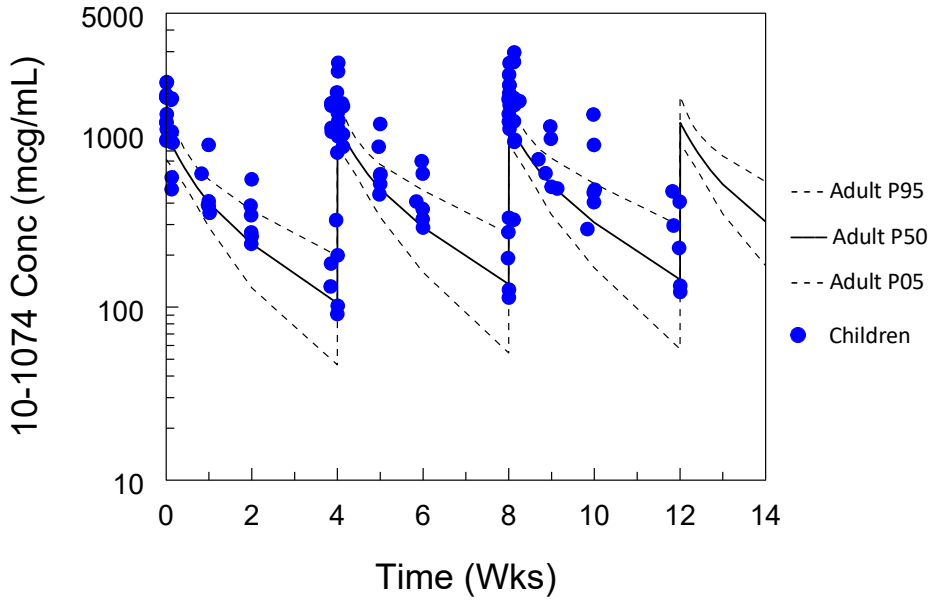
Participants in the 10-1074 arm received 30 mg/kg on Days 1, 28 and 56. The median (range) Cmax after the first dose was 1,632.5 mcg/mL (1,174.0-1,998.6 mcg/mL). The median Cmaxs were in the same range after the second and third doses (1,484.6 and 2,063.6 mcg/mL). The median pre-dose (28 days post previous dose) concentrations were 155.2 mcg/mL (91.3-318.6 mcg/mL), 232.0 mcg/mL (113.6-328.5 mcg/mL) and 258.3 mcg/mL (122.4-467.3 mcg/mL) at Days 28, 56 and 84, respectively. All six participants had 10-1074 concentrations that greatly exceeded 7.5 mcg/mL throughout the study. The median AUC following the first dose (AUC_{0-28}) and across all three doses (AUC_{0-84}) were 11,461 mcg*d/mL (8,222-16,937 mcg*d/mL) and 37,505 mcg*d/mL (32,485-66,034 mcg*d/mL), respectively. All participants exhibited average concentrations that exceeded 250 and 350 mcg/mL through Days 28 and 84 (Weeks 4 and 12) (Table 2; Figure 8).

Table 2: Median 10-1074 Concentrations for 6 Participants in the PK Step

$Cmax_{Dose1}$ (mcg/mL)	$Cmax_{Dose2}$ (mcg/mL)	$Cmax_{Dose3}$ (mcg/mL)	$Pre\text{-}Dose_{C28D}$ (mcg/mL)	$Pre\text{-}Dose_{C56D}$ (mcg/mL)	$Pre\text{-}Dose_{C84D}$ (mcg/mL)	AUC_{0-84D} (mcg*d/mL)
1,632.5	1,484.6	2,063.6	155.2	232.0	258.3	37,505

Following SMC review of these data on December 12, 2019, a recommendation was made to keep the 10-1074 dose the same at 30 mg/kg every 4 weeks for the remainder of the study.

Figure 8. 10-1074 Concentrations in Children in the PK Step compared to Adult Values (10-1074 adult percentiles from the Nussenzweig 584/MCA-906 study)



8. Section 2.3.1. Specific Aim 1 has been revised to read: VRC01LS and 10-1074 will not be associated with serious adverse events, and median trough concentrations at the doses used will be above established target values ~~of 200 mcg/mL and 7.5 mcg/mL respectively~~.

9. Section 4. Study Design: The following subsections have been revised to read:

Step 1 (first 6 participants)

All Step 1-3 participants must be from the EIT Study. After approval, Step 1 will begin for a maximum of 6 participants, all of whom will have participated in the PK Step (3 from each PK Step group), and all of whom will have been >12 weeks from last bNAb dosing. ART is continued throughout Step 1, and all participants receive both 10-1074 and VRC01LS. ~~If~~ **Following a recommendation from the study team and SMC to increase the maintenance** dosing ~~recommendations do not change~~ based on the PK Step, a VRC01LS loading dose of 30 mg/kg will be given at the start of Step 1, followed by 1**5**~~0~~mg/kg dosing at each 4-weekly visit, and 10-1074 dosing will be at 30mg/kg at each 4-weekly visit. At Week 0, Week 4, and Week 8 doses of Step 1, PK testing for both bNAbs will occur prior to infusion, and at Weeks 0 and 4 only at end of infusion, 1 hour post-infusion, 1 day post-infusion, and 1 week post-infusion. HIV-1 RNA will be checked every 4 weeks in Step 1. The first 6 participants in Step 1 will continue in this Step, receiving both oral ART and dual bNAb infusions, until the second study team/SMC review occurs. This review of safety and PK data will occur within 8-**10** weeks of the 6th participant's Week 8 PK sampling.

SMC Review of Safety and PK Data from Step 1 (first 6 participants)

Safety, PK, and viral suppression data for the first 6 Step 1 participants will be reviewed within

8**-10** weeks by the study team, sponsor, and SMC. All PK data will be assessed by the study team based on pre-specified PK trough targets which are the same as those used in the PK Step **for 10-1074 (7.5 mcg/mL) at Day 56, and revised for VRC01LS based on data from the PK Step (160 mcg/mL at Day 56)**. *(No changes to the remainder of this subsection.)*

10. Section 5.5. The following sentence has been added to the end of this section. **The first 6 participants in Step 1 will be selected without consideration of individual results from the PK Step.**

11. Section 6.3. Schedule of Intervention and Dosing. The second paragraph has been revised to read:

After approval is granted to begin Step 1, 6 of the first 12 PK Step participants will begin dual bNAb treatment per the dosing and follow-up schedule in Table ~~2~~**4**. The first 6 Step 1 participants will include 3 participants from each group in the PK Step; these participants may be the first 6 PK Step participants, but this is not required. **In Step 1 dosing for VRC01LS is 30mg/kg load followed by 15mg/kg maintenance, and for 10-1074 30mg/kg.** These Step 1 participants will receive enhanced PK testing for both bNAbs at the Week 0 and Week 4 visits, with pre-infusion testing, testing at end of infusion, 1 hour post-infusion, 1 day post-infusion and 1 week post-infusion (all other PK testing for these participants will be trough pre-dose testing). A review of the safety and PK data for the first 2 dual bNAb doses will occur for these 6 participants. While awaiting this review (see Section 13.8), all 6 participants will remain in Step 1 per follow-up outlined in Table ~~2~~**4**, with 4-weekly dual bNAb treatment and ongoing oral ART. Additional enrollments will not occur until approval is granted. **If the PK data are in range and no dosing changes for the bNAbs are required, the dosing used in Step 1 will continue to be used for the remainder of the study unless new data emerges requiring additional review.**

12. Section 6.3.1. VRC01LS Dose Calculation Instructions. The first paragraph has been revised to read: VRC01LS will be dosed at 30mg/kg at the first infusion visit, followed by 10mg/kg maintenance at subsequent infusion visits **in the PK Step**. **Following a recommendation to increase the maintenance dosing of VRC01LS based on review of data from the PK Step, VRC01LS will be dosed at 30mg/kg at the first Step 1 infusion visit, followed by 15mg/kg maintenance at subsequent Step 1 infusion visits**.

13. Section 6.4.2. Preparation of 10-1074 IV Solution. This section has been revised to read:

To prepare 10-1074 IV infusion, the pharmacist will calculate the weight-based dose as in section 6.3.2 and remove the total number of vials needed as well as a 100 mL IV bag of Sodium Chloride for Injection USP, 0.9% from storage. The pharmacist, using aseptic technique, will add the appropriate amount of 10-1074 to the 100 mL IV bag of Sodium Chloride for Injection USP, 0.9%. After product preparation in IV bags, the prepared 10-1074 may be stored at 2°C to 8°C **for up to 6 hours** ~~up to 24 hours~~ or at **controlled** room temperature (~~maximum~~ **20°C to** ~~30~~**25**°C) for ~~a maximum of 8~~**up to 4** hours**.** ~~total including t~~**T**he infusion ~~time~~**of study product must be completed within the storage timeframe specified for the respective storage conditions**. Product may not be stored in direct sunlight. If stored at 2°C to 8°C, prepared product must be equilibrated at room temperature (maximum 30°C) for a minimum of 30 minutes prior to product administration. The pharmacist will label the infusion bag including a Beyond Use Date and time.

14. Section 13.8.1. Quarterly Reporting and SMC Triggers for Safety or Stopping Criteria. The following subsection has been revised to read:

Review of Safety and PK Data from Step 1 (first 6 participants)

Safety, PK, and viral suppression data for the first 6 Step 1 participants will be reviewed within 8**-10** weeks by the study team including its sponsor representatives, and SMC. All PK data will be assessed by the study team based on pre-specified PK trough targets which are the same as those used in the PK Step **for 10-1074 (7.5 mcg/mL) at Day 56, and revised for VRC01LS based on data from the PK Step (160 mcg/mL at Day 56)**. The review will confirm that PK targets are met **for the updated VRC01LS dosing and** during dual bNAb administration. *(No changes to the remainder of this paragraph.)*

15. New Tables and Figures (Table 1, Figure 4 and Figure 5 in Section 2.1.8.1.1.2.; Table 2 and Figure 8 in Section 2.1.8.2.1.2) have been included in the protocol and the subsequent Table and Figure numbers in the protocol document have been updated accordingly.

The above information will be incorporated into the next protocol version as necessary if the protocol is amended.

Date: October 4, 2019

RE: LETTER OF AMENDMENT #1 FOR DUAL BNAB TREATMENT IN CHILDREN Version 2.0, dated December 20, 2018

"A Clinical Trial to Evaluate the Impact of Broadly Neutralizing Antibodies VRC01LS and 10-1074 on Maintenance of HIV Suppression in a Cohort of Early-Treated Children in Botswana"

DAIDS-ES #38551

TO: Study Coordinators at Sites Participating in Dual BNAb Treatment in Children

FROM: Roger Shapiro, MD, MPH (Corresponding Principal Investigator)

The following information impacts the Dual BNAb Treatment in Children Study (DAIDS-ES#38551) and must be forwarded to your Institutional Review Board (IRB)/Ethics Committee (EC) as soon as possible for their information and review. This must be approved by your IRB/EC before implementation.

The following information may also impact the sample informed consent. Your IRB/EC will be responsible for determining the process of informing subjects of the contents of this letter of amendment.

Upon receiving final IRB/EC and any other applicable Regulatory Entity (RE) approval(s) for this LoA, sites should implement the LoA immediately. Sites are still required to submit a LoA registration packet to the DAIDS Protocol Registration Office (PRO) at the Regulatory Support Center (RSC). Sites will receive a registration notification for the LoA once the DAIDS PRO verifies that all the required LoA registration documents have been received and are complete. A LoA registration notification from the DAIDS PRO is not required prior to implementing the LoA. A copy of the LoA registration notification along with this letter and any IRB/EC correspondence should be retained in the site's regulatory files.

After a review of the study timeline, the study team is proposing to implement the following change:

1. Increase the upper age limit of eligibility for enrollment to Step 1 from 5 years to 7 years old.

This change will affect the following sections of the protocol. Additions are shown in **bold**. Deletions are shown in ~~strikethrough~~.

<u>Section 5.4.1:</u>

Inclusion Criteria for Entry into Step 1 (followed by participation in Steps 2-3):

1) EIT Study participant
2) On ART for at least 96 weeks
3) ≥96 weeks and <~~5~~**7** years of age at enrollment
4) HIV RNA <40 copies/mL for at least 24 weeks prior to entry**
5) Ability to remain in close study follow-up for at least 56 weeks
6) Willingness to receive IV infusions of bNAbs
7) Willingness to provide signed informed consent (by the parent/guardian)

Section 7.9. Timing of evaluations

Enrollment should occur at or after 96 weeks on ART, with an upper age limit of 5 years **for the PK Step (7 years for Step 1)**.

CLARIFICATION MEMO #1

DATE: April 1, 2019

TO: Tatelo Co-Principal Investigators, CRS Leaders, and CRS Coordinators

FROM: Tatelo Protocol Team

SUBJECT: Clarification Memo #1 to the Tatelo Protocol (DAIDS ES-38551), Version 2.0 dated December 20, 2018 entitled "A Clinical Trial to Evaluate the Impact of Broadly Neutralizing Antibodies VRC01LS and 10-1074 on Maintenance of HIV Suppression in a Cohort of Early-Treated Children in Botswana"

This clarification memo does not result in a change in the protocol informed consent document. The Division of AIDS does not require you to forward it to your Institutional Review Board (IRB)/Ethics Committee (EC); however, as always you must follow your IRB/EC policies and procedures. If IRB/EC review of clarification memos is required at your site, please submit this document for review.

Each site should file a copy of this clarification memo with the protocol for reference.

The protocol clarifications contained in this memo will be included in the next version of the Tatelo (DAIDS-ES 38551) protocol if it is amended at a future date.

The following clarifications to the Tatelo protocol (DAIDS-ES 38551), Version 2.0, dated December 20, 2018 have been made:

1. 10-1074 is now available in a 30ml vial, in addition to the 5ml vial indicated in Section 6.2 of the protocol. Either size vial may be used in the study, depending on availability.

2. Rachel Bowman's email address was inadvertently omitted from the study team roster contact details. Her email address is bowman@fstrf.org.

A Clinical Trial to Evaluate the Impact of Broadly Neutralizing Antibodies VRC01LS and 10-1074 on Maintenance of HIV Suppression in a Cohort of Early-Treated Children in Botswana (Dual bNAb Treatment in Children)

Phase I/II

A Collaboration of:

The Harvard School of Public Health AIDS Initiative

The Brigham and Women's Hospital

The Ragon Institute at Massachusetts General Hospital

The Ministry of Health of Botswana

IND#: 140909
DAIDS-ES 38551

Coordinating Centre:

Botswana-Harvard School of Public Health Partnership for HIV Research and Education

Princess Marina Hospital

Private Bag BO 320, Bontleng

Gaborone, Botswana

Tel: +267-390-2671, Fax: +267-390-1284

Funded by:

The National Institute of Allergy and Infectious Diseases (NIAID)

Co-Principal Investigators: Roger Shapiro (corresponding)
Daniel Kuritzkes
Mathias Lichterfeld

Co-Investigators: Kara Bennett
Edmund Capparelli
Marina Caskey
Ajibola Gbolahan
Molly Pretorius Holme
Michael Hughes
Shahin Lockman
Joseph Makhema
Kenneth Maswabi
Mogomotsi Matshaba
Sikhulile Moyo
Kathleen Powis
Xu Yu

A Clinical Trial to Evaluate the Impact of Broadly Neutralizing Antibodies VRC01LS and 10-1074 on Maintenance of HIV Suppression in a Cohort of Early-Treated Children in Botswana (Dual bNAb Treatment in Children)

DAIDS-ES 38551

Version 2.0
Dated December 20, 2018

PROTOCOL SIGNATURE PAGE

I will conduct the study in accordance with the provisions of this protocol and all applicable protocol-related documents. I agree to conduct this study in compliance with United States (US) Health and Human Service regulations (45 CFR 46); applicable U.S. Food and Drug Administration regulations; standards of the International Conference on Harmonization Guideline for Good Clinical Practice (E6); Institutional Review Board/Ethics Committee determinations; all applicable in-country, state, and local laws and regulations; and other applicable requirements (e.g., US National Institutes of Health, Division of AIDS) and institutional policies.

Signature of Investigator of Record: _____

Date signed: ____/____/____ (dd/mm/yy)

Name of Investigator of Record (printed): _____

TABLE OF CONTENTS

List of Figures and Tables

Study Team Roster

Co-Principal Investigators:

Roger Shapiro, MD, MPH (corresponding co-PI)
Department of Immunology and Infectious Disease
Harvard T.H. Chan School of Public Health
651 Huntington Ave, FXB 305AA
Boston, MA 02115
Phone: 617-771-0040 (cell)
Fax: 617-739-8348
Email: rshapiro@hsph.harvard.edu

Daniel Kuritzkes, MD
Chief, Division of Infectious Disease
Brigham and Women's Hospital
75 Francis Street
Boston, MA 02115
Phone: 617-768-8371
Fax: 617-768-8738
Email: dkuritzkes@partners.org

Mathias Lichterfeld, MD, PhD
Infectious Disease Division
Brigham and Women's Hospital/Massachusetts General Hospital
65 Landsdowne Street
Cambridge, MA 02139
Phone: 617-768-8399
Fax: 617-726-5411
E-mail: mlichterfeld@partners.org

Co-Investigators:

Kara Bennett, MS
Bennett Statistical Consulting, Inc.
21 Nolan Road
Ballston Lake, NY 12019
Phone: 518-727-1399
Email: karabstat@gmail.com

Edmund Capparelli, PharmD
Division of Clinical Pharmacology and Developmental Therapeutics
University of California, San Diego, San Diego, CA
Phone: 858-246-0009
Fax: 858-246-0025
Email: ecapparelli@ucsd.edu

Marina Caskey, MD
Rockefeller University
1230 York Avenue
New York, NY 10065
Phone: 646-962-8747
Email: mcaskey@mail.rockefeller.edu

Ajibola Gbolahan, MD
Botswana-Harvard Partnership, Gaborone, Botswana
Phone: 267-3902671
Fax: 267-3901284
Email: gajibola@bhp.org.bw

Molly Pretorius Holme, MSc
Department of Immunology and Infectious Disease
Harvard T.H. Chan School of Public Health
651 Huntington Ave, FXB 406a
Boston, MA 02115
Phone: 617-432-4377
Fax: 617-739-8348
Email: mpretori@hsph.harvard.edu

Michael Hughes, PHD
Director, Center for Biostatistics in AIDS Research
Department of Biostatistics
655 Huntington Avenue
Building II Room 439A
Boston, Massachusetts 02115
Phone: 617-432-2815
Fax: 617-739-1781
Email: mhughes@sdac.harvard.edu

Shahin Lockman, MD, MS
Brigham and Women's Hospital, Boston
Harvard T.H. Chan School of Public Health, Boston
Phone: 617-771-8780 (cell)
Fax: 617-739-8348
Email: slockman@hsph.harvard.edu

Joseph Makhema, MD
Botswana-Harvard Partnership, Gaborone, Botswana
Phone: +267-3902671
Fax: +267-3901284
Email: jmakhema@bhp.org.bw

Kenneth Maswabi, MD
Botswana-Harvard Partnership, Gaborone, Botswana
Phone: 267-3902671
Fax: 267-3901284
Email: kmaswabi@bhp.org.bw

Mogomotsi Matshaba, MD
The Botswana-Baylor Children's Clinical Center of Excellence
Princess Marina Hospital, Gaborone, Botswana
Tel: +267 3190083
Email: matshaba@bcm.edu

Sikhulile Moyo, MPH, PhD
Botswana-Harvard Partnership, Gaborone, Botswana
Phone: +267-3902671
Cell phone: +267 72113640
Email: smoyo@bhp.org.bw

Kathleen Powis, MD, MPH
Massachusetts General Hospital
Botswana-Harvard Partnership, Gaborone, Botswana
Phone: +267-74300105 / 617-947-9150 (cell)
Email: kpowis@mgh.harvard.edu

Xu Yu, MD, MSc
Ragon Institute of MGH, MIT and Harvard
400 Technology Square
Cambridge, MA 02139
Phone: 857-268-7004
E-mail: xyu@partners.org

Product Developer Representative
Lucio Gama, MS, PhD
Vaccine Research Center
National Institutes of Health
40 Convent Drive, Room 5502
Phone: 301-761-7580
Email: lucio.gama@nih.gov

Protocol Data Manager
Christina Reding, MPH
Frontier Science and Technology Research
Foundation, Inc.
4033 Maple Road
Amherst, NY 14226
Phone: 716-834-0900 x7339
Email: reding@fstrf.org

Laboratory Data Manager
Rachel Bowman, PhD
Frontier Science and Technology Research Foundation, Inc.
4033 Maple Road
Amherst, NY 14226
Phone: (716) 834-0900 x7375

Data Operations and IT
Coulson Kgathi
Botswana-Harvard Partnership, Gaborone, Botswana
Phone: +267-390-2671
Email: ckgathi@bhp.org.bw

Pharmacists:

Pharmacist
Tshepho Frank, BPharm
Botswana-Harvard Partnership, Gaborone, Botswana
Phone: +267-390-2671
Email: tfrank@bhp.org.bw

DAIDS Protocol Pharmacist
Lynette Purdue, PharmD
Division of AIDS
5601 Fishers Lane
Rockville, MD 20852
Phone: 240-627-3061
Email: lpurdue@niaid.nih.gov

Project Administrator
Ria Madison
Botswana-Harvard Partnership, Gaborone, Botswana
Phone: 267-3902671
Cell phone: 267-72109025
Fax: 267-3901284
Email: rmadison@bhp.org.bw

Study Funder
National Institutes of Health
National Institutes of Allergy and Infectious Diseases

Medical Officer
Patrick Jean-Philippe, MD
National Institute of Allergy and Infectious Diseases
5601 Fishers Lane, 8B21,
Rockville, MD 20852, United States
Phone: 240-292-4790
Fax: 240-627-3465
Email: jeanphilippep@niaid.nih.gov

Program Officer
Judi Miller
National Institute of Allergy and Infectious Diseases
5601 Fishers Lane, 8C21,
Rockville, MD 20852, United States
Phone: 1-240-292-4801
Email: jmillera@niaid.nih.gov

Study Management

All questions concerning this protocol, including issues regarding:

Clinical medical management, toxicity management, concomitant medications, laboratory tests, and/or forms development should be sent via e-mail to rshapiro@hsph.harvard.edu.

Glossary

ADA	anti-drug antibody testing
ADCC	antibody-dependent cell-mediated cytotoxicity
AE	adverse events
ART	antiretroviral therapy or treatment
ATI	analytic treatment interruption
AUC	area under the curve
BHP	Botswana-Harvard AIDS Institute Partnership
BHHRL	Botswana-Harvard HIV Reference Laboratory
bNAb	Broadly neutralizing monoclonal antibody
CBV	Combivir
CHO	Chinese hamster ovary
CNS	central nervous system
CRF	case report form
CRPMC	Clinical Research Products Management Center
CTL	cytotoxic T-lymphocyte
DAIDS	Division of AIDS, National Institutes of Health
DBS	dried blood spot
ddPCR	digital droplet polymerase chain reaction
EAE	Expedited adverse event (reporting)
EFV	efavirenz
EIT	Early Infant Treatment Study
FDA	Food and Drug Administration
IRB	institutional review board
IV	intravenous
3TC	lamivudine
LFT	liver function test
LPV	lopinavir
LPV/r	lopinavir/ritonavir
MTB	Mycobacterium tuberculosis
MTCT	mother-to-child transmission
NIAID	US National Institute of Allergy and Infectious Diseases
NIH	US National Institutes of Health
NNRTI	nonnucleoside reverse transcriptase inhibitor
NRTI	nucleoside reverse transcriptase inhibitor
NVP	nevirapine
OHRP	US Office of Human Research Protection
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
PI	protease inhibitor or Principal Investigator
PK	pharmacokinetics
PMTCT	prevention of MTCT
RT	reverse transcriptase
SES	socioeconomic status
SMC	Safety Monitoring Committee
TDF	tenofovir disoproxil fumarate
ZDV	zidovudine

1. PROTOCOL SUMMARY

1.1. Title: A Clinical Trial to Evaluate the Impact of Broadly Neutralizing Antibodies VRC01LS and 10-1074 on Maintenance of HIV Suppression in a Cohort of Early-Treated Children in Botswana

1.1.1. Short title: Dual bNAb Treatment in Children

1.2. Sample Size: Up to 40 children

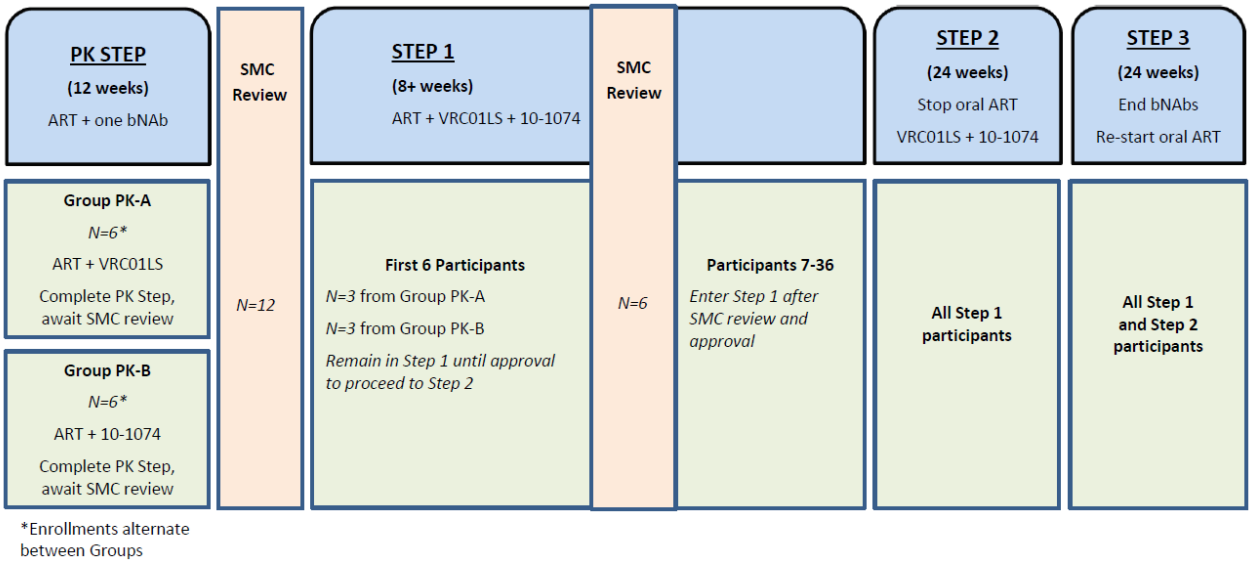
1.3. Study Population:

- HIV-infected children enrolled in the Early Infant Treatment (EIT) Study who are at least 96 weeks of life and meet entry criteria;
- In the PK Step only, HIV-infected children who are not enrolled in the EIT Study who are at least 96 weeks of life and meet entry criteria

1.4. Participating Sites: Gaborone CRS #31833 and Francistown CRS #31891

1.5. Study Design: This study will be a phase I/II, multi-site clinical trial of dual treatment with two broadly neutralizing monoclonal antibodies (bNAbs), VRC01LS and 10-1074, offered to HIV-1 infected virally suppressed children. There will be 4 Steps in the protocol: PK Step, Step 1, Step 2, and Step 3. In the PK Step, antiretroviral treatment (ART) is continued and 12 study participants will undergo safety and PK testing, 6 for each bNAb used in the study (10-1074 and VRC01LS). In Step 1, ART is continued and dual bNAb treatment occurs, with PK confirmation of dual bNAb dosing for the first 6 participants in Step 1. In Step 2, ART is withdrawn and dual bNAb maintenance treatment occurs. In Step 3, dual bNAbs will be discontinued and participants will be re-started on ART.

Figure 1: Study Schematic



1.6. Study Duration: 56-98 weeks

1.7. Study Intervention:

PK Step

The first 12 participants in the study will undergo the PK Step, to allow for detailed safety and PK assessment of each bNAb alone. In the PK Step, while continuing ART, participants 1, 3, 5, 7, 9, 11 will receive VRC01LS at Week 0, Week 4, and Week 8 as a single intravenous (IV) dose (30 mg/kg load, then 10mg/kg maintenance); safety and PK measurements will occur at end of infusion, 1 hour post-infusion, 1 day post-infusion, 1 week post-infusion, 2 weeks post-infusion, and 4 weeks post-infusion. Participants 2, 4, 6, 8, 10, 12 will receive 10-1074 at Week 0, Week 4, and Week 8 as a single IV dose (30mg/kg), and safety and PK measurements will also occur at end of infusion, 1 hour post-infusion, 1 day post-infusion, 1 week post-infusion, 2 weeks post-infusion, and 4 weeks post-infusion. In the PK Step, it is anticipated that all 12 participants will be from the Early Infant Treatment (EIT) Study; however, in the event that 12 participants from EIT are not available at the time the PK Step begins, participants 9-12 may be recruited from other virally suppressed HIV-infected children. These additional, non-EIT children would not be eligible for further study steps after completing the PK Step. In the event that a participant discontinues bNAb during the PK Step, the participant may be replaced to allow for PK data to be obtained from 12 participants total.

SMC Review of Safety and PK Data from PK Step

After all 12 PK Step participants complete the PK Step, safety, PK, and viral suppression data will be reviewed within 8 weeks by the study team, sponsor, and SMC (earlier review by the SMC may be triggered based on pre-specified safety parameters). Participants will not continue to be dosed with bNAbs while awaiting SMC review, but will continue ART. All PK data from the PK Step will be assessed based on pre-specified PK trough targets; peak values will be assessed, but primary guidance for peak values will be based on toxicity. The study team will determine if the pre-specified trough targets have been achieved, and present this information to the SMC. The SMC will review safety data utilizing pre-defined safety criteria. If safety criteria are met, and if the PK data are in the pre-specified range, approval to begin Step 1 may be granted by the SMC. If the dosing and frequency of bNAbs established in the PK Step is acceptable, it will continue to be used for the first 6 participants in Step 1 (see additional safety review below). If a change in dosing is recommended by experts on the study team (or by the SMC, if it does not accept the team's recommendation), adjustments will occur and IRB and study sponsor approval will be sought regarding required protocol changes prior to starting Step 1. The recommendations by the study team or by the SMC may include early termination of the study, if warranted by the safety/PK data, or a re-design of the study as approved by the study sponsors and regulatory boards.

Step 1 (first 6 participants)

All Step 1-3 participants must be from the EIT Study. After approval, Step 1 will begin for a maximum of 6 participants, all of whom will have participated in the PK Step (3 from each PK Step group), and all of whom will have been >12 weeks from last bNAb dosing. ART is continued throughout Step 1, and all participants receive both 10-1074 and VRC01LS. If dosing recommendations do not change based on the PK Step, a VRC01LS loading dose of 30 mg/kg will be given at the start of Step 1, followed by 10mg/kg dosing at each 4-weekly visit, and 10-1074 dosing will be at 30mg/kg at each 4-weekly visit. At Week 0, Week 4, and Week 8 doses of Step 1, PK testing for both bNAbs will occur prior to infusion, and at Weeks 0 and 4 only at end of infusion, 1 hour post-infusion, 1 day post-infusion, and 1 week post-infusion. Post-infusion PK blood draws will occur at the end of and 1-hour

after the completion of both infusions, if both are administered on the same day. HIV-1 RNA will be checked every 4 weeks in Step 1. The first 6 participants in Step 1 will continue in this Step, receiving both oral ART and dual bNAb infusions, until the second study team/SMC review occurs. This review of safety and PK data will occur within 8 weeks of the 6th participant's Week 8 PK sampling.

SMC Review of Safety and PK Data from Step 1 (first 6 participants)

Safety, PK, and viral suppression data for the first 6 Step 1 participants will be reviewed within 8 weeks by the study team, sponsor, and SMC. All PK data will be assessed by the study team based on pre-specified PK trough targets which are the same as those used in the PK Step. The review will confirm that PK targets are met during dual bNAb administration. Safety criteria will be reviewed by the SMC, also using the same criteria as for the PK Step. If safety criteria are met, and if the PK data for both bNAbs are in the pre-specified range, the SMC may approve entry into Step 2 for the first 6 participants and enrollment of additional participants into Step 1. If the PK data are in range and no dosing changes for the bNAbs are required, the dosing will continue to be used for the remainder of the study unless new data emerges requiring additional review. If a change in dosing is recommended by experts on the study team (or by the SMC, if it does not accept the team's recommendation), IRB and study sponsor approval will be sought regarding required protocol changes. The recommendations by the study team or by the SMC may include early termination of the study, if warranted by the safety/PK data, or a re-design of the study as approved by the study sponsors and regulatory boards.

Step 1 (all subsequent participants)

After approval to proceed is obtained by the SMC, additional participants will enroll directly into Step 1 and receive dual bNAbs and ART for 8 weeks. All study visits and follow-up will be the same as for the first 6 Step 1 participants, with the exception of PK testing; only trough PK testing will be required for these participants, beginning at Week 4 of Step 1.

Step 2 (all participants)

Following a successful Step 1 review, Step 2 may begin. Step 2 will begin after a participant has spent at least 8 weeks in Step 1. For the first 6 participants, Step 2 will begin at a scheduled bNAb dosing visit (i.e. 4 weeks from last Step 1 bNAb dosing), and for all subsequent participants it will begin at the 8 Week visit date for Step 1 (which becomes the Week 0 Step 2 visit). Participants with ongoing viral suppression throughout Step 1 will undergo withdrawal of ART and will continue maintenance 10-1074 (30mg/kg) and VRC01LS (10mg/kg) treatment for up to 24 weeks in Step 2. HIV-1 RNA will be checked at all visits in Step 2, which occur weekly from Weeks 1-4 in Step 2, and every 2 weeks thereafter. Qualitative HIV DNA will be checked at each visit by DBS. If a participant experiences a single viral rebound $\geq$400 copies/mL in Step 2, ART will be re-started (and VRC01LS and 10-1074 will be discontinued). A single viral rebound is defined as an HIV RNA result ≥400 copies/mL at any Step 2 visit, without need for confirmation. If HIV RNA becomes ≥400 copies/mL prior to the end of Step 2, all evaluations ordinarily scheduled at "Week 24" will be performed immediately prior to ART re-initiation. If HIV-1 RNA is ≥40 copies/mL but <400 copies/mL, or if Qualitative DNA turns from negative to positive, virologic monitoring tests will be repeated *weekly*. Clinical examination will occur at each study visit.

Step 3 (all participants)

All participants will end Step 2 after 24 weeks, and enter Step 3. In Step 3, both 10-1074 and VRC01LS will be discontinued, and ART will be re-started. Follow-up visits in Step 3 will occur at 4,

12, and 24 weeks.

1.8. Primary Objectives

- **Specific Aim 1:** To conduct an interventional clinical trial to determine the safety, pharmacokinetics, dosing and antiviral efficacy of up to 24 weeks of maintenance VRC01LS and 10-1074 immunotherapy in early-treated HIV-1 infected children in Botswana**.**
- **Specific Aim 2:** To evaluate effects of treatment with VRC01LS and 10-1074 on the size and cellular composition of residual viral reservoirs.
- **Specific Aim 3:** To investigate the influence of VRC01LS and 10-1074 treatment on the magnitude and quality of antiviral innate and adaptive immune responses.

1.9. Primary Endpoints and Analyses

Aim 1: The primary endpoints will be (1) the frequency and severity of treatment-associated adverse events, and (2) the proportion of children who maintain HIV-1 plasma RNA <400 copies/mL (and <40 copies/mL), after initiating VRC01LS and 10-1074 infusions (24 weeks after withdrawal of standard ART). Secondary endpoints will include pharmacokinetic profiles of all children who receive VRC01LS and 10-1074 and growth characteristics (height and weight z-scores) of virally suppressed children receiving bNAbs.

Aim 2: Viral reservoirs during treatment with VCR01LS and 10-1074 will be evaluated in blood samples (PBMCs) using a novel single-template, near full-length viral sequencing assay that provides information on the complete spectrum of HIV-1 DNA copies integrated into host chromosomes. This approach will allow us to evaluate defective vs. intact HIV-1 genomes, antiretroviral drug and cytotoxic T-lymphocyte (CTL) escape variants and longitudinal viral sequence evolution in total CD4 T cells as well as in phenotypically-complex subsets of CD4 T cells known to be critical for viral reservoir stabilization. Cell-associated HIV-1 RNA and plasma RNA will be simultaneously analyzed and sequenced. If rebound occurs during analytic treatment interruption (ATI), viral reservoir parameters will be correlated to viral rebound kinetics, to pharmacokinetic measurements of bNAbs, and to antiviral immune parameters determined in Specific Aim 3.

Aim 3: We will use comprehensive phenotypic and functional assays to profile effects of VRC01LS and 10-1074 treatment on biomarkers of immune activation and on innate cellular immune responses, coupled with RNA-Seq-based gene expression studies in selected immune cell subsets. In addition, the evolution of neutralizing antibody breadth and the frequency and functionality of HIV-1-specific T cells will be analyzed, and combined with viral sequencing data (generated in specific aim 2) to identify viral immune escape.

2. INTRODUCTION

2.1. Background

2.1.1. Non-ART viral suppression strategies are a priority for children. Long-term viral suppression with ART is difficult to maintain over the course of a lifetime, and significant toxicities to ART may accumulate with time. Novel strategies that maintain HIV viral suppression while allowing time off 3-drug ART are needed, and proof-of-concept studies to demonstrate the feasibility of such a strategy – and to study its impact on viral reservoir, immune responses, and clinical outcomes – are of high priority. The practical benefits of an ART-sparing intervention may reduce direct ART toxicities during critical periods of growth and development [1]. Long-term side effects and toxicities from 3-drug

ART are only beginning to be understood, particularly in areas of Africa where many children who received ART in childhood are now reaching adolescence. Even with newer and more tolerable ART regimens, toxicities that might be ameliorated by an ART-sparing strategy include impaired growth and endocrine effects [2, 3], lipodystrophy [4], hematologic toxicities [5], renal toxicity [6, 7], bone density effects [8-10], cardiovascular disease [11-13], and possible neurodevelopmental effects [14]. Finally, strategies that allow time off ART are highly desirable for many patients and caregivers for reasons related to treatment fatigue, travel, stigma, or other factors [15].

2.1.2. Viral reservoir suppression may be possible with broadly neutralizing monoclonal antibodies (bNAbs). Broadly neutralizing monoclonal antibodies can suppress HIV-1 RNA and may help to deplete residual viral reservoirs [16-19]. VRC01, a recombinant human immunoglobulin G1 (IgG1) monoclonal antibody produced in a Chinese Hamster Ovary (CHO) cell line that targets the CD4 binding site of gp120, has demonstrated activity in reducing plasma viremia among ART-untreated individuals [16], and delaying viral rebound after periods of ART interruption compared with historical controls in chronically infected adults [20, 21]. The long-acting VRC01LS formulation improves tissue levels and may improve efficacy [22]. The bNAb 10-1074 targets the V3 glycan supersite on the HIV-1 envelope (Env) protein, which is a different Env binding site from VRC01. This potent monoclonal antibody has achieved 1.52 log HIV RNA reductions when used in chronically infected adults [23]. Combination use of two separate bNAbs which act at different binding sites has not been previously evaluated in a cohort of low-reservoir, early-treated children, and – like combined potent ART – has a high probability of successful maintenance of viral suppression.

Although chronically-infected adults receiving either VRC01 or 10-1074 did not achieve long-term viral suppression off ART, there are important differences between these prior studies and the proposed combination study in early-treated children, including: 1) previous trials did not use a combined approach to prevent the development of resistance to each bNAb, which is widely regarded to be critical for suppressive treatment with bNAbs (as has been established with ART) [24, 25], and which is supported by some non-human primate data [26]; 2) viral reservoir burden is higher in chronically infected adults than in very early treated children; 3) chronically infected adults have pre-existing resistance to VRC01 and 10-1074 (from prior immune responses to the virus) which allow for escape [20, 21], and this viral resistance would not be expected in early-treated children; 4) the prior studies employed only minimal overlap of bNAbs and ART, and a longer overlap period may reduce viral reservoir sufficiently to allow for a longer period off ART, and 5) immune effects of bNAbs in children with a developing immune system may be different than in adults, and may facilitate induction of more potent autologous cellular and humoral immune responses against HIV-1. Other monoclonal antibodies targeting HIV co-receptors have demonstrated the ability to maintain viral suppression in a majority of adult participants for over a year [27] or during the period of antibody administration [28].

2.1.3. Viral reservoirs can be reduced with bNAbs. bNAbs have been associated with reduction in viral reservoirs, particularly when used in combination with ART. In non-human primates, combination bNAb treatment has led to 4- to 10-fold reduction in HIV copies in PBMCs, lymph nodes, and the GI tract [29]. The bNAb 3BNC117, which also targets the CD4 binding site, was shown to accelerate clearance of infected cells in animal models [19]. Although one study of VRC01 administered to adults on suppressive ART did not alter markers of viral reservoir [30], that study administered only two doses of VRC01 to participants on long-term suppressive ART, and detectable changes in markers for the latent reservoir would not be expected. Data using VRC01LS in non-human primates demonstrated improved protection against HIV infection during rectal challenge, perhaps mediated by enhanced mucosal localization and more robust antibody-dependent cell-mediated cytotoxicity (ADCC) [22]. It is plausible that viral reservoir dynamics of early treated children differ sufficiently from adults with chronic infection prior to treatment, and that the design of the proposed study also differs sufficiently, to hypothesize potential viral reservoir reduction as measured in blood samples from the

combination of VRC01LS and 10-1074 in early treated children.

2.1.4. VRC01LS and 10-1074 resistance may be limited in early treated children. Among perinatally HIV-infected children, founder viruses have been susceptible to VRC01 [31, 32] because VRC01 binds a highly conserved epitope (the CD4 binding site) [33][27]. The likelihood of viral susceptibility within this restricted reservoir is a critical difference between the study population and adults with chronic infection, and will be further evaluated in this study. No data are currently available regarding resistance to 10-1074 in a pediatric population with early ART; this study will provide the first data for this population. Preliminary viral sequencing data from the EIT study children (who will be the same children eligible for this intervention) have demonstrated no concerning mutations for 10-1074 at positions 324, 326, 327 (M. Lichterfeld, unpublished data, 2018). Viral reservoir size is another consideration for resistance in children, and has been closely linked to age at ART initiation. IMPAACT P1030, which evaluated viral reservoir following ART initiation between 4 weeks and 6 months of age, demonstrated that after 2 years of viral suppression proviral HIV DNA remained detectable in all children but was correlated with the time to viral treatment/suppression [34]. Recent data support conservation of founder virus and lack of viral diversification with early-ART treatment [35]. Our preliminary data from the EIT Study in Botswana supports and extends these findings. Preliminary results from the first children in this study who started ART in the first week of life shows lack of HIV DNA detectability to a limit of 5 copies/million cells for the majority of children.

2.1.5. bNAbs may enhance immune responses. Unlike ART, bNAbs have the potential to engage host immune cells in viral defense. bNAbs have demonstrated the ability to enhance and broaden autologous humoral immune responses, possibly through stimulation of B cells or dendritic cells by viral antigen-antibody complexes [36, 37]; this may allow for children to develop improved antiviral immune responses that help control or limit viral rebound upon withdrawal of ART. ART from infancy limits the infant HIV-1 antibody response, and most early treated children lack long-lasting HIV-1-specific antibodies [38]. Thus, immune modulation using bNAbs may benefit children who started treatment early and for that reason have very limited autologous immune responses to HIV-1. This immunologic benefit may be more long-lasting than the bNAb treatment itself (potentially restricting viral rebound during periods of non-adherence to ART later in life). bNAbs may also enhance clearance of viral reservoir cells through antibody-dependent cellular cytotoxicity (ADCC) [22], which may contribute to natural viral control or delay viral rebound following ART interruption [29]. Finally, emerging data from experimental animal models with non-human primates suggest that bNabs may facilitate the development of more functional HIV-1-specific cellular immune responses, and support the development of an improved antiviral immune profile.

2.1.6. Early childhood may be the ideal time period for immunologic interventions directed against the HIV-1 reservoir. Our proposed intervention will occur after at least 96 weeks on ART in children who began ART within days after birth. In addition to the advantage of a long period on ART to maximally reduce viral reservoir by attrition of infected cells of the early infant immune system, this is a period of immune quiescence in children. This period of life has been called a “honeymoon period” [39, 40] when the immune system is well-adapted to control a variety of infections – possibly including HIV – without immunopathology. ADCC may be supported by the use of a bNAb among children in this age range, whereas it may not be sufficiently developed in younger infants.

2.1.7. Safety considerations for VRC01 and 10-1074: Passive polyclonal antibody products have been used for treatment and prophylaxis with excellent safety profiles over many decades. These agents include hepatitis B immunoglobulin (HBIG), Respigam®, Cytogam®, HIVIg, and IVIG. In a large study of IVIG to prevent infection in preterm infants published in 2011, adverse events were rare and did not differ between IVIG and placebo [41].

VRC01 has an extensive safety record when used in adult clinical trials (N=over 840 participants) [16, 21, 30, 42], and adverse events leading to VRC01 or placebo discontinuation have been limited to one participant with chest discomfort and one with rash. VRC01 has been used in pediatric clinical trials [43, 44], and there have been no safety concerns reported. IMPAACT P1112 is a Phase I trial enrolling HIV-1-exposed infants at birth at high risk of transmission. It is determining the PK and safety of a single dose of VRC01 to reduce HIV-1 transmission to infants. VRC01 has been well tolerated at 20mg/kg (N=13) and 40mg/kg subcutaneously (SC) (N=13). There have been eight grade 3 or higher events, all from causes that were not attributed to VRC01 (neutropenia, elevated total bilirubin, elevated creatinine, abdominal distension, and episodes of bronchiolitis). No systemic reactions were attributed to VRC01. Local infusion-related reactions (mild or moderate erythema, induration or edema) did occur in 15 infants; none were serious and almost all resolved within four hours of injection. Pain (grade 1) at the injection site was reported in only two infants. There have been no other adverse events attributed to VRC01. VRC01LS differs by 2 amino acids from VRC01 (which modifies binding characteristics and extends the half-life), and it is currently also being used in children in IMPAACT P1112. In preclinical non-human primate experiments, VRC01LS administered IV at doses from 0.02 to 20 mg/kg (n=26), or SC at a dose of 10 mg/mL (n=6), had no indication of local reactogenicity, anaphylactoid reaction, or any systemic disease [22]. In human studies, 39 adults have received VRC01LS at different doses delivered intravenously or subcutaneously in clinical studies, and there have been no serious adverse events reported [45].

A tissue cross-reactivity study for VRC01LS was performed by Charles River Laboratories, Frederick, MD, to determine the potential cross-reactivity of VRC01LS with cryosections of selected neonatal human tissues. No VRC01LS cross-reactivity was observed in any of the neonatal human tissues examined. Similarly, no tissue cross-reactivity of toxicologic relevance was identified for VRC01LS in adult tissues [46].

In pre-clinical studies of 10-1074, twice weekly IV administration of 10-1074 at doses of 4, 15, and 60mg/kg/injection as well as the combination IV dose of 10-1074 and 3BNC117 (each 60mg/kg once weekly), and twice weekly doses of 10-1074 at 60 mg/kg/injection via the subcutaneous route, for a 25-day dosing period were considered well-tolerated in male and female Sprague Dawley rats. Test item-related findings were consistent with an immune response to repeated administration of a foreign protein primarily in subcutaneously-dosed animals, as evidenced by hindpaw swelling, liver and spleen organ weight changes; as well as microscopic liver and spleen changes including mononuclear cell infiltrate and sinusoid histiocytosis/dilatation in the liver, and lymphoid hypercellularity, sinusoid histiocytosis and extramedullary hematopoiesis of the spleen, in all dose groups. These changes were either fully or nearly completely recovered by the end of the 45-day recovery period [47].

The bNAb 10-1074 has been used in adults with a reassuring safety profile. No grade 3 or 4 or serious treatment-related adverse events, or notable laboratory abnormalities, occurred during a total of 5,447 patient days of follow-up [23]. As of August 2017, 10-1074 had been administered to 76 research participants, at doses ranging from 3 mg/kg to 30 mg/kg; 44 of these participants were HIV infected. Protocol MCA-885 was an open label, dose-escalation, Phase 1 study to evaluate safety, pharmacokinetics and antiretroviral activity of 10-1074 in 33 HIV-uninfected and HIV- infected individuals. No grade 3, 4 or serious adverse events considered possibly related to 10-1074 were reported, and no clinically significant treatment-related changes in laboratory parameters occurred during study follow up. Of 11 infusion-related events that were considered at least possibly related adverse events (AEs), no related grade 3 or 4 events were observed. The most common adverse event reported under protocol MCA-885 was headache (N=5/33). One participant experienced malaise/fatigue, and one had elevated bilirubin.

A tissue cross-reactivity study for 10-1074 was performed on a full panel of tissues from humans and

rats. Although 10-1074 showed cytoplasmic binding in some tissues, this finding was considered of little to no toxicologic significance [48]. No specific tissue cross-reactivity studies have been performed for 10-1074 to monitor for off-target events in pediatric tissues. However, the study procedures and monitoring in place for this study will be sufficient to identify almost all issues of clinical concern, in the unlikely event of differences between adult and pediatric tissue cross-reactivity.

IV administration will occur rather than SC because the SC volume would exceed the safety limits for children over ~ 10 kg given the current VRC01LS formulation of 100mg/ml. IV administration will also occur for 10-1074, which has not been used SC. IV formulations take longer to administer, but may be associated with fewer injection site reactions than SC administration.

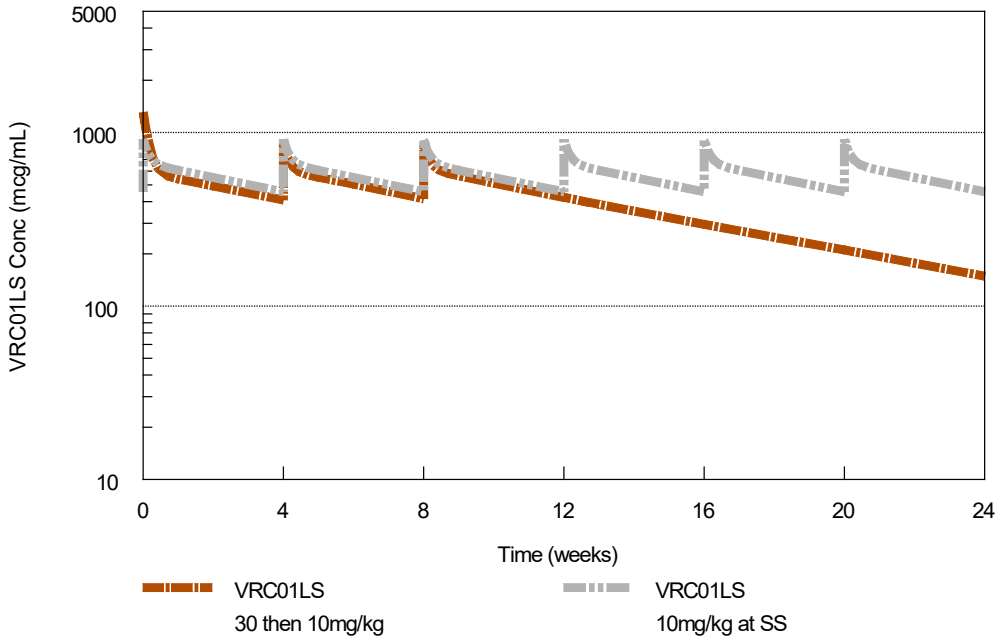
2.1.7.1. Safety considerations for combination of bNAbs: There are no indications to date for any safety considerations related to the combination of 2 different bNAbs. Data from at least 5 primate studies using combinations of bNAbs had no concerning safety signals from dual bNAb use [24, 49]. Two recent studies in HIV-infected and HIV-uninfected adults have evaluated 10-1074 in combination with 3BNC117 (which uses a similar CD4 binding site as VRC01), and there were no safety concerns using this combination when administering the antibodies in sequence. These studies have enrolled 25 HIV-infected and 24 HIV-uninfected participants, and in total there have been 9 AEs at least possibly related to the infusions, and no serious AEs have been reported to date in either protocol (Rockefeller University, unpublished data, 2018) [50].

2.1.8. VRC01LS and 10-1074 PK and dosing considerations:

2.1.8.1. PK Considerations for VRC01LS. For VRC01LS, target plasma levels for this study are based on preclinical studies of VRC01 that demonstrate over 91% of viral isolates tested across clades are neutralized at an ID_{50} of 50 µg/ml. VRC01 PK results in HIV-infected and uninfected patients have been published [16, 30], and a 40 mg/kg dose has been chosen for adults to maintain levels >50 µg/ml for 4 weeks, which leads to an overall median value closer to 100 µg/mL. VRC01LS has similar activity to VRC01, and these well-tolerated target concentrations can therefore be extrapolated. In HIV-infected adults receiving 40 mg/kg IV of VRC01, mean maximum serum concentrations were 1600 µg/ml ± standard deviation 230 µg/ml (n=5) after the first infusion, and 1500 µg/ml ±400 µg/ml after the second dose 28 days later. Mean 28-day trough serum concentrations were 57 µg/ml ±19 µg/ml (n=5) after the first dose, and 89 µg/ml ±40 µg/ml after the second dose (~ day 56). Similar results were reported for HIV-uninfected participants.

These PK and safety data for VRC01, coupled with results from adult studies of VRC01LS (VRC 606), allow for dosing estimation for children in the current study. In addition, VRC01LS dosing considerations have been described for children in IMPAACT P1112 [43]. VRC01LS differs by 2 amino acids from VRC01, and is expected to have a similar peak level with a longer half-life and improved concentration at mucosal surfaces. VRC01LS population PK parameters can be obtained from a PK analysis of study VRC606 [45] to generate a typical profile for a two-year-old given VRC01LS 30mg/kg loading dose IV followed by two maintenance doses of 10mg/kg (Figure 2).

Figure 2: VRC01LS Simulations - 2 year old

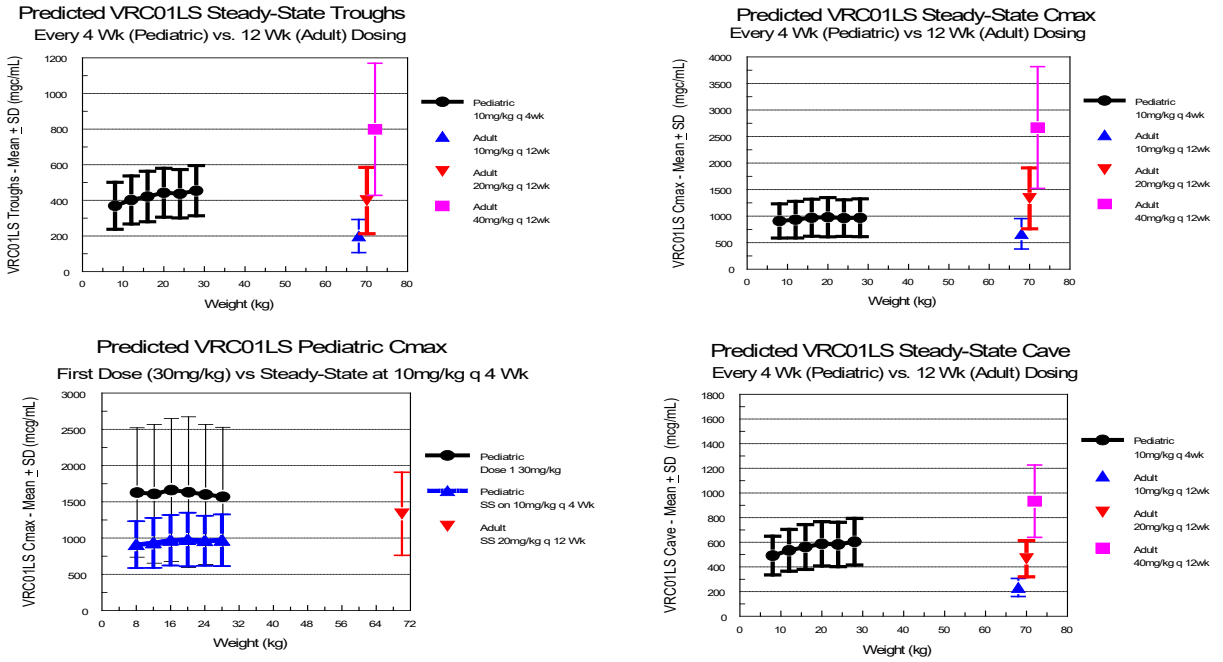


Conc: concentration; SS: steady state

Following administration of the 30mg/kg loading dose, the C_{max} and troughs are not expected to accumulate much with repeat 10 mg/kg dosing, with the expected steady-state trough concentrations with 10mg/kg IV greater than 400 µg/mL. We expect significantly higher initial VRC01LS concentrations than will be seen in infants from P1112 (40mg/kg SC), due to the lower slow and incomplete absorption following SC administration but the C_{max} with 10mg/kg every 4 weeks is still expected to be below the C_{max} of ~2200 µg/mL seen after 40mg/kg IV from VRC606 in adults. Additional data from P1112 is expected in 2018, and may be used to guide dosing further if needed.

The impact of weight on bNAb exposure metrics (C_{trough}, C_{max}, C_{ave}) is modest over the range of participant weights expected in the proposed study (Figure 3). Differences in exposure metrics at the weight extremes are about 20% or less for VRC01LS. The VRC01LS C_{max} modeled after the first dose (30mg/kg) is greater than at steady-state on 10mg/kg q4 weeks, but within the range seen in adults. Therefore, no stratification of dose, based on age or weight, is necessary for the current study.

Figure 3: VRC01LS Troughs, C_{max}, and C_{ave} by Body Weight



2.1.8.1.1. Pharmacokinetic Targets for VRC01LS

The VRC01LS target, 3rd dose trough (C84D), is derived from the multiple-dose PK results from adults in VRC606 incorporating expected PK differences due to age and weight in the study population [45, 50, 51]. Adult trough concentrations following multiple doses of 20mg/kg every 12 weeks are 150-300 mcg/mL and predicted adult average steady-state concentrations are 400-550 mcg/mL. The target for acceptable C84D trough concentrations for this study corresponds to achieving C84D that are greater than adults following 20mg/kg IV every 12 weeks and which also corresponds to the majority of subjects having average VRC01LS concentrations at or slightly above those seen in the adults. The pediatric dose of 10mg/kg every 4 weeks is predicted to achieve similar average steady-state concentrations to adults (20mg/kg every 12 weeks) of about 500-650 mcg/mL. With more frequent every-4-week dosing, pediatric peak-trough fluctuation will be reduced. Thus steady-state troughs are expected to be 50% higher and C_{maxs} lower than adults receiving VRC01LS 20mg/kg every 12 weeks. With VRC01LS's very long half-life, it would take at least 6 months of "maintenance" dosing to reach steady-state and steady-state will not be achieved in the PK Step of this study. Therefore, VRC01LS will be initiated as a loading dose of 30mg/kg IV followed by 10mg/kg IV every 4 weeks to rapidly achieve concentrations near predicted steady-state. The VRC01LS concentration 28 days after the third dose (second 10mg/kg dose), C84D, will be used to assess adequacy of the dosing regimen. Specifically, if the median C84D VRC01LS concentration is above 200 mcg/mL (~80% of the predicted adult steady-state trough), then VRC01LS dosing will be deemed adequate from a PK perspective.

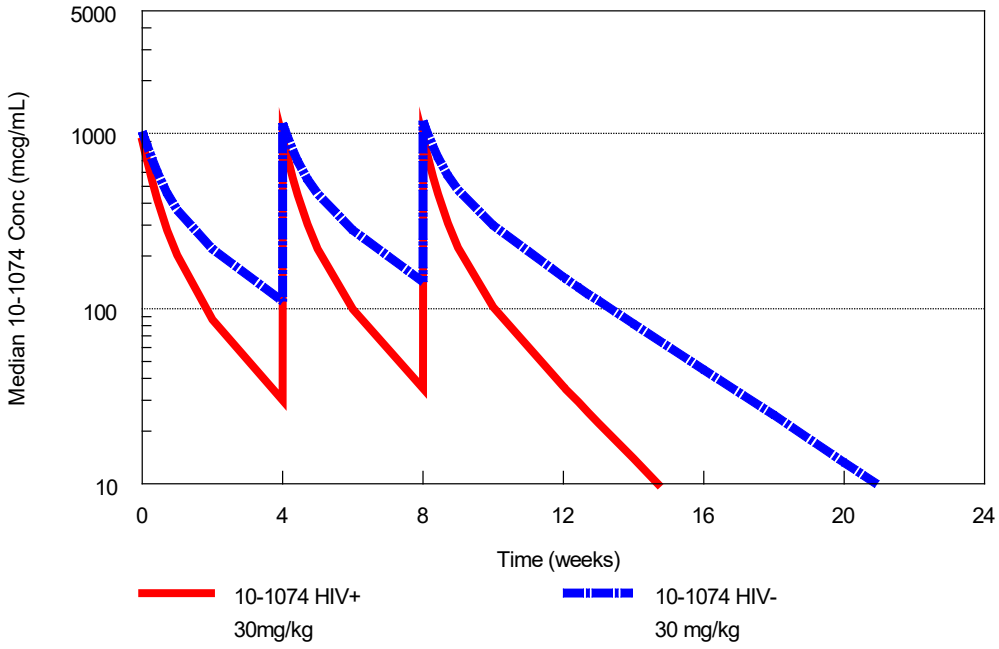
2.1.8.2. PK Considerations for 10-1074.

10-1074 has an average IC50 of 0.37 mcg/ml among 182 sensitive strains tested. For 10-1074, target plasma levels for this study are based on adult data presented by Caskey et al. [23]. This open-label phase 1 clinical trial was performed among 14 HIV-uninfected and 19 HIV-infected individuals who received a single IV infusion of 10-1074 at doses of 3, 10 or 30 mg/kg. 10-1074 serum levels were

determined by TZM.bl neutralization assay, and the dose of 30 mg/kg was associated with the maximal drop in HIV RNA of 1.52 log copies/mL. Viral nadir was reached at an average of 10.3 days (range 7-25 days). Serum half-life of 10-1074 was significantly different for individuals with and without HIV-1: for HIV-infected viremic participants, the half-life was 12.8 days, and for HIV-uninfected participants it was 24 days. It is expected that HIV-infected participants *with complete HIV RNA suppression* such as the children eligible for the proposed study will have serum half-life similar to those who are HIV-uninfected, and therefore dosing considerations for this study are based on the longer reported half-life of 24 days (and will also be evaluated in detail). It is also notable that pre-established mutations at V3-loop positions were associated with more rapid viral rebound, and that individuals lacking such mutations had longer time until rebound and low concentrations of 10-1074 (as low as 6.5µg/mL) at the time of rebound. Children in our proposed study do not have evidence of V3 loop mutations in archived virus, in sequencing performed to date (M. Lichterfeld, unpublished data, 2018).

Based on the data from Caskey et al., using extracted raw 10-1074 concentration data from the supplement files allows a population- PK analysis. This analysis reveals a similar elimination half-life to the published values from the non-compartmental analysis, with more rapid elimination in HIV-infected than HIV-uninfected subjects and a modest dose effect on elimination. In addition, this model can generate predicted profiles based on the proposed dosing regimen using a 2-year-old, 12.5 kg subject for the simulations, incorporating allometric scaling for size effects on PK parameters. This modeling is shown in Figure 4.

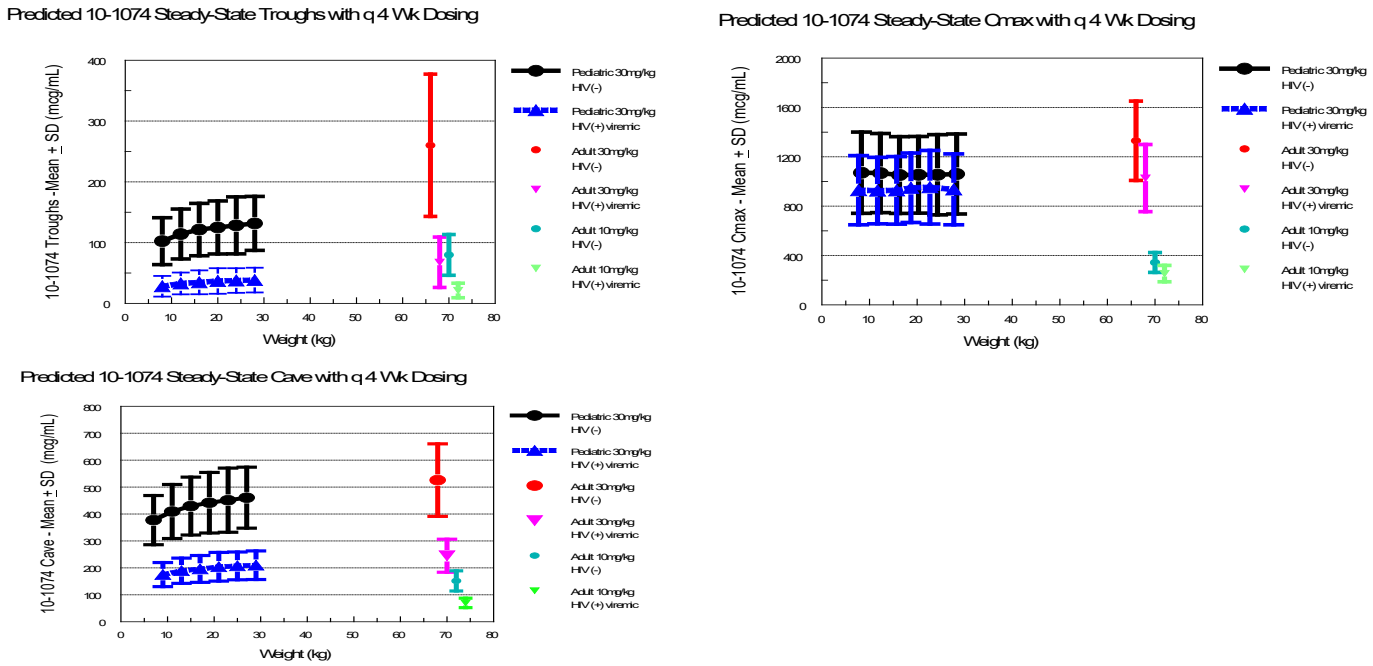
Figure 4: 10-1074 Simulations - 2 year old



Assuming no PK change with repeat dosing, the typical troughs after 2 doses in HIV-infected viremic subjects are ~30 µg/mL while those in HIV-uninfected are slightly over 100 µg/mL. Slight accumulation is expected after the second dose but the PK after 2 doses will be near steady-state.

The impact of weight on bNAb exposure metrics (C_{trough}, C_{max}, C_{ave}) are modest over the range of participant weights expected in the proposed study (Figure 5). Differences in exposure metrics at the weight extremes are about 30% or less for 10-1074. The impact of body weight modeled is greater for 10-1074 than VRC01LS and most pronounced on C_{trough}, as expected due to the shorter $t_{1/2}$ for 10-1074. However, expected bNAb exposure metrics are within the range of those seen or currently being studied in adults. Therefore, no stratification of dose, based on age or weight, is necessary for the current study.

Figure 5: 10-1074 Troughs, C_{max}, and C_{ave} by body weight



2.1.8.2.1 Pharmacokinetic Targets for 10-1074

The 10-1074 target for this study will also be based on the trough after the 3rd dose, C84D. Based on analysis of the PK data from Caskey et al., the expected average concentrations in HIV infected adults with 30mg/kg at steady-state are 150-300 mcg/mL. Single doses of 30 mg/kg and 10 mg/kg in adults exhibited good safety and short-term virologic activity–greater than 1 log HIVRNA reduction. Using a 30 mg/kg dose in young children is expected to be safe and result in 10-1074 concentrations that are about 25% lower than in adults. However, the pediatric concentrations are still expected to exceed those seen in adults following 10mg/kg by several fold. Since 10-1074 has a much shorter half-life than VRC01LS, no “loading” dose is needed to accelerate achievement of steady-state concentrations. Based on safety and tolerability following 30mg/kg in adults, 30mg/kg will be the pediatric dosage used in the current study. Pediatric 10-1074 third dose troughs, C84D, with this dosage are anticipated to be lower at steady-state than expected in adults on 30mg/kg but higher than in adults on 10mg/kg. Therefore, the acceptable target for C84D 10-1074 in this study will be a median that exceeds that expected from 10mg/kg in adults (7.5mcg/mL). The pediatric 30mg/kg dose is expected to result in nearly all of the participants maintaining trough concentrations above the target of 7.5 mcg/mL.

2.1.8.3. Co-administration. Regarding PK considerations for the co-administration of VRC01LS and 10-1074 (in a serial manner), animal data and initial human data suggest no PK concerns when using dual bNAbs. In data recently presented by Cohen et al. at CROI 2018 [50], PK results for the combination of 10-1074 and 3BNC117 were nearly identical to those found when each product was administered alone. This combination closely resembles our combination of 10-1074 with VRC01LS, and provides good assurance that no dosing adjustments will be required when using combination therapy. Step 1 of our study will also closely evaluate for any PK changes with dual bNAb administration in the first 6 participants who receive dual bNAb treatment. Despite the low suspicion that dual bNAb administration will alter PK parameters, the study team and SMC will evaluate for changes from the pre-specified PK targets (as listed above) for both VRC01LS and 10-1074 in these 6 participants to make a decision regarding further enrollment into Step 1 and starting Step 2. Trough PK values will be collected in all subsequent participants and will be stored for later analysis.

2.2. Rationale

2.2.1. Proof of Concept: The EIT cohort provides an ideal low-reservoir pediatric population in which to test whether viral suppression can be maintained for up to 24 weeks with dual bNAb therapy. Children in this cohort will have received at least 96 weeks of ART from very early in life, and will receive 8 weeks of overlapping ART plus VRC01LS/10-1074 prior to stopping ART for up to 24 weeks. Our design is practical in its approach, aiming to demonstrate as a proof-of concept that in a low-reservoir pediatric population dual bNAb therapy alone may be sufficient to maintain viral suppression for 24 weeks. If at least 30% of participants remain virally suppressed after 6 months of dual bNAb treatment following ART cessation, this will be a significant difference from prior cohorts such as the CHER Study where <6% of children treated in the first year of life remained virally suppressed by 6 months off ART in the absence of an intervention [52]. Our study design also allows for detailed study of viral reservoir changes as measured in blood samples during the addition of bNAbs to ART and following transition to bNAb maintenance; it accounts for ethical concerns by offering all participants a potentially effective intervention in a highly controlled manner to maintain viral suppression, while still ensuring interpretable study endpoints; and it is affordable and efficient because of the existing EIT study cohort.

2.2.2. Characterizing the Impact of VRC01LS and 10-1074 on the Infant Virologic Reservoir: We will apply state-of-the art virologic studies to quantify and characterize the viral reservoir in study participants; blood samples. These assays include quantitative digital droplet PCR (ddPCR) measurement of integrated (proviral) HIV-1 DNA, cell-associated HIV-1 RNA, and plasma HIV-1 RNA measured by a highly sensitive single-copy assay [53]. In addition, we will quantify the number of intact proviruses by use of a novel single-genome amplification strategy that combines limiting dilution PCR of near-full-length genomes with next-gen (Illumina) sequencing of the clonal amplicons. This approach will allow us to identify phylogenetic associations between viral sequences longitudinally collected during the proposed study, and is highly informative for characterizing viral sequence evolution and escape mutations in response to bNabs, autologous cellular immune responses or antiretroviral agents. In addition, this assay is more feasible than performing virus outgrowth assays for identifying genome-intact and replication-competent virus, given limitations on blood volume that can be obtained from young children, and helps to track clonal expansion of virally infected cells based on complete sequence identity of viral DNA products generated from discrete cells.

2.2.3. Unique Opportunity for Immunologic Evaluation: A major innovative component of this study is the ability to evaluate immunomodulatory effects of bNAbs on innate and adaptive immune responses in a group of pediatric HIV-1 patients specifically selected to have minimal residual viral reservoirs. So far, investigations of immune effects mediated by bNAbs have been restricted to animal

models or adult HIV-1 patients [36, 37]. While these studies suggested that bNAbs may be able to affect the breadth, magnitude and potency of antiviral humoral and cellular immune responses, immunological effects of bNAbs in the context of a developing immune system in children, specifically in those with an extremely low reservoir of virally infected cells, at present remain entirely unknown. We hypothesize that controlled exposure to low-level viremia during bNAb treatment (in the absence of ART) will support the evolution of highly functional autologous antiviral immune responses that may, in conjunction with direct antiviral effects of the bNAbs, allow for viral control to be maintained for a prolonged time period off conventional ART. In addition, our studies will, for the first time in pediatric patients, allow for detailed profiling of associations between antiviral immune responses and the size and cellular composition of viral reservoir cells in the setting of bNAb treatment.

2.3. Study Hypotheses

2.3.1. Specific Aim 1: VRC01LS and 10-1074 will not be associated with serious adverse events, and median trough concentrations at the doses used will be above established target values of 200 mcg/mL and 7.5 mcg/mL respectively. At least 70% of early-treated low-reservoir children will remain with HIV-1 RNA <400 copies/mL after 24 weeks of receiving VRC01LS and 10-1074 maintenance.

2.3.2. Specific Aim 2: Measurable decline in viral reservoir as measured in blood samples will occur in some children with the addition of dual bNAb therapy to ART. Viral reservoir size prior to receiving ART and prior to receiving VRC01LS and 10-1074, as determined by cell associated HIV-1 DNA and other assays, is inversely correlated to viral rebound kinetics (if rebound occurs on bNAb maintenance). If present, viral rebound will not exceed 1,000 copies/mL prior to re-initiation of ART (and acute retroviral syndrome will not occur). Delayed HIV-1 RNA and DNA suppression patterns in early life will correlate with viral rebound during the ATI. Baseline HIV from most children will be sensitive to VRC01LS and 10-1074, and a decrease in sensitivity will be detectable among children who experience viral rebound.

2.3.3. Specific Aim 3: The breadth of humoral immune responses to HIV-infection will increase following VRC01LS and 10-1074 use. Markers of cellular immune activation may increase on bNAb maintenance. Cell-mediated immune responses to HIV-1 will be preferentially detectable among children with viral rebound.

3. PRIMARY OBJECTIVES

Specific Aim 1: To conduct an interventional clinical trial to determine the safety, pharmacokinetics, dosing and antiviral efficacy of up to 24 weeks of maintenance VRC01LS and 10-1074 immunotherapy in early-treated HIV-1 infected children in Botswana. This uncontrolled, open-label clinical trial will be conducted at two sites in Botswana, using established clinical research infrastructure provided by the Botswana-Harvard Partnership (BHP). The primary endpoints of this study will be the frequency and severity of treatment-associated serious adverse events, and the proportion of children who maintain HIV-1 plasma RNA <400 copies/mL (and <40 copies/mL) 32 weeks after initiating dual bNAb treatment (24 weeks after withdrawal of standard ART). Secondary endpoints will include pharmacokinetic profiles of all children who receive VRC01LS and 10-1074 and growth characteristics of virally suppressed children receiving bNAbs.

Specific Aim 2: To evaluate effects of treatment with VRC01LS and 10-1074 on the size and cellular composition of residual viral reservoirs. Viral reservoirs during treatment with bNAbs will be evaluated in blood samples using a novel single-template, near full-length viral sequencing assay

that provides information on the complete spectrum of HIV-1 DNA copies integrated into host chromosomes. This approach will allow for evaluation of defective vs. intact HIV-1 genomes, antiretroviral drug and CTL escape variants and longitudinal viral sequence evolution in total CD4 T cells as well as in phenotypically-complex subsets of CD4 T cells known to be critical for viral reservoir stabilization. Cell-associated HIV-1 RNA and plasma RNA will be simultaneously analyzed and sequenced. Viral reservoir parameters will be correlated to viral rebound kinetics during ATI, to pharmacokinetic measurements of VRC01LS and 10-1074, and to antiviral immune parameters determined in Specific Aim 3.

Specific Aim 3: To investigate the influence of VRC01LS and 10-1074 treatment on the magnitude and quality of antiviral innate and adaptive immune responses. Data from clinical trials in HIV-1-infected adults show that bNAbs can profoundly influence autologous HIV-1 immune responses. We will use comprehensive phenotypic and functional assays to profile effects of bNAb treatment on biomarkers of immune activation and on innate cellular immune responses, coupled with RNA-Seq-based gene expression studies in selected immune cell subsets. In addition, the evolution of neutralizing antibody breadth and the frequency and functionality of HIV-1-specific T cells will be analyzed, and combined with viral sequencing data (generated in Specific Aim 2) to identify viral immune escape.

4. STUDY DESIGN

PK Step

The first 12 participants in the study will undergo the PK Step, to allow for detailed safety and PK assessment of each bNAb alone. In the PK Step, while continuing ART, participants 1, 3, 5, 7, 9, 11 will receive VRC01LS at Week 0, Week 4, and Week 8 as a single intravenous (IV) dose (30 mg/kg load, then 10mg/kg maintenance); safety and PK measurements will occur at end of infusion, 1 hour post-infusion, 1 day post-infusion, 1 week post-infusion, 2 weeks post-infusion, and 4 weeks post-infusion. Participants 2, 4, 6, 8, 10, 12 will receive 10-1074 at Week 0, Week 4, and Week 8 as a single IV dose (30mg/kg), and safety and PK measurements will also occur at end of infusion, 1 hour post-infusion, 1 day post-infusion, 1 week post-infusion, 2 weeks post-infusion, and 4 weeks post-infusion. In the PK Step, it is anticipated that all 12 participants will be from the EIT Study; however, in the event that 12 participants from EIT are not available at the time the PK Step begins, participants 9-12 may be recruited from other virally suppressed HIV-infected children. These additional, non-EIT children would not be eligible for further study steps after completing the PK Step. In the event that a participant discontinues bNAb during the PK Step, the participant may be replaced to allow for PK data to be obtained from 12 participants total.

SMC Review of Safety and PK Data from PK Step

After all 12 PK Step participants complete the PK Step, safety, PK, and viral suppression data will be reviewed within 8 weeks by the study team, sponsor, and SMC (earlier review by the SMC may be triggered based on pre-specified safety parameters). All PK data from the PK Step will be assessed based on pre-specified PK trough targets; peak values will be assessed, but primary guidance for peak values will be based on toxicity. The study team will determine if median trough concentrations are above the pre-specified targets (see Section 2.1.8), and present this information in a written report to the SMC. In addition, outlier concentrations will be presented and reviewed; the study team expects no more than 2 participants to have trough concentrations below 115 mcg/mL for VRC01LS and no more than 2 participants to have trough concentrations below 5mcg/mL for 10-1074. The SMC and

the study team will review any cases of outlier PK concentrations and make individual recommendations about whether a child with these levels should proceed to Steps 1-3. The SMC will review safety data utilizing pre-defined safety criteria defined in the protocol (see Section 13.8). If safety criteria are met, and if the PK data are in the pre-specified range, approval to begin Step 1 may be granted by the SMC. If the dosing and frequency of bNAbs established in the PK Step is acceptable, it will continue to be used for the first 6 participants in Step 1 (see additional safety review below). If a change in dosing is recommended by experts on the study team (or by the SMC, if it does not accept the team's recommendation), adjustments will occur and IRB and study sponsor approval will be sought regarding required protocol changes prior to starting Step 1. The recommendations by the study team or by the SMC may include early termination of the study, if warranted by the safety/PK data, or a re-design of the study as approved by the study sponsors and regulatory boards.

Step 1 (first 6 participants)

All Step 1-3 participants must be from the EIT Study. After approval, Step 1 will begin for a maximum of 6 participants, all of whom will have participated in the PK Step (3 from each PK Step group), and all of whom will have been >12 weeks from last bNAb dosing. ART is continued throughout Step 1, and all participants receive both 10-1074 and VRC01LS. If dosing recommendations do not change based on the PK Step, a VRC01LS loading dose of 30 mg/kg will be given at the start of Step 1, followed by 10mg/kg dosing at each 4-weekly visit, and 10-1074 dosing will be at 30mg/kg at each 4-weekly visit. At Week 0, Week 4, and Week 8 doses of Step 1, PK testing for both bNAbs will occur prior to infusion, and at Weeks 0 and 4 only at end of infusion, 1 hour post-infusion, 1 day post-infusion, and 1 week post-infusion. HIV-1 RNA will be checked every 4 weeks in Step 1. The first 6 participants in Step 1 will continue in this Step, receiving both oral ART and dual bNAb infusions, until the second study team/SMC review occurs. This review of safety and PK data will occur within 8 weeks of the 6th participant's Week 8 PK sampling.

SMC Review of Safety and PK Data from Step 1 (first 6 participants)

Safety, PK, and viral suppression data for the first 6 Step 1 participants will be reviewed within 8 weeks by the study team, sponsor, and SMC. All PK data will be assessed by the study team based on pre-specified PK trough targets which are the same as those used in the PK Step. The review will confirm that PK targets are met during dual bNAb administration. Safety criteria for the Step 1 data will be reviewed by the SMC, using similar criteria as for the PK Step (see Section 13.8). If safety criteria are met, and if the PK data for both bNAbs are in the pre-specified range, the SMC may approve entry into Step 2 for the first 6 participants and enrollment of additional participants into Step 1. If the PK data are in range and no dosing changes for the bNAbs are required, the dosing will continue to be used for the remainder of the study unless new data emerges requiring additional review. If a change in dosing is recommended by experts on the study team (or by the SMC, if it does not accept the team's recommendation), IRB and study sponsor approval will be sought regarding required protocol changes. The recommendations by the study team or by the SMC may include early termination of the study, if warranted by the safety/PK data, or a re-design of the study as approved by the study sponsors and regulatory boards.

Step 1 (all subsequent participants)

After approval to proceed is obtained by the SMC, additional participants will enroll directly into Step 1 and receive dual bNAbs and ART for 8 weeks. All study visits and follow-up will be the same as for the first 6 Step 1 participants, with the exception of PK testing; only trough PK testing will be required for these participants, beginning at Week 4 of Step 1.

Step 2 (all participants)

Following a successful Step 1 review, Step 2 may begin. Step 2 will begin after a participant has spent at least 8 weeks in Step 1. For the first 6 participants, Step 2 will begin at a scheduled bNAb dosing visit (i.e. 4 weeks from last Step 1 bNAb dosing), and for all subsequent participants it will begin at the 8 Week visit date for Step 1 (which becomes the Week 0 Step 2 visit). Participants with ongoing viral suppression throughout Step 1 will undergo withdrawal of ART and will continue maintenance 10-1074 (30mg/kg) and VRC01LS (10mg/kg) treatment for up to 24 weeks in Step 2. HIV-1 RNA will be checked weekly from Weeks 1-4 in Step 2, and every 2 weeks thereafter. Qualitative HIV DNA will be checked at each visit by DBS. If a participant experiences a single viral rebound ≥400 copies/mL in Step 2, ART will be re-started (and VRC01LS and 10-1074 will be discontinued). A single viral rebound is defined as an HIV RNA result ≥400 copies/mL at any Step 2 visit, without need for confirmation. If HIV RNA becomes ≥400 copies/mL prior to the end of Step 2, all evaluations ordinarily scheduled at "Week 24" will be performed immediately prior to ART re-initiation. If HIV-1 RNA is ≥40 copies/mL but <400 copies/mL, or if Qualitative DNA turns from negative to positive, virologic monitoring tests will be repeated *weekly*. Clinical examination will occur at each study visit.

Step 3 (all participants)

All participants will end Step 2 after 24 weeks, and enter Step 3. In Step 3, both 10-1074 and VRC01LS will be discontinued, and ART will be re-started. Follow-up visits in Step 3 will occur at 4, 12, and 24 weeks.

5. STUDY SITES AND STUDY POPULATION

5.1. Clinical Sites

This protocol will be implemented at two NIAID-approved BHP clinical research sites in Botswana: Gaborone (CRS #31833) and Francistown (CRS #31891). The Botswana-Harvard School of Public Health AIDS Initiative Partnership (BHP) was established in 1996 as a signed agreement between the Ministry of Health (MOH) of Botswana and the Harvard School of Public Health AIDS Initiative. BHP now maintains one of the largest research laboratories for HIV/AIDS-related work in Africa. Multiple studies enrolling more than 7,000 participants have been conducted at BHP, including HIV vaccine, prevention of mother-to-child transmission (PMTCT), ART trials (Phase I through Phase III), as well as observational studies; most of these studies have been NIH-funded. In 2006, BHP was selected as a Division of AIDS (DAIDS) "Clinical Trials Unit" (CTU) for the AIDS Clinical Trials Group (ACTG) and the International Maternal Pediatric Adolescent AIDS Clinical Trials (IMPAACT) group, and has enrolled to three studies of the HIV Prevention Trials Network (HPTN). BHP works in close collaboration with the MOH in the conduct of clinical trials.

5.2. Laboratory Sites

Laboratory techniques will be performed according to the most current approved protocols at each reference laboratory.

5.2.1. Botswana

Testing of samples collected at Gaborone:

HIV DNA PCR, hematology, chemistries, CD4+ cell counts, HIV-1 RNA measurements, and plasma/cell separation will be carried out at the Botswana-Harvard HIV Reference Laboratory (BHHRL), adjacent to Princess Marina Hospital in Gaborone.

Testing of samples collected at Francistown:

Samples will be processed at the National HIV Reference Laboratory in Francistown and either shipped to BHHRL in real-time, or run in real-time in Francistown, or stored at -70ºC for later shipment to BHHRL.

Testing of samples at collaborating institutions:

Samples may occasionally be sent between BHP sites or to designated DAIDS-approved back-up laboratory sites. For assays that cannot be performed at BHHRL, samples from Gaborone and Francistown will be processed and stored at -70ºC and later shipped to either Brigham and Women's Hospital in Boston, Massachusetts General Hospital / Ragon Institute in Boston, or to another designated facility for specialized testing. PK and anti-drug antibody (ADA) testing will be performed at the Vaccine Research Center in Bethesda, Maryland, at Duke University in Durham, North Carolina, and at Dartmouth University in Hanover, New Hampshire.

5.2.2. Brigham and Women's Hospital, Boston: The Kuritzkes Laboratory at Brigham and Women's Hospital is a research and diagnostic virology laboratory. The laboratory conforms to Good Laboratory Practices for the performance of research assays applied to samples from clinical trials.

5.2.3. Massachusetts General Hospital / Ragon Institute, Boston: The Ragon Institute at Massachusetts General Hospital (MGH), Massachusetts Institute of Technology (MIT) and Harvard is located in Cambridge, MA. The Center includes laboratory-based clinical investigators, clinic-based clinical researchers, and fellows who are conducting research in HIV virology, immunology, vaccine development, antiviral drug resistance, antiviral therapy, opportunistic infections, immunotherapy, AIDS-related malignancies and HIV epidemiology. There are several BL2+ containment rooms with a total of 80 laminar flow hoods and a vantage FACS cell sorter in large FLOW Cytometry CORE facility. There are adequate hoods, incubators, freezers, centrifuges, and other equipment necessary to conduct work with HIV positive specimens and to grow viral stocks and perform work on BL2+ level. To assess HIV-1 specific neutralizing antibody responses, additional immunologic testing of the samples shipped to MGH will be performed by the laboratory of Dr. Michael Seaman at Beth Israel Deaconess Medical Center in Boston.

5.2.4. PK Testing and ADA Testing for VRC01LS and 10-1074

5.2.4.1. Vaccine Research Center at NIH will perform PK testing and ADA testing for the VRC01LS. VRC01LS concentration in participant plasma samples will be quantified using the murine anti-VRC01 monoclonal antibody 5C9 as previously described [30]. Quantification of VRC01LS concentrations will be performed in 96-well plates on a Beckman Biomek-based automation platform (Beckman Coulter, Brea, CA, USA) utilizing the monoclonal antibody 5C9 for VRC01LS detection. If PK testing is required to confirm low/undetectable levels of standard ART during Step 2, it will be requested for selected specimens based on clinical responses, using the existing PK specimens sent to VRC.

5.2.4.2. The lab of Georgia Tomaras at Duke University will perform the PK testing for the 10-1074. Levels of 10-1074 will be determined by a validated sandwich ELISA, as previously described [54]. The serum concentration of 10-1074 will be calculated from the standard curve of 10-1074 run on the same plate using a 5-PL curve fitting algorithm.

5.2.4.3. The lab of Margaret Ackerman at Dartmouth University will perform the ADA testing for the 10-1074. The Ackerman laboratory has developed high throughput tools to evaluate antibody response to aid in therapeutic antibody and vaccine design and development. The ADA characterization assays are performed using a tiered testing approach to identify and define ADA responses that may arise during clinical trials. In short, drug products are conjugated with either biotin or a Sulfo-Tag label. Biotinylated and Sulfo-Tagged drug are then mixed with serum and if ADAs are present, they will act as a bridge between labeled forms of the drug. Biotinylated drug bridged to Sulfo-tagged drug can then be measured using the Meso Scale Diagnostics™ platform and a pre-established cut-point can be used to define positivity (Tier 1). If a sample is Tier 1 positive, a competition assay (Tier 2) with free drug is used to determine if the interaction between the drug and sample components is specific according to a reduction threshold determined during assay development. Finally, Tier 1 and Tier 2 positive samples will be titered to determine the relative magnitude of the ADA response.

5.3. Potential Study Participants

All participants receiving dual bNAb treatment will be recruited from the EIT Study. EIT is an ongoing NIAID-funded (U01AI114235) clinical trial of early infant diagnosis and treatment performed at BHP by the same investigative team (Multiple PIs Drs. Shapiro, Kuritzkes, and Lichterfeld). The study successfully launched in 2015, and has accrued 40 antepartum infected participants. *In utero* HIV infection has been identified in ~ 0.5% of infants screened at government maternity wards [55]. HIV-infected infants are offered enrollment in the study, and ART is started within 7 days of life (usually within 48 hours) if antenatal infection is identified, or before 57 days of life if intrapartum infection is identified. ART is provided through the Botswana government, and the initial ART regimen is nevirapine (NVP)/zidovudine (ZDV)/ lamivudine (3TC) for infants <2 weeks/40 weeks gestational age equivalent; thereafter the regimen is changed to lopinavir/ritonavir/ZDV/3TC. The aims of the study are to demonstrate that ART with NVP, ZDV, and 3TC can be safely initiated very early in life after diagnosis of HIV infection and will result in rapid viral decay in most infants, and to evaluate virologic and immunologic outcomes of very early ART in infancy.

A small number of children who are not enrolled in EIT may participate in the PK Step only of this study, if additional participants are needed to complete the PK step with 12 participants in a timely fashion. These children will go off-study after the PK Step and will not be eligible to participate in Steps 1-3.

5.4. Inclusion / Exclusion Criteria

We plan to enroll only children on long-term (for at least 96 weeks) suppressive ART initiated within 7 days of birth (or those with peripartum infection who initiated ART within 57 days of life), i.e. EIT cohort participants, in Steps 1-3; non-EIT children may participate in the PK Step only. Specific criteria are as follows:

5.4.1. Inclusion Criteria

Inclusion Criteria for PK Step*:

1) On ART for at least 96 weeks
2) ≥ 96 weeks and <5 years of age at enrollment
3) HIV RNA <40 copies/mL** for at least 24 weeks prior to entry
4) Ability to remain in close study follow-up for at least 12 weeks
5) Willingness to receive IV infusions of bNAbs
6) Willingness to provide signed informed consent (by the parent/guardian)

*It is anticipated that all children in PK Step will be from EIT Study; however, up to 4 HIV+ children outside the PK Study (age range 2-5 years) may participate in the PK Step if otherwise eligible and if EIT children are unavailable.
** All EIT children have HIV RNA drawn at least every 12 weeks. For non-EIT children or in the event of missing values, minimum HIV RNA criteria include lack of known detectable HIV RNA ≥40 copies/mL in past 36 weeks (note: viral suppression confirmed from tests with <400 copies/mL as threshold does not constitute "known detectable HIV RNA"), plus documented suppression to <40 copies/mL within 4 weeks of PK Step entry.

Inclusion Criteria for Entry into Step 1 (followed by participation in Steps 2-3):

1) EIT Study participant
2) On ART for at least 96 weeks
3) ≥96 weeks and <5 years of age at enrollment
4) HIV RNA <40 copies/mL for at least 24 weeks prior to entry**
5) Ability to remain in close study follow-up for at least 56 weeks
6) Willingness to receive IV infusions of bNAbs
7) Willingness to provide signed informed consent (by the parent/guardian)

** All EIT children have HIV RNA drawn at least every 12 weeks. In the event of missing values, minimum HIV RNA criteria include lack of known detectable HIV RNA ≥40 copies/mL in past 36 weeks, plus documented suppression to <40 copies/mL within 4 weeks of Step 1 entry.

5.4.2. Exclusion Criteria:

1) Medical condition making survival for at least 32 weeks unlikely
2) Active tuberculosis or malignancy
3) Actively breastfeeding
4) Previous receipt of VRC01/VRC01LS or 10-1074 (except during the PK Step)

It is anticipated that this group of participants will have a limited viral reservoir, based on previous work showing that early infant treatment limits, but does not prevent, establishment of the HIV reservoir [56]. We hypothesize that a smaller viral reservoir in these children will improve the chances for maintaining viral suppression with dual bNAbs. We believe that adding additional inclusion criteria based on immunologic parameters or a lower HIV-1 RNA or DNA threshold may limit participation in the study without clear data suggesting that stricter parameters are required for a successful intervention. Stricter criteria, especially those based on non-standard tests available only in specialized laboratories, might unnecessarily restrict the applicability and interpretability of data generated from this intervention. In addition, preliminary data from the first 10 children in the EIT study suggest that similar low viral reservoir (median ~ 5 copies/million PBMCs) occurred at 84-96 weeks even among children with brief periods of viral rebound (BHP, unpublished data, 2018).

5.5. Recruitment Process

Children who enrolled in the EIT study and have reached at least 96 weeks of life and meet entry criteria are eligible for the intervention (and 4 additional children outside of the EIT Study may be eligible for the PK Step only). All children enrolled in the EIT Study will be eligible for screening for the proposed intervention after 84 weeks of ART (and may enter the intervention at or after 96 weeks of ART). Screening evaluation and testing will be performed as part of the routine visits in the EIT Study. By definition, all participants in the EIT Study meet certain baseline criteria to be part of the EIT Study. For *in utero* infected children, this includes being HIV DNA PCR positive within 96 hours of life and starting ART before 7 days of life. For intrapartum infected children, it includes being HIV DNA PCR negative by 96 hours of life, but HIV positive and on ART before 57 days of life.

To allow for careful monitoring of participant safety, no more than two children will enroll per week for the entire study (regardless of site of enrollment). We anticipate that mothers/caregivers for almost all eligible children (90-100%) will choose to have their children participate in the intervention study. We anticipate this high acceptability based on their current engagement in the EIT study, the potential future benefits to HIV-infected children if the intervention is effective, and the interest expressed by mothers/caregivers in potential alternatives to daily ART dosing. We therefore anticipate a sample size of 20-36 EIT children for the intervention study (if 35-40 children are available at or after 96 weeks from EIT, 0-5 mothers/caregivers decline, and 4-10 are ineligible). An additional 4 non-EIT children may participate in the PK Step in the event that fewer than 12 EIT children are immediately available for this study step at the time it is enrolling (i.e., if fewer than 12 EIT children have reached the 96 week eligibility window at the time the PK Step is enrolling, or if a greater number than anticipated are ineligible or do not consent). Thus, the total possible number of participants in all study steps is 36 if all PK Step participants are from EIT, but may be as high as 40 if 4 non-EIT participants are enrolled in the PK Step.

To enroll non-EIT children in the PK Step, study staff will liaise with staff at local pediatric HIV clinics to identify potentially eligible children. The parent/guardian will be given information about the study, and if interested, study staff will meet with the parent/guardian to provide further education about the study and to obtain informed consent.

5.6. Study Consent

Consent will be obtained from the parent/guardian. Assent of the children involved in this study will not be sought because they are too young to be capable of providing assent. The protocol-specific consent forms describe the study products to be used and all aspects of protocol participation (See Section 15). Elements of informed consent are included as described in Title 45, CFR Part 46 and Title 21 CFR, Part 50, and in the International Conference on Harmonisation (ICH) E6, Good Clinical Practice: Consolidated Guidance 4.8. In addition to IRBs and all regulatory agencies with oversight of the study, a BHP Community Advisory Board will review the consent forms before they are finalized. This review will include issues of cultural competence, local language considerations, and the level of understandability.

The consent process will begin at or after the 84-week EIT study visit at the EIT Study Clinic. Separate consent forms will be used for the PK Step and for the intervention study (Steps 1-3). EIT participants enrolled in the PK Step will remain in the EIT Study (EIT visits will be performed at the time of PK Step visits, if required). EIT participants enrolled in Steps 1-3 will permanently exit the EIT Study at the time of Step 1 entry. However, in all cases this will be a seamless transition for the

participants. Care will be provided by the same study team, and consent will be obtained to share all data and records as needed between the two studies.

5.7. Reimbursement

We will offer reimbursement for participant transport (or will provide patient transport), and 100-150 Botswana Pula (approximately $11.00-16.50) per attended scheduled visit to compensate for participant time commitment. We will also offer reimbursement for unscheduled visits that are requested of the participant for follow-up tests. This compensation rate is slightly higher than other BHP studies because of the intensity of follow-up for each child, the need for a blood draw at most visits, the need for IV infusion, and because of the critical importance of maintaining close contact with each participant.

5.8. Participant Retention, Missed Visits, Study Withdrawal

We anticipate 100% of children enrolled will be retained in the cohort, as has been the case for the EIT Study. Resources will be devoted to cohort retention, transport provided as needed, and care providers will be compensated fairly for their time at a rate approved by the Botswana IRB. Most women who enroll infants in BHP studies have a cell phone number or a family member with a cell phone number. We will perform home visits if requested (for visits other than bNAb administration) and will perform calls and home visits for any missed study visit. If bNAb administration is not possible within a study visit window, ART will be re-started. Likewise, if a parent/guardian requests withdrawal from the intervention during the bNAb-only phase, ART will be re-started.

6. STUDY PRODUCT AND INTERVENTIONS

6.1. VRC01LS Description, Supply, and Storage

VRC01LS is a recombinant human immunoglobulin G1 (IgG1) monoclonal antibody produced in a CHO cell line that binds to the CD4 binding site of gp120. The antibody was originally isolated from an adult infected with HIV-1 who maintained viral control without ART. Investigators at the Vaccine Research Center (VRC) isolated and screened memory B lymphocytes from peripheral blood mononuclear cells (PBMC) of HIV-1-infected donors to identify this antibody clone. VRC01 was found to neutralize more than 90% of genetically diverse heterologous strains of HIV-1 at a concentration of 50 mcg/mL, and more than 70% at a concentration of 1 µg/mL. VRC01LS is modified by 2 amino acids to have longer half-life than VRC01.

VRC01LS is manufactured under cGMP using a stably transfected CHO cell line, purified, and the drug product vials filled and labeled at the VRC, Vaccine Clinical Material Program operated by Leidos Biomedical Research, Inc., Frederick, MD. VRC01LS will be shipped from the NIAID Clinical Research Products Management Center (CRPMC). VRC01LS is a sterile, aqueous buffered solution filled into 10 or 3 mL single-dose glass vials. Each vial contains a volume of 6.25 ± 0.1 mL or 2.25 ± 0.1 mL at a concentration of 100 ± 10 mg/mL VRC01LS in formulation buffer containing 25 mM Sodium Citrate, 50 mM Sodium Chloride, and 150 mM LArginine Hydrochloride at pH 5.8. The vials contain a clear, colorless to yellow liquid essentially free of visible particles; some opaque or translucent particles may be present. The formulation buffer is composed of 25 mM sodium citrate, 50

mM sodium chloride, and 150 mM L-arginine hydrochloride at pH 5.8. The vials are intended for single use only and thus contain no preservative.

At the CRPMC, VRC01LS is to be stored in a freezer at -35^{0}C to -15^{0}C. In study site pharmacies, VRC01LS is to be stored in a freezer with a temperature range of -45°C to -10°C (-49°F to 14°F). Following thaw, VRC01LS vials may be stored for up to 24 hours at controlled room temperature (maximum 27°C) and/or up to 4 weeks at 2°C to 8°C. Product may not be stored in direct sunlight. If stored at 2°C to 8°C, vials must be equilibrated at controlled room temperature (maximum 27°C) for a minimum of 30 minutes and may be held at room temperature for up to 8 hours prior to product preparation.

Sodium Chloride for Injection USP, 0.9% will be purchased by BHP pharmacists from pharmaceutical suppliers.

Any empty vials, unused portion of entered vials, or unused IV solution that contains study product will be discarded in a biohazard containment bag and incinerated or autoclaved in accordance with institutional or pharmacy policy.

6.2. 10-1074 Description, Supply, and Storage

10-1074 is a recombinant, fully human IgG1 bNAb targeting the V3 loop and surrounding glycans on the HIV-1 envelope. It was cloned from a patient infected with HIV-1 clade A virus. 10-1074 is expressed in CHO cells and purified using standard methods.

The 10-1074 to be used for this study was manufactured by Mass Bio under contract to the NIAID and will be provided for the study by DAIDS. 10-1074 will be shipped from the NIAID CRPMC. 10-1074 is supplied as single-use 20 mg/mL solution for IV injection in a 5ml volume of buffered solution composed of sodium phosphate, potassium phosphate, potassium chloride, sodium chloride and polysorbate 80 with a pH of 7.0. Sodium Chloride for Injection USP, 0.9% will be purchased by BHP pharmacists from pharmaceutical suppliers.

Vials of 10-1074 must be stored or shipped at 2-8°C.

Any empty vials, unused portion of entered vials, or unused IV solution that contains study product will be discarded in a biohazard containment bag and incinerated or autoclaved in accordance with institutional or pharmacy policy.

6.3. Schedule of Intervention and Dosing

The first 12 eligible and consenting participants will receive 3 doses of a single bNAb (participants 1, 3, 5, 7, 9, 11 receive VRC01LS, and 2, 4, 6, 8, 10, 12 receive 10-1074); dosing for VRC01LS is 30mg/kg load followed by 10mg/kg maintenance, and for 10-1074 30mg/kg. Oral ART will continue during the PK Step. For the PK Step, PK measurements will occur at end of infusion, 1 hour post-infusion, 1 day post-infusion, 1 week post-infusion, 2 weeks post-infusion, and 4 weeks post-infusion (Table 1). Pre-infusion PK testing will also occur at Weeks 4 and 8. After the PK Step, monitoring will occur as outlined in 13.8.

After approval is granted to begin Step 1, 6 of the first 12 PK Step participants will begin dual bNAb treatment per the dosing and follow-up schedule in Table 2. The first 6 Step 1 participants will include

3 participants from each group in the PK Step; these participants may be the first 6 PK Step participants, but this is not required. These Step 1 participants will receive enhanced PK testing for both bNAbs at the Week 0 and Week 4 visits, with pre-infusion testing, testing at end of infusion, 1 hour post-infusion, 1 day post-infusion and 1 week post-infusion (all other PK testing for these participants will be trough pre-dose testing). A review of the safety and PK data for the first 2 dual bNAb doses will occur for these 6 participants. While awaiting this review (see Section 13.8), all 6 participants will remain in Step 1 per follow-up outlined in Table 2, with 4-weekly dual bNAb treatment and ongoing oral ART. Additional enrollments will not occur until approval is granted.

After approval is granted from this second review, the first 6 Step 1 participants will enter Step 2 and discontinue ART, provided they have been in Step 1 for at least 8 weeks and have remained with viral suppression <40 copies/mL (Table 3). Additional participants will also enroll in Step 1 at this time (both those who previously participated in the PK Step, and new participants). These participants may also proceed to Step 2 and discontinue ART after 8 weeks in Step 1, if they remain with viral suppression <40 copies/mL. A participant who experiences a confirmed viral rebound ≥40 copies/mL in Step 1 will discontinue bNAbs, complete visit procedures equivalent to those outlined for Step 1 Week 8, and enter Step 3.

All participants in Step 2 will continue with monthly dual bNAb maintenance for up to 24 weeks. HIV-1 RNA will be checked at least every 4 weeks while on ART in Step 1, and at every visit in Step 2 (weekly from Weeks 0-4 of Step 2, and every 2 weeks from Week 6-24 of Step 2). Qualitative HIV DNA will be checked at each Step 2 and Step 3 visit by dried blood spot (DBS). Clinical examination will occur at each study visit, except for the 1-day post-infusion PK visits. If participants experience a single viral rebound ≥400 copies/mL in Step 2, ART will be re-started (and VRC01LS and 10-1074 will be discontinued) without need for confirmation. If HIV RNA becomes ≥400 copies/mL in Step 2 prior to the Week 24 visit, all “Week 24” evaluations will be performed immediately prior to ART re-initiation. If HIV-1 RNA is ≥40 copies/mL but <400 copies/mL, or if qualitative DNA turns from negative to positive, virologic monitoring tests will be repeated *weekly*. After 24 weeks in Step 2, or if HIV RNA ≥400 copies/mL, participants will discontinue bNAbs, re-start ART and enter Step 3 (Table 3). Participants who enter Step 3 early due to viral rebound will have additional weekly visits until HIV RNA returns to <40 copies/mL. Routine Step 3 study visits will occur at weeks 4, 12, and 24.

6.3.1. VRC01LS Dose Calculation Instructions:

VRC01LS will be dosed at 30mg/kg at the first infusion visit, followed by 10mg/kg maintenance at subsequent infusion visits.

Prior to preparation of the first infusion (enrollment and Step 1 entry visit), a new prescription for VRC01LS will be sent to the pharmacy. The prescription must contain the participant’s weight based upon the participant’s weight at the most recent visit where weight was measured. Subsequent visit weights (based upon the participant’s weight at the most recent visit where weight was measured) must be communicated to the pharmacy in writing prior to the day of the infusion visit. Any changes in weight of more than 10% (between the prior weight and the weight on the day of the infusion visit) will require an updated visit weight communication to the pharmacy in writing so that product can be prepared based on that weight change.

6.3.2. 10-1074 Dose Calculation Instructions:

10-1074 will be dosed at 30mg/kg. Prior to preparation of the first infusion (enrollment and Step 1 entry visit), a new prescription for 10-1074 will be sent to the pharmacy. The prescription must contain the participant’s weight based upon the participant’s weight at the most recent visit where weight was

measured. Subsequent visit weights (based upon the participant's weight at the most recent visit where weight was measured) must be communicated to the pharmacy in writing prior to the day of the infusion visit. Any changes in weight of more than 10% (between the prior weight and the weight on the day of the infusion visit) will require an updated visit weight communication to the pharmacy in writing so that product can be prepared based on that weight change.

6.4. Preparation of Study Product Intravenous (IV) Solution

IV infusion will be used for this study because the product formulations and volumes required for children over ~ 10 kg are not practical for subcutaneous (SC) injection.

6.4.1. Preparation of VRC01LS IV Solution: To prepare VRC01LS IV infusion, the pharmacist will calculate the weight-based dose as in section 6.3.1 and remove the total number of vials needed as well as a 100 mL IV bag of Sodium Chloride for Injection USP, 0.9% from storage. The pharmacist, using aseptic technique, will add the appropriate amount of VRC01LS to the 100 mL IV bag of Sodium Chloride for Injection USP, 0.9%. Typically 50–100mL of additional volume may be added to a 100mL bag of normal saline. Each pharmacist should test the capacity of the brand of saline bags that will be used at the site to confirm the capacity to add additional volume. The pharmacist will label the infusion bag including a Beyond Use Date and time.

VRC01LS is a highly concentrated protein solution and may develop white, opaque to translucent particles after thawing. When particles are observed, they may disappear after a few hours at room temperature or storage at 2°C to 8°C.

The following instructions apply to thawing VRC01LS vialed product:
1. Thaw vials for a minimum of 1 hour at controlled room temperature (maximum 27°C) after removing from freezer.

2. Keep the material at controlled room temperature (maximum 27°C) during the entire preparation period, up to the maximum storage times described in the Section 6.1.

3. Prior to preparation for administration, vials should be swirled for 30 seconds with sufficient force to resuspend any visible particles, yet avoiding foaming. DO NOT SHAKE THE VIALS. If particles are observed, return the vials to 2°C to 8°C storage. If the particles redissolve within the maximum storage times described in Section 6.1, the vials may be used for product preparation. **If particles continue to be observed, do not use the vialed product for IV administration.** Refrigerated product must be equilibrated at controlled room temperature (maximum 27°C) for a minimum of 30 minutes before preparation and must be used within 8 hours of any subsequent return to room temperature.

4. If the thawed material is not administered within 24 hours of thaw, follow the storage information provided in Section 6.1.

The following instructions apply to VRC01LS Prepared Product (IV Bag and Syringe):
After product preparation in IV bags, the prepared VRC01LS may be stored at 2°C to 8°C up to 24 hours or at room temperature (maximum 30°C) for a maximum of 8 hours total including the infusion time. Product may not be stored in direct sunlight. If stored at 2°C to 8°C, prepared product must be equilibrated at room temperature (maximum 30°C) for a minimum of 30 minutes prior to product administration.

6.4.2. Preparation of 10-1074 IV Solution: To prepare 10-1074 IV infusion, the pharmacist will calculate the weight-based dose as in section 6.3.2 and remove the total number of vials needed as well as a 100 mL IV bag of Sodium Chloride for Injection USP, 0.9% from storage. The pharmacist, using aseptic technique, will add the appropriate amount of 10-1074 to the 100 mL IV bag of Sodium Chloride for Injection USP, 0.9%. After product preparation in IV bags, the prepared 10-1074 may be stored at 2°C to 8°C up to 24 hours or at room temperature (maximum 30°C) for a maximum of 8 hours total including the infusion time. Product may not be stored in direct sunlight. If stored at 2°C to 8°C, prepared product must be equilibrated at room temperature (maximum 30°C) for a minimum of 30 minutes prior to product administration. The pharmacist will label the infusion bag including a Beyond Use Date and time.

6.5. Study Product Administration

At all infusion visits during dual bNAb treatment in Step 1 and Step 2, the designated order for bNAb administration will be 10-1074 first and VRC01LS second. When both study products are administered there will be a gap of approximately 10-15 minutes (longer if necessary) during which the second infusion will be set up and vital signs will be measured.

6.5.1. 10-1074 Administration: The solution will typically be administered IV over about 30 minutes. The total time needed to administer the dose maybe longer based on factors such as participant tolerance.

The clinician responsible for administration will check the IV bag label and confirm that the participant identifier is correct, that the weight on the bag label is within 10% of the participant's current actual weight, and that the beyond use date/time has not been reached prior to administration.

A 0.2 or 0.22 micron in-line filter infusion set must be used for IV administration (see SOP for Infusion instructions).

6.5.2. VRC01LS Administration: The solution will typically be administered IV over about 30 minutes. The total time needed to administer the dose may be longer based on factors such as participant tolerance.
The clinician responsible for administration will check the IV bag label and confirm that the participant identifier is correct, that the weight on the bag label is within 10% of the participant's current actual weight, and that the beyond use date/time has not been reached prior to administration.

An in-line filter infusion set must be used for IV administration (see SOP for Infusion instructions). In-line filters must comply with the following specifications: 1.2 micron PES (polyethersulfone) filter membrane, DEHP-free, latex-free (equivalent to Braun #473994 filter extension set). When the in-line filter is added to the tubing, prime the administration set. Flush the administration set with about 30 mL or appropriate volume of normal saline at the end of product administration.

6.6. Concomitant Medications: ART Management

During the PK Step, Step 1, and Step 3, ART will be provided as part of the Botswana ART program (Masa Program), but may be dispensed by study staff as per prior agreements between BHP and the Botswana Ministry of Health. ART is standard of care for HIV-infected children in Botswana and will not be considered study drug. At the time of enrollment, all children in this study will continue the ART regimen being provided through the EIT Study, and will re-start ART with that same regimen in Step 3

as long as there is no clinical contraindication. During the PK Step, Step 1, and Step 3, the ART regimen may be changed if required. All ART management decisions will be per Botswana ART guidelines and/or by study physicians made with guidance from specialists.

7. STUDY PROCEDURES AND EVALUATIONS

7.1. Clinical Evaluations and Procedures

Study enrollment and follow-up for participants will be performed at the study clinic when possible, or at home visits if requested by caregivers for some visits (other than for bNAb administration). Children will be followed as outlined below in Table 1, Table 2, and Table 3.

Table 1 refers to special procedures for the PK Step to evaluate single bNAb administration. Table 2 describes Step 1 (the start of dual bNAb therapy) for the first 6 participants and for all additional participants. Table 3 outlines procedures for Step 2 when participants receive dual bNAb therapy without ART for 24 weeks, and procedures for the period of follow-up after dual bNAb therapy is discontinued and ART is re-started (Step 3).

Table 1: Follow-up and laboratory testing for the PK Step (first 12 participants)

	PK Step												
Study week	0	0+1 day	1wk	2wks	4wks	4wks +1day	5wks	6wks	8wks	8wks+ 1 day	9wks	10wks	12wks
10-1074	X[$] / 0				X[$] / 0				X[$] / 0				
VRC01LS	0 / X[$]				0 / X[$]				0 / X[$]				
Exam≠	X		X	X	X		X	X	X		X	X	X
Baseline Characteristics/ Demographics/SES	X												
Adherence Assessment	X		X	X	X		X	X	X		X	X	X
Acceptability Assessment	X												X
HIV RNA[∞]	X				X				X				X
CBC^	X		X				X				X		X
CD4/CD8	X				X				X				X
Chem / LFTs^	X		X[#]				X[#]				X[#]		X
Stored Plasma	X				X				X				X
Stored PBMC	X				X				X				X
HIV-1 Qualitative DNA	X		X		X		X		X				X
Proviral Genome Sequencing	X												X
HIV-1 DNA Analysis by ddPCR[%]	X				X				X				X
Immune Profiling	(X)												(X)
ELISA	X												X
PK Testing	X*	X	X	X	X*	X	X	X	X*	X	X	X	X
ADA Testing	X												X
Blood Volume (mL)	12	2	4	2	10	2	4	2	10	2	3	2	12

SES, socioeconomic status

Laboratory test time-points are optional when marked in parentheses: (X).

[≠] Participants will be weighed at least monthly, and at each visit preceding an infusion visit.

[$] Participants 1, 3, 5, 7, 9, and 11 will receive VRC01LS only (30mg/kg at Week 0, then 10 mg/kg at Week 4 and 8), and participants 2, 4, 6, 8, 10, and 12 will receive 10-1074 only (30mg/kg).

[∞] May be drawn up to 4 weeks prior to Week 0 visit.

^ CBC includes: hemoglobin (g/dl), Hematocrit (%), RBC (million/mm^3), MCV (microns), WBC (10^3/ cu mm), differential WBC, absolute neutrophil count (ANC), Platelets (10^3/cu mm). Chemistry includes: electrolytes, BUN, creatinine, glucose, albumin. LFTs include ALT, AST, total and direct bilirubin. Any draw that includes electrolytes should be performed with a butterfly needle when possible, to reduce the chances of hemolysis; specimens considered hemolyzed should be repeated at the next regularly scheduled visit. If clinical concern for hyperkalemia exists, hemolyzed specimens should be repeated as soon as possible.

[#] LFTs only

[%] CD4 T cell subsets will be considered if sufficient PBMC volume available.

* PK testing will occur pre-infusion (at Weeks 4 and 8), at the end of infusion and again 1 hour post-infusion on the day of administration.

Table 2: Follow-up and laboratory testing for Step 1 (all participants)

	Step 1: ART + dual bNAbs							Possible additional visits for first 6 participants in Step 1[$]											
Study week	0	0+ 1[&]	1	4	4+ 1[&]	5	8[$]	10*	12*	14*	16*	18*	20*	22*	24*	26*	28*	30*	32*
VRC01LS	X			X			X		X		X		X		X		X		X
10-1074	X			X			X		X		X		X		X		X		X
Exam	X		X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Baseline Characteristics/ Demographics/ SES (if required)	X																		
Adherence Assessment	X		X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Acceptability Assessment	X						X												
HIV RNA[≠]	X[∞]			X			X		X		X		X		X		X		X
CBC^	X		X			X		X		X		X		X		X		X	
CD4/CD8	X							X		X		X		X		X		X	
Chem / LFTs^	X		X[#]			X[#]		X[#]		X[#]		X[#]		X[#]		X[#]		X[#]	
Stored Plasma	X			X			X		X		X		X		X		X		X
Stored PBMC	X			X			X		X		X		X		X		X		X
HIV-1 Qualitative DNA	X			X			X		X		X		X		X		X		X
Proviral Genome Sequencing**	X						X												
HIV-1 gag DNA Analysis by ddPCR[%]	X						X				X				X				X
Immune Profiling	(X)			(X)			X				X		(X)		X				X
ELISA	X						X				X				X				X
PK Testing[&]	X[&]	X[&]	X[&]	X[&]	X[&]	X[&]	X		X		X		X		X		X		X
ADA Testing[≠]	X																		
Blood Volume (mL)	12	2	4	9	2	4	11	4	8	4	9	4	8	4	9	4	8	4	9

SES, socioeconomic status

Laboratory test time-points are optional when marked in parentheses: (X).

[≠] If a participant has a confirmed viral load result ≥40 copies/mL during Step 1, he/she will discontinue bNAbs, complete visit procedures equivalent to those outlined for Step 1 Week 8 and enter Step 3.

[∞] May be drawn up to 4 weeks prior to Week 0 visit.

[&] For first 6 participants, PK draws will occur pre-infusion at Week 0, and also for both bNAbs at end of infusion at Week 0, 1 hour post-infusion at Week 0, Week 0 + 1 day after infusion, Week 1, Week 4 pre-infusion, at end of infusion at Week 4, 1 hour post-infusion at Week 4, Week 4 + 1 day after infusion, and Week 5. All subsequent PK testing will be pre-dose trough testing only. Other participants will not have PK on Day 0 or Week 1 or Week 5, but will have pre-dose trough testing at the visits noted beginning at 4 weeks.

[$] Step 2 testing schedule for "Step 2 Week 0" supersedes Step 1 schedule on the day of entry into Step 2.

* Additional Step 1 visits may be required only for the first 6 participants while awaiting SMC review of safety and PK data. In the unlikely event that a participant reaches the Week 32 Step 1 visit prior to the conclusion/recommendation of the SMC review, Week 30 and Week 32 visit procedures will be repeated alternately every two weeks, if feasible, until a decision on Step 2 is reached.
** In the event of plasma rebound ≥400 copies/mL, viral sequencing from plasma samples will also be considered.
[%] CD4 T cell subsets will be considered if sufficient PBMC volume available.
^ CBC includes: hemoglobin (g/dl), Hematocrit (%), RBC (million/mm^3), MCV (microns), WBC (10^3/ cu mm), differential WBC, absolute neutrophil count (ANC), Platelets (10^3/cu mm). Chemistry includes: electrolytes, BUN, creatinine, glucose, albumin. LFTs include ALT, AST, total and direct bilirubin. Any draw that includes electrolytes should be performed with a butterfly needle when possible, to reduce the chances of hemolysis; specimens considered hemolyzed should be repeated at the next regularly scheduled visit. If clinical concern for hyperkalemia exists, hemolyzed specimens should be repeated as soon as possible.
[#] LFTs only
[≠]ADA testing will not be done on the first 6 participants in Step 1 who had this testing during the PK Step.

Table 3: Follow-up and laboratory testing for Step 2 and Step 3 (all participants)

	Step 2: Dual bNAbs alone															Step 3: ART alone			
Study week	0	1	2	3	4	6	8	10	12	14	16	18	20	22	24[#]	0[≠]	4	12	24
VRC01LS	X				X		X		X		X		X		X				
10-1074	X				X		X		X		X		X		X				
Exam	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X
Adherence Assessment	X																X	X	X
Acceptability Assessment	X								X						X				X
HIV RNA*	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X
CBC^		X				X		X		X		X		X			X		X
CD4/CD8	X					X		X		X		X		X	X		X	X	X
LFTs		X				X		X		X		X		X			X		
Stored Plasma	X		X		X	X	X		X		X		X		X			X	X
Stored PBMC	X		X		X	X	X		X		X		X		X			X	X
HIV-1 Qualitative DNA*	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X
Proviral Genome Sequencing**	X														X				**X**
HIV-1 DNA Analysis by ddPCR[%]	X				X		X				X				X				X
Immune Profiling	X		(X)		X				(X)		X		(X)		X				X
ELISA	X				X		X				X				X				X
PK Testing (pre-dose trough)	X				X[&]		X[&]		X[&]		X[&]		X[&]		X[&]				
ADA Testing																			X
Discontinue ART	X																		
Re-start ART																X			
Blood Volume (mL)	10	7	8	6	9	10	9	8	8	8	9	8	8	8	12	0	8	8	11

Laboratory test time-points are optional when marked in parentheses: (X).
* If HIV RNA remains <400 copies/mL; if HIV RNA becomes ≥400 copies/mL during the period of bNAbs alone, ART will be resumed, and follow-up schedule modified per protocol. If HIV RNA ≥40 copies/mL and <400 copies/mL, or if qualitative DNA turns from negative to positive, HIV RNA will occur weekly.
# If HIV RNA becomes ≥400 copies/mL prior to the Step 2 Week 24 visit, all "Week 24" evaluations will be performed (but bNAbs will not be administered) immediately prior to ART re-initiation. Follow-up will then occur weekly until HIV-1 RNA returns to <40 copies/mL, with testing that will include adherence assessment, exam, HIV-1 RNA, qualitative HIV DNA, stored plasma and stored PBMC. Thereafter, visits will occur as routine Step 3 visits at Weeks 4, 12, and 24 (as applicable depending on study week when re-suppression occurs), with events as per Table 3. If weekly follow-up during the period of re-suppression coincides with one of these scheduled visits, the testing protocol for that visit week will be observed.
** Genome sequencing will be performed from PBMC. In the event of plasma rebound ≥400 copies/mL, viral sequencing from plasma samples will also be considered.
% CD4 T cell subsets will be considered if sufficient PBMC volume available.
^ CBC includes: hemoglobin (g/dl), Hematocrit (%), RBC (million/mm^3), MCV (microns), WBC (10^3/ cu mm), differential WBC, absolute neutrophil count (ANC), Platelets (10^3/cu mm).
& May include PK testing for the presence of drugs from the participant's prior Step 1 ART regimen, for a subset of HIV-1 RNA-suppressed children.
≠ Participants will enter Step 3 on the same day as completing the Step 2 Week 24 visit.

7.2. Monitoring during Step 2

Transition to treatment with VRC01LS and 10-1074 alone (Step 2) will be performed in a closely monitored setting with frequent visits and HIV RNA testing to ensure that ART is re-started within days of viral rebound and that HIV-1 RNA is quickly re-suppressed after ART re-start (see also Sections 7.7.2 and 13.8.1). There will be minimal risk of loss-to-follow-up during this critical period, as we have extensive experience finding/retaining these same participants in the EIT Study.

7.3. Participant Sampling

Samples will be obtained as outlined in Table 1, Table 2, and Table 3. No maternal samples will be requested (but are available from EIT study). Please see below discussion of blood volume limits.

7.4. Specimen Management

All specimens will be sent to the BHP Laboratory in Gaborone or to the National HIV Reference Laboratory in Francistown. Complete blood counts, chemistries, HIV RNA, Qualitative HIV DNA and CD4 cell counts will be performed at these or at BHP/NIAID designated back-up laboratories. HIV RNA testing will be performed with expedited testing and reporting (<7 days). Validated laboratory values will be entered at the BHP Data Center or directly uploaded from the laboratory to the Data Center.

Stored specimens will be shipped periodically to Dr. Kuritzkes' and Dr. Lichterfeld's lab in Boston. Samples for VRC01LS PK/ADA testing will be sent to the Vaccine Research Center in Bethesda. Samples for 10-1074 PK testing will be sent to Dr. Tomaras' lab at Duke University, and samples for 10-1074 ADA testing will be sent to Dr. Ackerman's laboratory at Dartmouth University.

Initial laboratory data review will evaluate data completeness, data checks against the clinical database (e.g., dates of samples/results), assessment for outliers and interaction with the testing

laboratories regarding assay limits of detection and the potential impact of low blood volumes and low event counts (especially immune cell subsets) on the testing, results and analyses.

7.5. Biohazard Containment

Transmission of HIV and other blood borne pathogens can occur through contact with contaminated needles, blood, and blood products. Respiratory pathogens such as Mycobacterium tuberculosis (MTB) are transmitted by inhalation of droplet nuclei. Appropriate blood, secretion, and respiratory precautions will be employed by all personnel in the administration of IV infusion, collection of clinical samples and the shipping and handling of all clinical samples and isolates for this study, as currently recommended by the Centers for Disease Control and Prevention in the United States, the WHO internationally and the National Institutes of Health.

All protocol specimens will be shipped using packaging that meets requirements specified by the International Air Transport Association Dangerous Goods Regulations for UN 3373, Biological Substance, Category B, and Packing Instruction 650. Culture isolates, if obtained in this study, are to be shipped as specified for UN 2814 Category A Infectious Substances.

7.6. Blood Volumes

Blood volumes are an important consideration for this protocol, and will be minimized to the greatest extent possible at every visit. We will follow NIH Clinical Center guidance for maximal allowed blood volumes allowed in research. The NIH recommends a limit of 5 mL/kg per single blood draw and a limit of 9.5 mL/kg in any 8-week period. We will draw no more than 12 mL at any draw during the study and <12 mL at most visits. If additional clinical blood draws occur, study staff will account for these draws and make adjustments in the volume drawn for the study if needed. However, given that all children will be at least 96 weeks of age, we expect them to be over 10 kg (which is ~ 5th percentile). By NIH Clinical Center guidance, a 10 kg child would be able to have up to 50 mL of blood per draw (we will only draw 12 mL maximum), and up to 95 mL in an 8-week period (the maximum in this study would be far lower). Thus, we believe that the blood draw volumes for the study are well-within safety parameters, even if a child is at 5th percentile or lower in weight.

7.7. Schedule of Evaluations

7.7.1. Evaluations in the Setting of Maintained Viral Suppression:

All visits will assess for diagnoses, signs and symptoms of any Grade.

Adherence to ART during the PK Step, Step 1, and Step 3 will be monitored through an Adherence Assessment form, on which information such as date of last dose of ARV, any missed doses of ARVs, and reasons for missed doses will be recorded.

Acceptability of the infusions will be assessed by asking questions about tolerability and willingness to opt for procedures involving infusions in the future.

<u>PK Step Week 0 Visit</u>

- Confirmation of signed and dated PK Step consent form
- Perform physical exam

- Continue ART
- Baseline Characteristics/Demographics/Socioeconomic Status (SES) Data Collection
- Adherence/Acceptability Assessments
- 10-1074 or VRC01LS given
- Samples obtained for: HIV-1 RNA, hematology, CD4/CD8, chemistry/LFTs, stored plasma and PBMCs for HIV proviral DNA quantification (including by ddPCR), qualitative HIV DNA PCR, whole genome sequencing, immune profiling (optional), ELISA, PK testing (at end of infusion and 1-hour post-infusion), ADA testing.
- Set up visit for next day for "Week 0 + 1 day" post-dose PK testing

PK Step Week 0 + 1 day post-single bNAb infusion Visit
- Continue ART
- Samples obtained for: PK testing

PK Step Week 1 Visit
- Perform physical exam
- Continue ART
- Adherence Assessment
- Samples obtained for: hematology, LFTs, qualitative HIV DNA PCR, PK testing

PK Step Week 2 Visit
- Perform physical exam
- Continue ART
- Adherence Assessment
- Samples obtained for: PK testing

PK Step Week 4 Visit
- Perform physical exam
- Continue ART
- Adherence Assessment
- 10-1074 or VRC01LS given
- Samples obtained for: HIV-1 RNA, CD4/CD8, stored plasma and PBMCs for HIV proviral DNA quantification (including by ddPCR), qualitative HIV DNA PCR, PK testing (pre-infusion, at end of infusion and 1-hour post-infusion)
- Set up visit for next day for "Week 4 + 1 day" post-dose PK testing

PK Step Week 4 + 1 day post-single bNAb infusion Visit
- Continue ART
- Samples obtained for: PK testing

PK Step Week 5 Visit
- Perform physical exam
- Continue ART
- Adherence Assessment
- Samples obtained for: hematology, LFTs, qualitative HIV DNA PCR, PK testing

PK Step Week 6 Visit
- Perform physical exam
- Continue ART
- Adherence Assessment
- Samples obtained for: PK testing

PK Step Week 8 Visit
- Perform physical exam
- Continue ART
- Adherence Assessment
- 10-1074 or VRC01LS given
- Samples obtained for: HIV-1 RNA, CD4/CD8, stored plasma and PBMCs for HIV proviral DNA quantification (including by ddPCR), qualitative HIV DNA PCR, PK testing (pre-infusion, at end of infusion and 1-hour post-infusion)
- Set up visit for next day for "Week 4 + 1 day" post-dose PK testing

PK Step Week 8+1 Visit
- Continue ART
- Samples obtained for: PK testing

PK Step Week 9 Visit
- Perform physical exam
- Continue ART
- Adherence Assessment
- Samples obtained for: hematology, LFTs, PK testing

PK Step Week 10 Visit
- Perform physical exam
- Continue ART
- Adherence Assessment

Samples obtained for: PK testing

PK Step Week 12 Visit
- Perform physical exam
- Continue ART
- Adherence/Acceptability Assessments
- Samples obtained for: HIV-1 RNA, hematology, CD4/CD8, chemistry/LFTs, stored plasma and PBMCs for HIV proviral DNA quantification (including by ddPCR), qualitative HIV DNA PCR, whole genome sequencing, immune profiling (optional), ELISA, PK testing, ADA testing

---

Step 1 Week 0 Visit
- Confirmation of signed and dated consent form (for enrollment to Steps 1-3)
- Perform physical exam
- Continue ART
- Baseline Characteristics/Demographics/SES Data Collection (if required)
- Adherence/Acceptability Assessments
- VRC01LS and 10-1074 given

- Samples obtained for: HIV-1 RNA, hematology, CD4/CD8, chemistry/LFTs, stored plasma and PBMCs for HIV proviral DNA quantification (including by ddPCR), qualitative HIV DNA PCR, whole genome sequencing, immune profiling (optional), ELISA; for the first 6 participants only PK testing pre-infusion, at end of infusion and 1-hour post infusion; ADA testing
- Set up visit for next day for "Day 1 post- dual dose" PK testing (first 6 participants only)

Step 1 Week 0 + 1 day post-bNAb infusion visit (first 6 only)
- Continue ART
- Samples obtained for: PK testing

Step 1 Week 1 Visit
- Perform physical exam
- Continue ART
- Adherence Assessment
- Samples obtained for: hematology, LFTs, PK testing

Step 1 Week 4 Visit
- Perform physical exam
- Continue ART
- Adherence Assessment
- VRC01LS and 10-1074 given
- Samples obtained for: HIV-1 RNA, stored plasma, stored PBMC for immune profiling (optional), qualitative HIV DNA PCR, PK testing

Step 1 Week 4 + 1 day Visit (first 6 only)
- Continue ART
- Samples obtained for: PK testing

Step 1 Week 5 Visit
- Perform physical exam
- Continue ART
- Adherence Assessment
- Samples obtained for: hematology, LFTs, PK testing

Step 1 Week 8-32 Visits (if remaining in Step 1)
- Perform physical exam
- Adherence Assessment
- Acceptability Assessment (at Week 8)
- Continue ART while remaining in Step 1
- VRC01LS and 10-1074 given at Weeks 8, 12, 16, 20, 24, 28, 32
- Samples obtained for: HIV-1 RNA monthly (at Weeks 8, 12, 16, 20, 24, 28, 32); hematology, CD4/CD8, and LFTs (at Weeks 10, 14, 18, 22, 26, and 30); stored PBMC/plasma monthly (at Weeks 8, 12, 16, 20, 24, 28, and 32), qualitative HIV DNA PCR monthly (at Weeks 8, 12, 16, 20, 24, 28, and 32), whole genome sequencing (Week 8), quantitative HIV DNA PCR from stored PBMC every 8 weeks (at Weeks 8, 16, 24, and 32), immune profiling (at Weeks 8, 16, 20 (optional), 24, and 32), ELISA (at Weeks 8, 16, 24, and 32), PK testing (at Weeks 8, 12, 16, 20, 24, 28, and 32).

Step 2 Week 0 (must coincide with a Step 1 Week 8, 12, 16, 20, 24, 28, or 32 Visit)

- Perform physical exam
- Discontinue ART
- Adherence Assessment
- Acceptability Assessment
- VRC01LS and 10-1074 given
- Samples obtained for: HIV-1 RNA, CD4/CD8, stored plasma/PBMC, qualitative DNA PCR, whole genome sequencing, quantitative HIV DNA PCR from stored PBMC, immune profiling, ELISA and PK testing

Step 2 Non-infusion Visits (Weeks 1, 2, 3, 6, 10, 14, 18, 22)

- Perform physical exam (all visits)
- Samples obtained for: HIV-1 RNA (all visits), hematology (at Weeks 6, 10, 14, 18, and 22), CD4/CD8 (at Weeks 6, 10, 14, 18, and 22), LFTs (at Weeks 1, 6, 10, 14, 18, and 22), stored plasma/PBMC (only at non-infusion visits at Weeks 2 and 6), qualitative DNA PCR (all visits), immune profiling (optional at Week 2)

Step 2 Infusion Visits (Weeks 4, 8, 12, 16, 20)

- Perform physical exam (all visits)
- Acceptability Assessment (Week 12)
- VRC01LS and 10-1074 given
- Samples obtained for: HIV-1 RNA (all visits), stored plasma/PBMC (all visits), qualitative DNA PCR (all visits), quantitative HIV DNA PCR from stored PBMC (at Weeks 4, 8, 16), immune profiling (at Weeks 4, and 16, optional at Weeks 12 and 20), ELISA (at Weeks 4, 8, 16), PK testing (at Weeks 4, 8, 12, 16, 20)

Step 2 Week 24 Visit (or last visit off ART)

- Perform physical exam
- Acceptability Assessment
- VRC01LS and 10-1074 given (if visit at 24 weeks, but not if last Step 2 visit for rebound)
- Samples obtained for: HIV-1 RNA, CD4/CD8, stored plasma/PBMC, qualitative DNA PCR, whole genome sequencing, quantitative HIV DNA PCR from stored PBMC, immune profiling, ELISA, PK testing
- Enter Step 3 (Step 3 Week 0 = Day of ART re-start)

Step 3 Week 0 (occurs the same day as completion of Step 2 Week 24 evaluations)

- Re-start ART

Step 3 additional weekly visits for participants who enter Step 3 with HIV RNA ≥400 copies/mL; repeat weekly until HIV RNA returns to <40 copies/mL, also complete Routine Step 3 Visits)

- Perform physical exam
- Adherence Assessments
- Samples obtained for: HIV-1 RNA, Qualitative DNA PCR, plasma and stored PBMC

Step 3 Weeks 4, 12, 24 (Routine)

- Perform physical exam
- Adherence Assessment
- Acceptability Assessment (Week 24)
- Labs: HIV-1 RNA, hematology (at Weeks 4 and 24 only), CD4/CD8 (all visits), LFTs (at Week 4 only), stored plasma/PBMC (at Weeks 12 and 24 only), qualitative DNA PCR, whole genome sequencing (at Week 24 only), quantitative HIV DNA PCR, and immune profiling (at Week 24 only), CD4/CD8 (all visits), ELISA at Week 24, ADA testing at Week 24.

7.7.2. Evaluations in the Event of Viral Rebound: If viral rebound ≥400 copies/mL occurs and ART is re-started per protocol, the "Step 2 Week 24" visit schedule will occur (except without bNAb administration), and the participant enters Step 3. Follow-up will then occur with additional weekly visits in Step 3 until HIV-1 RNA returns to <40 copies/mL, with testing that will include exam, adherence assessment, HIV-1 RNA, Qual DNA PCR, stored plasma and stored PBMC (8 mL blood draws). Thereafter, follow-up will continue with routine Step 3 visits at Weeks 4, 12, and 24 (as applicable depending on study week when re-suppression occurs). Laboratory collections for these visits will be as per Table 3. The scheduled visit laboratory schedule will be followed when it coincides with weekly follow-up visits to monitor viral re-suppression. HIV resistance testing will be performed for clinical purposes if viral suppression has not been achieved 12 weeks following ART re-initiation.

7.8. End of Study Follow-Up

Children will be followed up for a minimum of 56 weeks, and a maximum of 98 weeks for those who start in the PK step and continue (or longer in unlikely event that Step 1 is extended beyond 16 weeks for the first 6 participants).

7.9. Timing of evaluations

Enrollment should occur at or after 96 weeks on ART, with an upper age limit of 5 years.

Scheduled evaluations

Every attempt should be made to conduct the visit as close as possible to the target visit date, and barring that, within the target visit window. Visit windows remain open until the mid-way point to the next scheduled evaluation, at which point the visit is marked as missed and a contact would be counted as the next scheduled evaluation. However, if a visit window is missed, every effort should still be made to conduct the laboratory evaluations and clinical evaluations that were required at the missed visit at the time of the next clinical contact, even if this falls outside of the specified visit window, with the exception of PK testing. The study team may check on participants by telephone between visits.

Visits will occur at the weeks indicated whenever possible. A visit may be counted as completed if attendance is either early (if performed no sooner than halfway between the previous visit target date and the target date for the upcoming scheduled visit) or late (no later than halfway between the target date for the scheduled visit and the target date for the next scheduled visit). Thus, a child is always eligible for a scheduled visit when seen in the clinic. However, the clinic staff may use their discretion to either perform visits as "unscheduled" if far from the target date and a reliable participant, or to count a visit as "scheduled" and perform all required tasks (see "Ill visits" below). Infusion visits that

are not completed within 14 days of the scheduled visit will trigger review by the protocol team and, if in Step 2, weekly HIV RNA testing will occur.

Missed visits
If a scheduled visit is missed then the next attended visit should include major monitoring parameters from the missed visit (within allowable blood draw ranges). If a visit is missed, the schedule of visits should not be "reset" but should remain as if the visit were not missed.

Ill visits
If an ill visit occurs and the participant will not be able to attend the next scheduled visit, the ill visit can substitute for the scheduled visit at the discretion of the study physician. In such a case, all information/tests scheduled for the routine visit should be obtained and reported.

If an ill visit is not considered a scheduled visit, clinical events that meet reporting criteria for the study should be noted in the patient medical record (non-source document) and also recorded on an unscheduled contact CRF, and other applicable CRFs. The participant should be reminded to attend the next regularly scheduled study visit. Should the ill visit constitute a serious adverse event that requires expedited adverse event (EAE) reporting, the EAE form should be completed and submitted to the Data Centre within the specified time frame.

Premature discontinuation of study treatment
If the single bNAb needs to be discontinued during the PK Step the child will go off Step after completing the visit procedures equivalent to Week 12 of the PK Step. If the participant is permanently discontinuing study treatment in the PK Step he/she will go off-study. In general during participation in Step 1 or Step 2, if a child needs to discontinue study treatment for any reason, he/she will complete final visit evaluations for the Step and enroll to Step 3.

7.10. Laboratory Testing Panels in Botswana

Samples will be stored for up to 10 years after the study is closed with IRBs of record, if consent for this is granted.

Plasma HIV-1 RNA
All evaluations are to be done using approved viral load assays at the BHP study laboratory in Gaborone or at the HIV Reference Laboratory in Francistown. Testing may occur by approved laboratory techniques, and may occur in conjunction with the Botswana Masa Program using the Roche Taqman platform. If the approved method for HIV RNA detection in Botswana is modified, the study may utilize newly approved platforms (including point-of-care testing) but will also continue with the "gold standard" testing throughout the study.

Hematology
Hemoglobin, hematocrit, red blood cells (RBC), mean corpuscular volume (MCV), white blood cell count (WBC), differential WBC (neutrophils, lymphocytes, eosinophils, monocytes, and basophils), absolute neutrophil count (ANC), and platelets.

Blood Chemistries / LFTs
Sodium, potassium, chloride, glucose, bicarbonate, BUN, creatinine, ALT, AST, and bilirubin (total and direct).

CD4+/CD8+

Determinations of CD4+ and CD8+ cell counts and subset percentage evaluations should be performed at the approved BHP laboratories in Gaborone or Francistown, throughout the course of the study. All CD4+ and CD8+ cell count results will be recorded on the CRFs.

Stored Plasma/Cells

Stored plasma will be performed per laboratory SOP, either from the PBMC separation process or upon direct processing of whole blood for HIV RNA testing. When PBMC processing is not performed, cells will be stored separately after processing for plasma. Stored plasma will be used for analysis of ADA at designated laboratories (VRC, Duke).

Stored PBMC

Whole blood will be processed for PBMCs per standard laboratory protocols. PBMCs will be separated into 2 equal aliquots and frozen until shipped to Boston. Plasma remaining from PBMC separation will be used for HIV RNA testing and ELISA testing when applicable, and the remainder will be stored.

HIV-1 DNA PCR (Qualitative)

For Qualitative DNA PCR, whole blood will be obtained by dried blood spot (DBS). Testing may occur by approved laboratory techniques, including the Roche Taqman platform. Selected Qual DNA PCR tests may occur at the BHP laboratory on PBMC samples (of approximately 1 million PBMCs), if volume permits. The Cepheid platform for rapid HIV DNA testing may also be utilized for DBS or whole blood testing as an adjunct to the Taqman platform.

Proviral Genome Sequencing

The number of intact proviruses will be quantified using a novel single-genome amplification strategy that combines limiting dilution PCR of near-full-length genomes with next-gen (Illumina) sequencing of the clonal amplicons, as described in our previous work [57]. Briefly, proviral DNA diluted to single genomes will be amplified with primers spanning near full-length HIV-1 clade C, followed by Illumina MySeq next-generation sequencing. Resulting contigs will be de novo assembled; subsequently, an analysis pipeline written in R coding language will be used to identify large deletions, hypermutations, premature stop codons and defects in the 5-LTR region. Sequences without detectable defects will be classified as “genome-intact”. Genome sequencing will be performed from PBMC. In the event of plasma rebound ≥400 copies/ml, viral sequencing from plasma samples will also be considered.

HIV DNA PCR (Quantitative)

Quantitative DNA PCR will occur by approved laboratory techniques from stored PBMCs, at the Kuritzkes laboratory in Boston. These assays include quantitative digital droplet PCR (ddPCR) measurement of integrated (proviral) HIV-1 DNA, cell-associated HIV-1 RNA, and plasma HIV-1 RNA measured by a highly sensitive single-copy assay.

Immune Profiling

Immune profiling assays performed at MGH/Ragon, and neutralization assays at BIDMC, are listed in 7.13.

HIV ELISA

HIV ELISA will be performed on plasma using test kits approved as part of the Botswana Masa Program, run at the BHP study laboratory.

PK Testing / ADA Testing

Vaccine Research Center at NIH will perform PK testing and ADA testing for the VRC01LS. VRC01 concentration in participant plasma samples will be quantified using the murine anti-VRC01 monoclonal antibody 5C9 for VRC01LS detection. The lab of Georgia Tomaras at Duke University will perform the PK testing for the 10-1074. Levels of 10-1074 will be determined by a validated sandwich ELISA. The lab of Margaret Ackerman at Dartmouth University will perform the ADA testing for the 10-1074 using a tiered testing approach.

7.11. Clinical Evaluations and Reporting Requirements

Children will undergo an examination at every study visit (except the 1-day post-infusion PK visits). Length and weight will be recorded at least monthly. All prior clinical and laboratory information from the EIT Study will be available for clinical and study purposes. All diagnoses, signs/symptoms will be recorded on CRF, as will WHO Stage III/IV HIV-related illnesses. Vaccinations, hospitalizations, and relevant concomitant medications (from a targeted list included in the electronic data capture system) are recorded at each follow-up visit. Adherence to and modifications of ART are recorded at each visit while participants are on ART. These are assessed with specific questions in the CRFs.

7.12. Medical Care and Referrals

General medical care of children should be provided through the study clinics. Vaccinations may be provided either at the study clinic or at government clinics. Children requiring hospitalization will be referred appropriately, and clinical decisions during hospitalization will be made by hospital staff. However, study staff will follow hospitalized children, and advise regarding ART decisions (and possibly other medical decisions) as appropriate.

7.13. Laboratory Procedures in Boston

Please reference the Laboratory Testing Documents for all virology research laboratory testing details. The following immunologic research assays will be conducted, according to standard protocols:

- Immune phenotyping of different leukocyte subsets, using flow cytometry or Cytometry by time-of-flight (CYTOF)
- Analysis of HIV-1-specific T cell responses and NK cells using cytokine secretion, proliferation and cytotoxicity assays
- Interferon-γ elispot assays for characterizing HIV-1-specific cytotoxic T cell responses
- Gene expression profiling assays in isolated T cells, using gene microarrays or RNA-Seq assays (if sufficient PBMC samples available)
- Immunogenetic assessments of HLA class I alleles
- Analysis of HIV-1-specific neutralizing antibody responses (in collaboration with Dr. Michael Seaman, BIDMC)
- HIV-1 DNA analysis and viral sequencing assays

8. ASSESSMENT OF SAFETY

8.1. Serious Adverse Events (SAEs)

SAEs are defined as an AE following any exposure to the study agent that:

- Results in death

- Is life-threatening (the term "life-threatening" refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.)
- Requires inpatient hospitalization or prolongation of existing hospitalization (hospitalization itself is not an AE, but is an outcome of the event). The following types of hospitalization do not require expedited reporting to DAIDS:
  - Any admission unrelated to an AE
  - Admission for diagnosis or therapy of a condition that existed before receipt of study agent(s) and has not increased in severity or frequency as judged by the clinical investigator.
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect, or
- Is an important medical event that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above.

Guidelines for reporting SAE's that require expedited reporting (EAE's) are summarized in an EAE SOP.

8.2. Adverse Event Reporting

All adverse events (Grade 1 or higher), including all infusion reactions, will be recorded on an Adverse Event Log. Potential infusion reactions will also be described on the Infusion Reaction form.

8.2.1. Expedited Adverse Event Reporting to DAIDS: Requirements, definitions and methods for expedited reporting of AEs are outlined in Version 2.0 of the DAIDS EAE Manual, which is available on the RSC website at https://rsc.niaid.nih.gov/clinical-research-sites/manual-expedited-reporting-adverse-events-daids.

The DAIDS Adverse Experience Reporting System (DAERS), an internet-based reporting system, must be used for expedited AE reporting to DAIDS. In the event of system outages or technical difficulties, expedited AEs may be submitted via the DAIDS EAE Form. For questions about DAERS, please contact **NIAID CRMS Support** at CRMSSupport@niaid.nih.gov. Site queries may also be sent from within the DAERS application itself.

Where DAERS has not been implemented, sites will submit expedited AEs by documenting the information on the current DAIDS EAE Form. This form is available on the RSC website: https://rsc.niaid.nih.gov/clinical-research-sites/paper-eae-reporting. For questions about expedited reporting, please contact DAIDS RSC (DAIDSRSCSafetyOffice@tech-res.com).

8.2.2. Reporting Requirements for this Study: The SAE Reporting Category, as defined in Version 2.0 of the DAIDS EAE Manual, will be used for this study.

VRC01LS and 10-1074 are the only study agents for which *expedited* reporting is required for SAEs. The Suspected Unexpected Serious Adverse Reactions (SUSAR) reporting category will be used in all other circumstances, until 30 days after study completion for each participant.

In addition to all SAEs meeting the above criteria, all grade 3 or higher serum sickness, grade 3 or higher urticarial or other hypersensitivity reactions, and grade 4 injection site reactions must also be reported as EAEs in this study.

8.2.3. Grading Severity of Events: The most current Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table) at the time that the study opens will be used, and is available on the RSC website at https://rsc.niaid.nih.gov/clinical-research-sites/daids-adverse-event-grading-tables.

8.2.4. Expedited AE Reporting Period: The *expedited* AE reporting period for this study is for 30 days following the final dose of VRC01LS and 10-1074.

After the protocol-defined AE reporting period, unless otherwise noted, only SUSARs as defined in Version 2.0 of the EAE Manual will be reported to DAIDS if the study staff become aware of the events on a passive basis (from publicly available information).

9. CLINICAL MANAGEMENT

9.1. Toxicity Management

VRC01LS and 10-1074 are monoclonal antibodies and unlikely to lead to toxicities other than possible local injection site reactions (See Section 2.1.7).

However, AEs of interest to be monitored for include:

- Constitutional symptoms, such as fever, changes in blood pressure, rigors/chills;
- Injection site reaction/extravasation changes, pruritus, urticaria, erythema, desquamation, ulceration;
- Serum sickness like syndromes as evidenced by fever, rash, arthralgia, arthritis, nephritis;
- Deposition of immune complexes in the kidneys leading to renal insufficiency;
- Anaphylaxis; Acute Respiratory Distress Syndrome, bronchospasm/wheezing, facial flushing;
- Cytokine release syndrome.

The most current Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table) should be used to report adverse events and is available on the RSC website at https://rsc.niaid.nih.gov/clinical-research-sites/daids-adverse-event-grading-tables.

This Toxicity Management section refers to management of toxicities that occur among children receiving VRC01LS and 10-1074. Additional details are provided in study-specific SOPs. Please also refer to specific SOPs and to Botswana ART guidelines for management of toxicities related to ART, which is used according to best standard of care in Botswana.

For the 12 PK Step participants, toxicity may be attributed to either bNAb alone in the PK Step. If the single bNAb needs to be discontinued during the PK Step the child will go off Step. In Steps 1 and 2, toxicity management should generally group both bNAbs together in assessment of relationship to the event, with the exception of reactions specific to each infusion.

9.1.1. Dosage Modification Instructions: VRC01LS and 10-1074 will not be dose-adjusted because of toxicity.

9.1.2. Management for Rashes, Urticaria and Hypersensitivity Reactions: All rashes are graded based on the DAIDS toxicity table (Corrected Version 2.1, July 2017).

Grade 1 or 2 rashes or urticaria
VRC01LS and 10-1074 may be continued with close observation, at the discretion of the study clinician. If an isolated Grade 1 or 2 rash does not resolve within 14 days of onset, further management should be discussed with the study team. Other potential causes of rash/urticaria should be investigated and treated, and potential causative agents (such as lotions/creams/soaps) should be discontinued.

Grade 3 rash with no definitive alternative explanation (Grade 3 rash should have vesicles or limited number of bullae or superficial ulcerations of mucous membrane):
Any products or non-essential medicines that could be causing the rash should be discontinued. ALT and FBC with differential should be drawn, and the child should be evaluated clinically approximately weekly, until the rash resolves to Grade 1 or lower. If the Grade 3 rash was ultimately deemed very likely due to another diagnosis or drug, then VRC01LS and 10-1074 may be continued, in consultation with a study PI/designee. However, if VRC01LS and 10-1074 were paused for possible hypersensitivity and no other explanation is apparent, they should be permanently discontinued and ART re-started (if paused).

Grade 4 rash (with mucosal involvement, or suspected hypersensitivity):
Immediately modify or hold VRC01LS and 10-1074 and refer to the hospital for further clinical care/management, including clinical management of ART resumption if paused, and seek guidance from a study PI/designee. If VRC01LS and 10-1074 were paused for possible hypersensitivity and no other explanation is apparent, they should not be re-started.

9.1.3. Guidelines for Managing Local Reactions / Infusion Site Reactions
Infusion site reactions should be managed as follows:

Grade 1-2: Continue administration of VRC01LS and 10-1074. Use alternate infusion site until event resolves to less than Grade 1.

Grade 3: Defer further VRC01LS and 10-1074 administration until event reviewed by the study team. Contact protocol team within 72 hours. Participant should be followed approximately weekly until resolution to Grade ≤1 or until stabilized and no longer in need of frequent monitoring, as determined by the site investigator.

Grade 4: Provide immediate clinical management and consult with protocol team. Permanently discontinue VRC01LS and 10-1074. Participant should be followed approximately weekly until resolution to Grade ≤1 or until stabilized and no longer in need of frequent monitoring, as determined by the site investigator.

9.1.4. Guidelines for Other Toxicities

In general, VRC01LS and 10-1074 may be continued for other toxicities that are Grade 1 or 2.

For Grade 3 toxicities, VRC01LS and 10-1074 may be continued or held, depending on the toxicity and potential relatedness to study drug.

Children who develop a symptomatic Grade 4 adverse event or toxicity felt to be related to VRC01LS and 10-1074 will have VRC01LS and 10-1074 discontinued with ART resumption, if it had been paused.

Children experiencing adverse events requiring permanent discontinuation of study treatment should be followed approximately weekly until resolution of the adverse event to Grade ≤1 or until stabilized and no longer in need of such frequent monitoring, as determined by the site investigator.

In cases in which VRC01LS and 10-1074 are held due to a toxicity, they may be resumed (with careful monitoring) when that toxicity has resolved to Grade 2 or lower if there is an alternate explanation for the event.

Any toxicity leading to non-receipt of either study product that extends beyond 14 days of the expected infusion visit will trigger review by the protocol team and, if in Step 2, weekly HIV-1 RNA testing will occur.

9.2. Participants Lost to Follow-up

Collecting any intention to move or change of address during each visit will minimize the loss of contact with participants. In case of absence at a scheduled visit, a study nurse or counselor will attempt to locate the parent/guardian and child by phone or home visit. Efforts will be made to keep all participants in study follow-up. In the event that consent is withdrawn by the parent/guardian, a final study visit / blood draw will be requested for the child at this time, and ART resumed (if paused) with appropriate referral for care.

9.3. Child Death

In the case of a child's death, a Death Form should be completed within 3 business days of the study team becoming aware of the death. If a child dies in a health facility, events prior to and at the time of death will be collected from hospital records and a verbal autopsy CRF will be completed. If death occurs outside the hospital setting, a verbal autopsy CRF will be completed upon interview of the parent or guardian. If the parent of a participant dies participant follow-up will continue throughout the study period if possible. The purpose of the study will be explained to the guardian of the child. During screening, study staff will ask the parent/guardian, where possible, to identify an individual with whom she would like the study team to share information about her baby's health in the event of her absence. This information will be documented on the locator form. Additional support may be sought from social services.

9.4. Criteria for Study Discontinuation

- Request by the parent/guardian to withdraw.
- The child's parent/guardian is judged by the investigator to be at significant risk of failing to comply with the provisions of the protocol as to cause harm to the child or to interfere with the validity of the study results. This may include the child missing 2 consecutive study visits (despite attempts to locate), at the investigator's discretion.
- At the discretion of the NIH or local Ministry of Health.
- At the recommendation of the SMC.

- The study may be discontinued at any time by NIH, the FDA, the IRB/ECs, Office of Human Research Protection (OHRP), Botswana Government or other government agencies as part of their duties to ensure that research participants are protected.

10. STATISTICAL CONSIDERATIONS

10.1. Statistical Considerations for Aim 1

10.1.1. Safety and PK evaluations: The first objective of Aim 1 is to determine the safety and PK parameters for VRC01LS and 10-1074. We will describe the cumulative proportion of children experiencing serious adverse events during follow-up, and the proportion of potentially bNAb-related events. We will describe the PK parameters in detail for participants in the PK Step, the first 6 participants in Step 1, and the proportion of children with trough VRC01LS and 10-1074 concentrations below defined trough ranges at Step 1 Weeks 4, and 8, and monthly during Step 2. We will also perform exploratory analyses correlating trough concentrations in plasma with HIV-1 RNA levels and other viral reservoir parameters.

10.1.2. Proportion Remaining Virally Suppressed: The next objective of Aim 1 is to evaluate the proportion of children in whom HIV RNA remains <400 copies/mL after 32 weeks of dual bNAb treatment (24 weeks off ART). With a sample size of 20 children (the minimum number expected), safety and PK evaluations will be descriptive, and there will be reasonable precision to estimate the proportion with HIV viral suppression. For example, if the observed proportion with HIV RNA <400 copies/mL is 70%, then this includes 46% to 88% with 95% confidence (based on exact confidence limits) and so there would be evidence that HIV RNA can be maintained suppressed in 46% or more of children. If the observed proportion is lower than 70%, this study will have value as proof-of-concept but is less likely to be of importance as an alternative treatment to ART. Based on data from the CHER Study [52] and from a randomized treatment interruption trial in Kenya [58], <6% of children treated in the first year of life are expected to remain virally suppressed by 6 months off ART. Thus, we believe that demonstrating at least 30% success at 6 months is of scientific interest for this proof-of-concept trial. If the observed proportion is 30% suppressed with an N=20, then a 95% confidence interval will be from 12% to 54% (based on exact confidence limits), and would indicate a biologic effect of the intervention. Thus, for an observed proportion of 30%, we will reasonably be able to rule out low true proportions (e.g. <12%) or high proportions (e.g. >54) and conclude that this strategy achieves suppression in a subset of individuals (even if it is not be useful for most treatment purposes). Accordingly, we have based study stopping criteria on the probability of achieving 30% success (see Section 13.8.1). This approach provides a balance between maximizing the scientific benefits of the study, while not allowing an excessive number of participants to experience viral rebound in the event that there is little treatment benefit from the dual bNAb strategy.

Although this proof-of-concept Phase I/II study will not have a control group, an observed proportion of at least 30% suppression on dual bNAb therapy would provide reasonable evidence of a positive effect of VRC01LS + 10-1074 in maintaining virologic suppression, as maintenance of HIV-1 RNA <400 copies/mL is unlikely in the absence ART, even for early-treated children. As noted above, data from children treated in the first year of life in CHER [52] and in Kenya [58] showed that few children remain suppressed to a threshold of <400 copies/mL at 6 months off ART. In CHER, only 2 of 183 children were suppressed to <400 copies/mL at 6 months (one of whom has remained suppressed for > 9 years, and the other rebounded at ~ 8 months). Additional data from Aims 2 and 3 that correlate viral reservoir (pre-ART, and pre- and post-bNAbs) and maintenance of viral suppression, and

associations between immune responses and viral control, will bolster interpretation of the specific effect of VRC01LS + 10-1074 on maintenance of viral suppression.

10.1.3. Dynamics of viral decay and rebound: Based on frequent HIV RNA monitoring after ART withdrawal, the kinetics of viral rebound (if rebound occurs) will be summarized descriptively. Analyses will assess viral suppression off-ART (yes vs. no) and time to HIV RNA ≥400 (and ≥40) copies/mL, and will evaluate associations of these metrics with pre-ART and on-ART virologic and immunologic measures including immune activation and anti-HIV immune responses (binary regression; rank correlations analyzing infants with sustained undetectable HIV RNA off-ART as the lowest rank).

10.1.4. Growth assessments: Child weight and height will be measured at least every month, and WHO standardized Z-scores will be evaluated for changes over time (beginning with pre-intervention data from the EIT study), and compared with data from prior studies for children on continuous ART. Although neurodevelopmental assessments would be of interest, the small number of children in this cohort and lack of an appropriate comparator group would preclude meaningful information from being obtained from standard neurodevelopmental testing.

10.2. Data Analysis and Statistical Considerations for Specific Aim 2

10.2.1. Virologic Assays: Results of virologic assays will be expressed as copies/mL, copies/million PBMC or copies/million CD4+ cells, or full-length genome-equivalents, as appropriate. Assay limits of detection will be calculated based on the volume of plasma or the number of cells tested. For phylogenetic studies, we will perform sequence alignments using MUSCLE [59]. Phylogenetic distances between sequences will be examined using ClustalX-generated neighbor joining algorithms [60]. Genome-intact viral sequences will be evaluated for the presence of viral escape mutations to antiretroviral agents, to HIV-1-specific CD8 T cell-mediated immune pressure (based on CTL epitope mapping data conducted in SA3) and to VCR01LS and 10-1074-mediated immune activity. For calculation of HIV-1 DNA changes in CD4 T cell populations, Tobit regression for censored data [61] will be applied using a standard Markov-Chain Monte Carlo Method as described in [62], applying non-informative normally distributed priors. Differences in HIV-1 DNA content between different CD4 cell populations within the same study persons will be analyzed using the Wilcoxon signed rank test for paired data. Biomarkers of viral reservoir size will be correlated with PK levels (trough levels and AUC levels) of VRC01LS and 10-1074, and with viral rebound kinetics after discontinuation of standard antiretroviral therapy.

10.2.2. Potential pitfalls and alternative approaches: Each of the virologic techniques described above are well established in our laboratory and should not pose technical problems. The major challenges that may arise include limited assay sensitivity due to limited recovery of CD4 T cells or plasma due to limitations on sampling in small children, and the possibility that very early treatment initiation may have reduced reservoir size to undetectable levels. Another potential limitation is that sampling of plasma and circulating CD4+ T cells may not reflect the reservoir in lymphoid tissues. Although negative results in these assays do not imply absence of any residual tissue reservoir, as illustrated by the “Mississippi baby” [63, 64] and the “Boston patients” [65], absence of detectable measures of the reservoir will still be informative if they remain negative during the period of maintenance therapy with VRC01LS and 10-1074 alone. Likewise, a detectable change in any one of the several reservoir measures during the period of bNAb maintenance therapy will be highly informative.

10.3. Data analysis and interpretation for Specific Aim 3

10.3.1. Analysis of flow cytometry/CyTOF data: Mass spectrometry and flow cytometry data will be stored as FSC files, and analyzed according to standard protocols, using FlowJo software or using "Spanning tree progression analysis of density normalized events" (SPADE), a computational algorithm specifically developed to analyze and visualize high-dimensional mass spectrometry data generated by multidimensional CyTOF and flow cytometry data [66]. CyTOF and flow cytometry data will be analyzed and visualized using dot plots, histograms, heatmaps, principal component analysis (PCA) or as consensus tree structures reflecting cellular hierarchies based on immunophenotypic characteristics.

10.3.2. Analysis of functional immunological assays: Mean and standard deviation of functional immune parameters will be calculated, as previously demonstrated in the investigators' work [67-69]. We will consider paired T- tests or nonparametric Wilcoxon tests for comparison between baseline and on-study immunological results.

10.3.3. Analysis of gene expression profiles: Differences in gene expression intensity among baseline (prior to study treatment) and subsequent timepoints will be detected using the empirical Bayes (eBayes) adjusted t-test, as implemented in the LIMMA package of R. Detection of p-values will be corrected for multiple hypothesis testing using calculation of false discovery rates (FDR). For further analysis, principal component analysis, hierarchical clustering or gene set enrichment analysis (GSEA) will be performed, as described in our previous work [70].

10.3.4. Analysis of neutralizing breadth: Viral neutralization titers (50% inhibitory dose [ID_{50}]) against tier 1-3 viruses in the presence of patient plasma will be calculated as the plasma dilution at which relative light units (RLU) are reduced by 50% compared to the number of RLU in virus control wells after subtraction of background RLU counts in cell control wells. ID_{50} levels will be longitudinally compared between baseline and on-study timepoints.

10.3.5. Associations between immune parameters and clinical/virological characteristics: Correlations will be calculated between biomarkers of HIV reservoir size (total HIV DNA, genome-intact HIV DNA, plasma RNA) and (i) proportions of individual leukocyte and CD4 memory T cell subsets, (ii) phenotypic markers of T, B, NK cell and dendritic/monocyte cell markers, (iii) proportions and functions of HIV-specific CD4 and CD8 T cells, NK cells, and neutralizing antibody breadth and (iv) gene expression patterns in specific immune cell subsets. Correlations will be cross-sectionally determined at pre-bNAb infusions and at selected timepoints during the subsequent study, using Spearman correlations. In addition, correlations between immunological and virological parameters across multiple timepoints will also be calculated, using generalized estimated equations for repeated measures (GEEs). Associations between changes of specific HIV-1 reservoir biomarkers during given time intervals and corresponding changes in immunologic measures will also be analyzed. In addition, possible associations between immune responses and viral rebound kinetics after antiretroviral treatment interruption will also be explored.

11. DATA HANDLING AND RECORD KEEPING

11.1. Data Management Responsibilities

Data will be entered by clinical study staff onto standardized case report forms (either paper or electronic) at the sites. Electronic data capture will occur using Medidata Rave, supported by Frontier

Science (please see Human Subjects Protections Section 13 for a description of data storage and access plans, and security of the data). A data manager will incorporate basic error checking capability to minimize data entry errors and to allow rapid querying of the sites for illogical or missing data. The data manager will generate monthly accrual reports as well as periodic reports (approximately 3-monthly) that will summarize basic demographic data and reported aggregate diagnoses, to allow real-time review of enrollment/data.

11.2. Source Documents

The following data items will be considered source documents:

- **Signed Consent Form**
- **Certified Copies of Clinical records:** photocopies of clinical records, including hospitalization notes (where necessary) will be made at every visit and all copies will be certified as true copies of the original by the person making the photocopy. This will be documented by signing and dating a "certification of copy" stamp on the photocopy and those copies will be filed in the study participant's file at the site.
- For any death of a study participant while on study.
    - Copies of death certificate when available.
    - Copies of hospital inpatient or outpatient/clinic records with information thought by the Study Physician to be directly related to the participant's death.
    - Copy of the completed verbal autopsy form.
    - Any laboratory or other clinical investigation/procedure report containing information directly related to the participant's death.

11.3. Quality Control and Assurance

1. The study physician will review all completed CRFs.
2. Study nurses and study physician will be responsible for the following:
    a. Weekly internal audits of participants' files
    b. Create checklist and follow-up logs
3. Administrative assistants are responsible for:
    a. Updating follow-up logs and appointment book
    b. Confirming the availability of checklist in the files. The checklist should include:
        i. Locator form filled and signed
        ii. Enrolment consent signed with time and date of consent
        iii. Laboratory information
        iv. Pharmacy information

Keyed data will be reviewed by a second person (either a study physician or study nurse) whenever feasible, to improve quality control.

If an error, inconsistency or incomplete data is identified, the CRF will be tagged "unverified" until the error is resolved. If the original staff member is not available to make corrections, the study physician or other research nurse will make corrections or completions on his/her behalf provided they have access to the correct information.

There are additional levels of quality control, which will occur at the Data Manager level (Frontier Science). Error checking is part of the RAVE data entry program and assists the keyer in identifying problems. The keyer is notified by the program feature of a logic check function that gives a detailed

explanation of the incomplete or inconsistent data. This allows for potential errors to be resolved prior to CRF completion.

12. CLINICAL SITE MONITORING

Site monitors under contract to the National Institute of Allergy and Infectious Diseases (NIAID) will visit participating clinical research sites to review participants records, including consent forms, CRFs, medical records (e.g., physicians' progress notes, nurses' notes, individual's hospital charts), and laboratory records to ensure protection of study participants, compliance with the EC/IRB approved protocol/amendments, and accuracy and completeness of records. The monitors will inspect sites' regulatory files to ensure that local regulatory requirements, in addition to U.S. Federal regulations, are being followed. They will also inspect sites' pharmacies to review product storage and management.

The site investigator will make study documents (e.g. consent forms, drug distribution forms, eCRFs) and pertinent hospital or clinic records readily available for inspection by the local IRB and the Harvard T.H. Chan School of Public Health IRB, the Botswana Ministry of Health, the site monitors, the FDA, the NIAID, the OHRP, and other local, U.S. or international regulatory entities for confirmation of the study data.

13. HUMAN SUBJECTS PROTECTIONS

Human subjects will be enrolled in this study. HIV-infected children who are study eligible will represent the population enrolled in this study. The health status of HIV-infected children participating in this study is expected to reflect that of optimally-treated HIV-infected children in Botswana. HIV-infected pediatric participants will receive the standard-of-care in accordance with Botswana National HIV treatment guidelines for at least 8 weeks from the date of bNAb treatment initiation, in addition to the bNAb intervention. Study participants will then undergo ART treatment interruption in Step 2 and will continue ongoing VRC01LS and 10-1074 infusions with close monitoring for up to 24 weeks. If viral load rebounds, ART will immediately be re-started. If there is no viral rebound, ART will be restarted after the final VRC01LS and 10-1074 infusions of the Step 2 Week 24 Visit (at Week 0 of Step 3).

Inclusion criteria for this study make it likely that only children with very low viral reservoir will undergo the dual bNAb intervention. All children will have started ART within the EIT study, with the majority from the antenatal cohort and having started <7 days of life, and all required to have maintained viral suppression <40 copies/mL for 24 weeks prior to enrollment.

13.1. Characteristics of study population

The study population will consist of HIV-infected children ≥96 weeks of life.

13.2. Sources of Research Material

Research material will consist of data obtained from questionnaires and from blood draws.

13.3. Participant Recruitment and Study Consent

Participants will be recruited from the EIT study as outlined above. For eligible HIV-infected children whose parents/guardians wish to enroll, written informed consent for the study will be obtained by study physicians or study nurses. Consent forms will be written in both Setswana and English and will be reviewed verbally with the parent/guardian by the study team. The consent process will describe the purpose of the study, the nature and duration of study follow-up, and the risks and benefits of participation, and will highlight the parent/guardian's freedom to decline or withdraw participation at any point without compromising her infant's future medical care. Up to 4 additional children from outside the EIT study may be recruited for the PK Step only, in the event that EIT children are not available for recruitment. These children may be recruited from Botswana government ART clinics or other treatment clinics in Botswana including the Botswana-Baylor Children's Clinical Center for Excellence.

Study investigators will prioritize optimizing participant education and informed consent procedures for the study. Participant understanding of scientific information, research methods, and human subjects rights can be enhanced through the following: 1) full, standardized disclosure of study-related information in both written and verbal formats, 2) minimizing the reading level and enhancing the visual display of written documents, 3) providing adequate time and opportunity for participants to read/hear about, reflect upon, ask questions about, and discuss the research.

13.4. Risks and Benefits

VRC01LS and 10-1074 are expected to be safe for children, and antibody-related adverse events other than possible injection site reactions or infiltrations are not anticipated. Risks also include the potential for viral rebound, and possibly acute retroviral syndrome. Increased seeding of the viral reservoir is also a possibility, although the clinical consequences of this are not known. Frequent viral load monitoring and early re-start of ART will minimize the potential risks of viral rebound and reservoir seeding to a very low level. The potential benefit for children in this study who remain virally suppressed during treatment with dual bNAbs is an extended period off ART with the potential for increased patient/caregiver satisfaction and reduced long-term toxicities, and the theoretical benefit of a broader immune response to HIV. There is also substantial scientific benefit from the knowledge gained, including benefit for other children in Botswana.

13.4.1. Risks

13.4.1.1 Stigma / Confidentiality / Coercion: Some parents/guardians of children enrolled in the study, or the children participants themselves might be subject to stigma or the perception of stigma if they participate in the study, especially regarding HIV status disclosure. However, most participants are already being followed in the EIT study and we are not aware of issues or concern about stigma from study clinic visits. The ability to remain off daily ART may *reduce* stigma in the household or community by moving all HIV-related treatment to the study clinic during the period of dual bNAb therapy. Measures to protect confidentiality of data are in place that include computer encryption and use of subject identifiers instead of personal identifiers, with limited/encrypted access to the linkage between subject and personal identifiers.

Coercion to enroll in the study will be avoided by the balanced consent process and the limited amount of travel and time reimbursement which is set at a level approved by the ethical review boards.

13.4.1.2. Blood Draws: Blood volumes are an important consideration for this protocol, and will be minimized to the greatest extent possible at every visit. A summary for multiple guidelines on this topic, published by WHO in the WHO Bulletin, is as follows: "Existing guidelines for blood sample volume limits (ranging from 1–5% of total blood volume within 24 hours and up to 10% of total blood volume over 8 weeks) are consistent with the limited evidence available on "minimal risk" to children. Lower limits for sick children are advisable, and a maximum of 3 ml/kg post-neonatally within 24 hours (3.8% of total blood volume), in line with United States policy, seems to be a reasonable guideline, although each study must be judged on its own merits and greater caution may be needed in children with illnesses that impair the replenishment of blood volume or hemoglobin [71]." The NIH Clinical Center also provides guidance for maximal allowed blood volumes allowed in research. The NIH recommends a limit of 5 mL/kg per single blood draw and a limit of 9.5 mL/kg in any 8-week period.

We will draw no more than 12 mL at any draw during the study, and less at most visits. If additional clinical blood draws occur, study staff will account for these draws and make adjustments in the volume drawn for the study if needed. However, given that all children will be at least 96 weeks of age, we expect them to be over 10 kg (which is ~ 5th percentile). By established limits above, a 10 kg child would be able to have up to 50 mL of blood per draw (we will only draw 12 mL), and up to 95 mL in an 8 week period (the maximum in this study would be much less). Thus, we believe that the blood draw volumes for the study are well-within safety parameters, even if a child is at 5th percentile or lower in weight.

13.4.1.3. VRC01LS and 10-1074: Passive polyclonal antibody products have been used for treatment and prophylaxis with excellent safety profiles over many decades. These agents include hepatitis B immunoglobulin (HBIG), Respigam®, Cytogam®, HIVIg, and IVIG. In a large study of IVIG to prevent infection in preterm infants published in 2011, adverse events were rare and did not differ between IVIG and placebo [41]. However, few children have received VRC01LS and none have received 10-1074, and while safety and PK data are available from children/adults, there are unknowns. Among children and adults who have participated in prior studies, only minor safety concerns were identified with these agents. Children will be closely monitored for these events. Review of detailed safety and PK testing will occur for all children enrolled in the PK Step and the first 6 children in Step 1 (see below Section 13.8).

13.4.1.4. Antiretroviral treatment interruption: Standard ART will be paused during this study after at least 8 weeks of overlap between standard ART and dual bNAb therapy. The BHP laboratory use the sensitive Roche Taqman assay, which has limit of quantification to <40 copies/mL to monitor HIV RNA in this period, and will also use Qualitative HIV DNA for those who are negative at study entry as a potentially more sensitive measure for viral rebound. Although a lower limit of detection is possible when large blood volumes are obtained (a limiting factor when treating children), the lower limit for clinically defining children who have reservoirs low enough to participate in this trial is unknown and we believe that using clinically available and interpretable cut-off of <40 copies/mL is appropriate. The goal of this study is to prevent viral rebound using VRC01LS and 10-1074 (i.e., these agents will serve as effective alternate therapy). However, even if viral rebound occurs, the risk is expected to be small. Analytic treatment interruptions have been performed safely in HIV-positive patients, and have been helpful in determining the extent of viremia, viral rebound and changes in immune function following initiation of a treatment modality, such as ART. Analytical treatment interruption (interruption until plasma viremia returns followed by re-initiation of ART, or re-initiation of ART shortly after return of plasma viremia) is different from structured treatment interruptions that have been studied as potential ART sparing treatment strategies. Episodic antiretroviral therapy guided by CD4+ counts in such structured treatment interruptions often involve intermittent and repeating cessation of therapy in viremic individuals for months at a time, and may be associated with increased risk of opportunistic

disease or death from any cause. We are not planning on sustained or episodic treatment interruptions in the setting of plasma viremia. Although studies in adults such as SMART [72] and Trivican [73] have led to concerns regarding treatment interruption, there are 2 reasons why the proposed strategy is expected to be safe if viral rebound does occur: 1) frequent monitoring of viral rebound, and 2) unique characteristics of children and safety of previous treatment interruption trials among children. In SMART, unchecked viremia for long periods of time lead to a pro-inflammatory state and immune dysregulation. By contrast, in the proposed study ART will be resumed upon confirmed detection of viremia, thus limiting the risk of uncontrolled viral replication. Interruption studies in children have not replicated concerning findings in adults [74-76], including the Bana Study performed in Botswana (personal communication, E. Lowenthal, 2016). Although drug resistance may be a concern with repeated interruption of NNRTI-based ART, resistance is not anticipated with a single interruption and resumption of PI-based ART [77], especially given the expectation that if viral rebound occurs in this population it will do so after all ART levels are undetectable. Finally, preliminary data from the first 10 children in the EIT study suggests that even those children who experience occasional viral rebound have low viral reservoir (median ~ 5 copies/million cells) at 84-96 weeks of life, suggesting that brief plasma rebounds may not impact viral reservoir to a large extent (BHP, unpublished data, January 2018).

The VRC01LS and 10-1074 maintenance in this study will be performed in a carefully monitored manner. ART withdrawal will be followed by weekly viral load monitoring for 4 weeks, and 2-weekly monitoring thereafter (however, if HIV RNA is 40-400 copies/mL, or if there is a newly positive qualitative DNA PCR, weekly HIV RNA monitoring will continue). Children will be restarted on ART (the same regimen as prior to interruption) if they have a single viral load ≥400 copies/mL. If patients experience viral rebound, they will be followed with viral load testing until they achieve viral suppression (<40 copies mL), at which time, routine clinical monitoring will be continued.

The risk of developing antiretroviral resistance after analytic treatment interruption is small, and similar to continuous therapy groups in large trial populations [78]. Although this study proposes a much more limited analytical treatment interruption, HIV resistance testing will be performed if viral suppression has not been achieved 12 weeks following ART re-initiation.

Children who rebound in the setting of VRC01LS and 10-1074 may exhibit resistance to these bNAbs, which usually occurs by selection for pre-existing resistance mutations (and is felt to be less likely in early-treated low reservoir children). If resistance occurs to one or both bNAbs, it would indicate that there is limited utility in using the same bNAb agent in future studies or treatment settings for the child.

13.4.2. Benefits: Results from this study may provide a proof-of-concept that may benefit other children in Botswana in the future, including those who may be unable to take ART or to remain suppressed on ART. There are no known individual benefits to study participants. However, it is possible that some may indirectly benefit from the potential to remain off ART for an extended period of time while viral suppression is closely monitored and maintained, with potential medical and psycho-social benefits that accompany this break from daily ART. It is also plausible that participants could potentially have improved immunologic responses to HIV if viral rebound does occur later in life at a time of non-adherence.

13.4.2.1. Time off ART: Given the unique characteristics of children in this study and the possibility that up to 70% might remain without viral rebound for up to 24 weeks, the potential advantages of identifying children who may remain off ART for long periods of time (and avoid potential ART toxicities) are substantial. Benefits of remaining off ART include patient preferences (including potential reduced stigma of taking daily medication), and the avoidance of possible long-term ART toxicities that include impaired growth and endocrine effects [2, 3], lipodystrophy [4], hematologic

toxicities [5], renal toxicity [6, 7], bone density effects [8-10], cardiovascular disease [11-13], and possible neurodevelopmental effects [14]. In addition, strategies that allow time off ART are highly desirable for many patients and caregivers for reasons related to treatment fatigue, travel, stigma, or other factors [15].

13.4.2.2. Immune Responses: Another potential benefit of bNAb use in children is the potential for immunologic recognition of HIV and avoidance of acute retroviral syndrome in the event that viral rebound occurs either during the study or at a later period in life. It is expected that early treated children will have almost no immune recognition of HIV, and therefore, at a future period in life where viral rebound occurs it may do so with high viral load and accompanying antiretroviral syndrome. VRC01LS and 10-1074 may enhance immune responses to HIV, and may therefore mitigate the possibility of antiretroviral syndrome for participants in this study.

13.4.2.3. Proof of Concept: Dual bNAb therapy with VRC01LS and 10-1074 may will help answer critically important questions for HIV curative research, including whether early-treated children can remain virally suppressed with dual bNAb therapy; what markers predict ongoing suppression or delayed viral rebound; and modification of immune responses by bNAbs. As bNAb research progresses, and as longer-acting agents are developed, bNAbs may become a viable strategy for viral suppression and for allowing time off ART for patients in Botswana and elsewhere. Such a strategy may be particularly important for children during times of growth and development, and during times when adherence to daily ART is challenging. For some children who cannot receive daily ART or who fail to suppress viral load on standard ART, a bNAb-based strategy may serve as a viable future alternative.

13.4.3. Risks/Benefits: The risks of the study are mitigated by the close monitoring that will occur. While there may be no direct benefit to individual participants in the study, there are several potential benefits for those who are able to maintain viral suppression during the dual bNAb therapy. There are several benefits associated with the proof-of-concept that may benefit HIV-infected children in the future.

13.5. Adherence to Human Subjects Requirements

Study participants will be recruited, enrolled, and followed in Botswana. Adherence to human subjects regulations will primarily be the responsibility of both Harvard T.H. Chan School of Public Health and Botswana staff. The BHP team will adhere with all requirements by:

- Developing a study protocol that is able to meet its stated research objectives, and thus reflect adequate risk-benefit ratios for human subjects.
- Specifying study procedures in the protocols that protect the rights and safety of human subjects.
- Developing an informed consent form that includes all elements of informed consent required by Federal regulations and accurately represents study requirements, risks, and benefits in language that is understandable to study participants.
- Including human subjects considerations and requirements in study training sessions.
- Monitoring adherence to protocol specifications and human subjects requirements.

This proposal will be submitted to the Institutional Review Boards (IRB) of the Botswana Ministry of Health (the Health Research and Development Committee, or HRDC, and to the Harvard T.H. Chan School of Public Health.

13.6. Procedures for Minimizing Potential Risks

1) Identification and disclosure of HIV Status: Children participating in our study (or their parents) may experience stigma from being thought to be HIV-infected. We have spent several years working to reduce the stigma concerns among participants in previous studies (including the EIT study, from which most participants will be drawn), and have had success in these efforts. We have dedicated health educators at each site, and we provide extensive counseling during the screening process and at all subsequent visits, as needed. We invite parents/guardians of participants to bring family members or significant others to the clinic if they wish to do so, for joint counseling. We have also helped to develop support groups in the community for HIV-infected individuals.

Any information provided by the participant and/or the participant's parent/guardian, including and especially their HIV status, will be subject to strict confidentiality. Special care will be exercised in all interactions with the participant and his/her parent/guardian to ensure that participation or non-participation in the study does not cause release of information.

The protection of information given in interviews is standard procedure. The identity of the participants will be only kept at the clinic sites in a locked facility, and in an encrypted database. Identifying information will be stored in separate locked file cabinets, and a numeric code will be assigned to the completed questionnaires.

2) Recruitment and Informed Consent: A parent/guardian will undergo an informed consent process and sign a written informed consent for their child's participation in this study. The study informed consent form will contain all of the required elements as outlined by 45 CFR, 46.116. Informed consent forms for BHP studies are translated into Setswana, and back-translated into English. These translations are approved by both IRBs at Harvard School of Public Health and at the Botswana Ministry of Health (the HRDC). In general, Setswana-speaking nurses conduct the informed consent process with the potential participant's parent/guardian, and physicians will review and discuss the process and answer questions after the nurse goes through the consent form. In a private setting, the nurse verbally reviews the contents of the entire informed consent form with the child's parent or legal guardian (regardless of literacy level). For more complicated studies, this process can take 1-2 hours, allowing sufficient time for the parent/guardian to ask questions. Schematics/diagrams are used when possible. Sometimes, informed consent occurs over two separate visits. At the end of the discussion regarding the study (and full review of the informed consent form), the parent/guardian is given the opportunity to read the form, to ask questions, etc. Then, the nurse and study physician usually review the parent/guardian's understanding of study purpose, procedures, risks, benefits, etc.—the most important elements of the study from a human subjects perspective—to ensure that the parent/guardian understands these prior to the parent/guardian and the study staff member who conducted the informed consent process signing the form.

3) Safety and toxicity and PK monitoring plans outlined in the protocol, and approved by IRBs, SMC and study sponsor, will ensure the early identification of events and safe resolution of any possible toxicities. Post-infusion monitoring for 2-4 hours will occur following the first infusion (4 hours for the first cohort receiving 10-1074 in the PK Step). If initial doses are tolerated 1-hour post-infusion monitoring will occur with subsequent infusions.

4) Blood Draws: The study will follow research criteria established by NIH for safe volumes of blood drawn in children. Study physicians will carefully review all clinical history and weights to be sure that established safe blood volumes drawn at each visit or over time are not exceeded and prioritize blood

draws, as needed, if limits are being approached based upon a child's weight. Physicians will monitor for signs and symptoms of anemia, including pallor, tachycardia, and scheduled full blood count. Close clinical monitoring for anemia will occur in all children.

13.7. Participation of Children, Women and Minorities

Children are included in this research plan and will comprise 100% of those enrolled in the study. BHP study sites are accessible to individuals drawn from different ethnic groups, all of whom have access to government clinics and who are offered HIV testing by the National Program to prevent MTCT and ART, if HIV-infected.

13.8. Monitoring and Interim Analyses

An independent, external Safety Monitoring Committee (SMC) will be established for this study, consisting of approximately 5 members, and approved by the study sponsor. Committee members will include those with expertise in HIV treatment trials and pediatrics. The primary role of the SMC is to help protect the safety of participants in the study. The reviews of interim data by the SMC will be triggered by criteria described below, but will occur at least every six months unless otherwise recommended by the SMC. The SMC will make recommendations to the study team and to the study sponsor. Following SMC reviews, the sponsor and study team will communicate and discuss the findings. A response plan will be developed by the study team, and sign off by the sponsor will be required to implement the response.

13.8.1. Quarterly Reporting and SMC Triggers for Safety or Stopping Criteria. Monthly safety and accrual monitoring by the protocol team will occur throughout the study. Reports will be provided to the study sponsor and IRBs quarterly. Regularly scheduled conference calls will occur between members of the study team (including 1 or more PIs) and the Medical Officer at least quarterly. Serious adverse events that are EAEs may trigger an early review by the SMC (SAE-category EAE reporting will be used for most of the study). The occurrence of any EAE will prompt immediate review by the PIs and Medical Officer. The attribution of relationship of an EAE to study product will be discussed and the relationship will be determined by the study team, taking into account the site and the Medical Officer's assessment of the event. Interpretation of 10-1074 or VRC01LS relationship to adverse events will be based on the type of event, the relationship of the event to the time of immunization, the known biology of the monoclonal antibody and the investigators' medical judgment. Gradation of relationship will use the following terminology: "not related", and "related." The occurrence of 2 EAEs determined to be related to study product will trigger an SMC review. This may lead to a recommendation for study discontinuation in consultation with the study team and sponsor. Due to the fact that Grade 3 neutropenia and anemia are common in children on ARVs, and the fact that hemolyzed specimens may falsely lead to Grade 3 hyperkalemia values, these toxicities will not be counted in the real-time safety review algorithm.

NOTE: If at any time during the study the triggers for SMC review are met, the study will be paused to new accruals and an SMC review performed within 7 days. The SMC will decide what additional data they would like to see and advise the study team on how to proceed.

Review of Data from PK Step

After all 12 PK Step participants complete the PK Step, safety, PK, and viral suppression data will be reviewed within 8 weeks by the study team including its sponsor representatives, and SMC. All PK

data from the PK Step will be assessed based on pre-specified PK trough targets; the study team will determine if median trough concentrations are above the pre-specified targets (see Section 2.8.1) and present this information in a written report to the SMC. In addition, outlier concentrations will be presented and reviewed; the study team expects no more than 2 participants to have trough concentrations below 115 mcg/mL for VRC01LS and no more than 2 participants to have trough concentrations below 5mcg/mL for 10-1074. The SMC and the study team will review any cases of outlier PK concentrations and make individual recommendations about whether a child with these levels should proceed to Steps 1-3. The SMC will review safety data utilizing pre-defined safety criteria defined as follows: 0 EAEs support study continuation; 1-2 EAEs related to study product but for different events warrant concern; >2 EAEs, or 2 of the same EAEs that are related to study product may lead to a recommendation for study discontinuation. If safety criteria are met, and if the PK data are in the pre-specified range (see Section 2.1.8), Step 1 will begin unless otherwise recommended by the SMC. If the dosing and frequency of bNAbs established in the PK Step is accepted, it will continue to be used for the first 6 participants in Step 1 (see additional safety review below). If a change in dosing is recommended by experts on the study team (or by the SMC, if it does not accept the team's recommendation), adjustments will occur and IRB and study sponsor approval will be sought regarding required protocol changes prior to starting Step 1. The recommendations by the study team or by the SMC may include early termination of the study, if warranted by the safety/PK data, or a re-design of the study as approved by the study sponsors and regulatory agencies.

Review of Safety and PK Data from Step 1 (first 6 participants)

Safety, PK, and viral suppression data for the first 6 Step 1 participants will be reviewed within 8 weeks by the study team including its sponsor representatives, and SMC. All PK data will be assessed by the study team based on pre-specified PK trough targets which are the same as those used in the PK Step. The review will confirm that PK targets are met during dual bNAb administration. Safety criteria for the Step 1 data will be reviewed by the SMC, using similar criteria as for the PK Step: 0 EAEs support study continuation; 1-2 EAEs related to study product but for different events warrant concern; >2 EAEs, or 2 of the same EAEs that are related to study product may lead to a recommendation for study discontinuation. If safety criteria are met, and if the PK data are in the pre-specified range (see Section 2.1.8), unless otherwise recommended by the SMC, the first 6 participants will enter Step 2 and enrollment of additional participants into Step 1 will continue. If the PK data are in range and no dosing changes for the bNAbs are required, the dosing will continue to be used for the remainder of the study unless new data emerges requiring additional review. If a change in dosing is recommended by experts on the study team (or by the SMC, if it does not accept the team's recommendation), IRB and study sponsor approval will be sought regarding required protocol changes. The recommendations by the study team or by the SMC may include early termination of the study, if warranted by the safety/PK data, or a re-design of the study as approved by the study sponsors and regulatory boards.

Review for Early Stopping Criteria in Step 2

The SMC will review safety and virologic outcome data every six months after the first participant enters Step 2 or on a frequency recommended by the SMC, but at least annually. During Step 2, as well as a possible early review triggered by the occurrence of EAEs as outlined above, early review by the SMC will be triggered if 2 participants are unable to achieve re-suppression of HIV-1 RNA to <40 copies/mL within 12 weeks after re-starting ART. Otherwise, time to re-suppression will be reported to the SMC as part of their regular reviews.

Earlier reviews will also be triggered if all seven of the first seven participants in Step 2 experience viral rebound or if 13 of the first 14 participants in Step 2 experience viral rebound during the 24 week period of ART interruption. As a guideline for evaluating the proportion of participants experiencing viral rebound, if a true success rate (i.e. no rebound within 24 weeks) of 30% seems unlikely, then the study team feels that termination of further enrollment to Step 2 and restart of ART among participants already enrolled would be appropriate. Specifically, if all of the first seven participants in Step 2 experience viral rebound before or at week 24 (so no successes), or 13 or more of the first 14 participants (so no or only one success), then termination of further enrollment to Step 2 and restart of ART among participants already enrolled may be appropriate. These criteria have been chosen on the basis that the observed rates would be unlikely (probability 0.10) if the true success rate is 30%. Conversely, observed rates meeting these criteria would be likely (probability 0.88) if the true success rate is 5% (a value chosen based on what was observed in the CHER [52] and Kenya studies [58]). Note, however, that the SMC will be presented with all available virologic outcome data for all participants who have been enrolled at the time that either of these triggers are met in order to inform their recommendations. No additional enrollments will occur during this period of SMC review.

Routine reports to the SMC will occur every 6 months unless otherwise recommended by the SMC. Built-in safety measures for the study include a plan to enroll slowly, with no more than two children enrolling per week. Additional reviews or an altered schedule of review or trigger for early review may be instituted at the discretion of the SMC. The SMC will make recommendations for the study team and Sponsor at each of these reviews regarding whether the study should continue as originally designed. Statistical analyses will be prepared in advance of each SMC meeting.

13.9. Biohazard Containment

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel in the drawing of blood, infusing antibodies, and shipping and handling of all specimens for this study, as currently recommended by the Centers for Disease Control and Prevention.

All infectious specimens will be sent using the ISS-1 SAF-T-PAK or equivalent package that is compliant to the International Air Transport Association Dangerous Goods Regulations Packing Instruction 602. Please refer to individual carrier guidelines (for example: Federal Express, Airborne, etc.) for additional specific instructions. Appropriate shipping permits will be obtained.

13.10. Vertebrate Animals

This project does not involve vertebrate animals.

14. ADMINISTRATIVE PROCEDURES

14.1. Protocol Registration

Prior to implementation of this protocol, and any subsequent full version amendments, the protocol and the protocol informed consent forms will be approved by the IRBs in Botswana and in Boston, and any other applicable regulatory entity. Upon receiving final approval, investigators will submit all required protocol registration documents to the DAIDS Protocol Registration Office (PRO) at the

DAIDS Regulatory Support Center (RSC). The DAIDS PRO will review the submitted protocol registration packet to ensure that all of the required documents have been received. Site-specific informed consent forms will be reviewed and approved by the DAIDS PRO, and sites will receive an Initial Registration Notification from the DAIDS PRO that indicates successful completion of the protocol registration process. A copy of the Initial Registration Notification should be retained in the site's regulatory files.

Upon receiving final IRB/EC and any other applicable regulatory entity approval(s) for an amendment, sites should implement the amendment immediately. Sites are required to submit an amendment registration packet to the DAIDS PRO at the RSC. The DAIDS PRO will review the submitted protocol registration packet to ensure that all the required documents have been received. Site-specific ICF(s) WILL NOT be reviewed and approved by the DAIDS PRO and sites will receive an Amendment Registration Notification when the DAIDS PRO receives a complete registration packet. A copy of the Amendment Registration Notification should be retained in the site's regulatory files.

For additional information on the protocol registration process and specific documents required for initial and amendment registrations, refer to the current version of the DAIDS Protocol Registration Manual, which is available on the RSC website: https://rsc.niaid.nih.gov/clinical-research-sites/daids-protocol-registration-policy-and-procedures-manual.

14.2. Regulatory Oversight

This study will be sponsored by the National Institute of Allergy and Infectious Diseases (NIAID) which is part of the United States National Institutes of Health (NIH). The study product VRC01LS is provided by the NIAID Vaccine Research Center (VRC). The study product 10-1074 is provided by the Division of AIDS (DAIDS). DAIDS within the NIAID will be responsible for regulatory oversight of this study. DAIDS will distribute safety-related information pertaining to the study products prior to and during the conduct of the study, in accordance with its sponsor obligations.

An IND application to the Division of Antiviral Drug Research, Center for Drug Evaluation and Research (CDER), FDA, will be submitted. To support the IND application, the investigator brochure for VRC-HIVMAB080-00-AB (VRC01-LS) and 10-1074 will be submitted to the FDA. These documents include all relevant information on physical, chemical and pharmaceutical properties of the study drugs, summarize results from non-clinical and clinical studies and list all toxicology and safety data. Cross-reference to the existing IND for VRC01LS (held by DAIDS) and 10-1074 (held by Rockefeller University) will also occur for the IND application of this clinical trial.

14.3. Study Implementation

This study will be conducted in accordance with the protocol, international good clinical practice guidelines, and all applicable US, and non-US country and local regulations. Study implementation will also be guided by SOPs. These SOPs will be updated and/or supplemented as needed to describe roles, responsibilities, and procedures for this study.

14.4. ClinicalTrials.gov

This protocol will be subject to the United States Food and Drug Administration Amendments Act of 2007 (FDAAA), including registration in ClinicalTrials.gov.

15. REFERENCES

1. McComsey GA, Leonard E. Metabolic complications of HIV therapy in children. *Aids* 2004,18:1753-1768.
2. Cames C, Pascal L, Diack A, Mbodj H, Ouattara B, Diagne NR*, et al.* Risk Factors for Growth Retardation in HIV-Infected Senegalese Children on Antiretroviral Treatment: The ANRS 12279 MAGGSEN Pediatric Cohort Study. *Pediatr Infect Dis J* 2016.
3. Yanovski JA, Miller KD, Kino T, Friedman TC, Chrousos GP, Tsigos C*, et al.* Endocrine and metabolic evaluation of human immunodeficiency virus-infected patients with evidence of protease inhibitor-associated lipodystrophy. *J Clin Endocrinol Metab* 1999,84:1925-1931.
4. Piloya T, Bakeera-Kitaka S, Kekitiinwa A, Kamya MR. Lipodystrophy among HIV-infected children and adolescents on highly active antiretroviral therapy in Uganda: a cross sectional study. *J Int AIDS Soc* 2012,15:17427.
5. Parkes-Ratanshi R, Katende D, Levin J, Wakeham K, Heiner G, Kamali A*, et al.* Development of Severe Anemia and Changes in Hemoglobin in a Cohort of HIV-Infected Ugandan Adults Receiving Zidovudine-, Stavudine-, and Tenofovir-Containing Antiretroviral Regimens. *J Int Assoc Provid AIDS Care* 2015,14:455-462.
6. Troya J, Bascunana J. Safety and Tolerability: Current Challenges to Antiretroviral Therapy for the Long-Term Management of HIV Infection. *AIDS Rev* 2016,18:127-137.
7. Hamzah L, Jose S, Booth JW, Hegazi A, Rayment M, Bailey A*, et al.* Treatment-limiting renal tubulopathy in patients treated with tenofovir disoproxil fumarate. *J Infect* 2017.
8. Tebas P, Powderly WG, Claxton S, Marin D, Tantisiriwat W, Teitelbaum SL*, et al.* Accelerated bone mineral loss in HIV-infected patients receiving potent antiretroviral therapy. *Aids* 2000,14:F63-67.
9. Post FA. Bone mineral density decline in patients on stable antiretroviral therapy. *Aids* 2017,31:727-729.
10. Chitu-Tisu CE, Barbu EC, Lazar M, Ion DA, Badarau IA. Low bone mineral density and associated risk factors in HIV-infected patients. *Germs* 2016,6:50-59.
11. Namuyonga J, Lubega S, Musiime V, Lwabi P, Lubega I. Cardiac Dysfunction Among Ugandan HIV-infected Children on Antiretroviral Therapy. *Pediatr Infect Dis J* 2016,35:e85-88.
12. Zhou DT, Oektedalen O, Shawarira-Bote S, Stray-Pedersen B. Changes in coronary heart disease risk profiles of HIV patients in Zimbabwe over 9 months: a follow-up study. *HIV AIDS (Auckl)* 2016,8:165-174.
13. Badie SM, Rasoulinejad M, Salehi M, Kochak HE, SeyedAlinaghi S, Manshadi SA*, et al.* Evaluation of Echocardiographic Abnormalities in HIV Positive Patients Treated with Antiretroviral Medications. *Infect Disord Drug Targets* 2016.
14. Lowick S, Sawry S, Meyers T. Neurodevelopmental delay among HIV-infected preschool children receiving antiretroviral therapy and healthy preschool children in Soweto, South Africa. *Psychol Health Med* 2012,17:599-610.
15. Ananworanich J. The Emerging Potential For Hiv Cure For Infants, Children, And Adults. 24th Conference on Retroviruses and Opportunistic Infections, Seattle, February 13–16, 2017. Abstract 12.
16. Lynch RM, Boritz E, Coates EE, DeZure A, Madden P, Costner P*, et al.* Virologic effects of broadly neutralizing antibody VRC01 administration during chronic HIV-1 infection. *Sci Transl Med* 2015,7:319ra206.
17. Caskey M, Klein F, Lorenzi JC, Seaman MS, West AP, Jr., Buckley N*, et al.* Viraemia suppressed in HIV-1-infected humans by broadly neutralizing antibody 3BNC117. *Nature* 2015,522:487-491.

18. **Schoofs T, Klein F, Braunschweig M, Kreider EF, Feldmann A, Nogueira L*, et al.* HIV-1 therapy with monoclonal antibody 3BNC117 elicits host immune responses against HIV-1. *Science* 2016,352:997-1001.**
19. **Lu CL, Murakowski DK, Bournazos S, Schoofs T, Sarkar D, Halper-Stromberg A*, et al.* Enhanced clearance of HIV-1-infected cells by broadly neutralizing antibodies against HIV-1 in vivo. *Science* 2016,352:1001-1004.**
20. **Bar KJ, Harrison LJ, Overton ET, et al. ACTG 5340: The effect of VRC01 on viral kinetics after analytic treatment interruption. Conference on Retroviruses and Opportunistic Infections (CROI), Boston, abstract 32LB, 2016.**
21. **Bar KJ, Sneller MC, Harrison LJ, Justement JS, Overton ET, Petrone ME*, et al.* Effect of HIV Antibody VRC01 on Viral Rebound after Treatment Interruption. *N Engl J Med* 2016,375:2037-2050.**
22. **Ko SY, Pegu A, Rudicell RS, Yang ZY, Joyce MG, Chen X*, et al.* Enhanced neonatal Fc receptor function improves protection against primate SHIV infection. *Nature* 2014,514:642-645.**
23. **Caskey M, Schoofs T, Gruell H, Settler A, Karagounis T, Kreider EF*, et al.* Antibody 10-1074 suppresses viremia in HIV-1-infected individuals. *Nat Med* 2017,23:185-191.**
24. **Stephenson KE, Barouch DH. Broadly Neutralizing Antibodies for HIV Eradication. *Curr HIV/AIDS Rep* 2016,13:31-37.**
25. **Bhiman JN, Lynch RM. Broadly Neutralizing Antibodies as Treatment: Effects on Virus and Immune System. *Curr HIV/AIDS Rep* 2017,14:54-62.**
26. **Nishimura Y, Gautam R, Chun TW, Sadjadpour R, Foulds KE, Shingai M*, et al.* Early antibody therapy can induce long-lasting immunity to SHIV. *Nature* 2017,543:559-563.**
27. **Maddon, P., et al., Pro140 Sc Monotherapy Provides Long-Term, Full Virologic Suppression In HIV Patients, In American Society for Microbiology Microbe Conference (ASM Microbe). 2016: Boston, MA.**
28. **Scheid JF, Horwitz JA, Bar-On Y, Kreider EF, Lu CL, Lorenzi JC*, et al.* HIV-1 antibody 3BNC117 suppresses viral rebound in humans during treatment interruption. *Nature* 2016,535:556-560.**
29. **Barouch DH, Whitney JB, Moldt B, Klein F, Oliveira TY, Liu J*, et al.* Therapeutic efficacy of potent neutralizing HIV-1-specific monoclonal antibodies in SHIV-infected rhesus monkeys. *Nature* 2013,503:224-228.**
30. **Ledgerwood JE, Coates EE, Yamshchikov G, Saunders JG, Holman L, Enama ME*, et al.* Safety, pharmacokinetics and neutralization of the broadly neutralizing HIV-1 human monoclonal antibody VRC01 in healthy adults. *Clin Exp Immunol* 2015,182:289-301.**
31. **Nakamura, K.J., et al., Coverage of primary mother-to-child HIV transmission isolates by second-generation broadly neutralizing antibodies. AIDS, 2013. 27(3): p. 337-46.**
32. **Fouda, G.G., et al., Postnatally-transmitted HIV-1 Envelope variants have similar neutralization-sensitivity and function to that of nontransmitted breast milk variants. Retrovirology, 2013. 10: p. 3.**
33. **Wu, X., et al., Rational design of envelope identifies broadly neutralizing human monoclonal antibodies to HIV-1. Science, 2010. 329(5993): p. 856-61.**
34. **Uprety, P., et al., Cell-Associated HIV-1 DNA and RNA Decay Dynamics During Early Combination Antiretroviral Therapy in HIV-1-Infected Infants. Clin Infect Dis, 2015. 61(12):1862-70.**
35. **Rolland M, Sanders-Buell E, Bose M, et. al. Resurgence Of Hiv-1 Founder Viruses Following Antiretroviral Treatment Interruption. 24th Conference on Retroviruses and Opportunistic Infections, Seattle, February 13–16, 2017. Abstract 299LB.**
36. **Ackerman ME, Alter G. Opportunities to exploit non-neutralizing HIV-specific antibody activity. *Curr HIV Res* 2013,11:365-377.**
37. **Bolton DL, Pegu A, Wang K, McGinnis K, Nason M, Foulds K*, et al.* Human Immunodeficiency Virus Type 1 Monoclonal Antibodies Suppress Acute Simian-Human Immunodeficiency Virus Viremia and Limit Seeding of Cell-Associated Viral Reservoirs. *J Virol* 2016,90:1321-1332.**

38. **Luzuriaga K, McManus M, Catalina M, Mayack S, Sharkey M, Stevenson M*, et al.* Early therapy of vertical human immunodeficiency virus type 1 (HIV-1) infection: control of viral replication and absence of persistent HIV-1-specific immune responses. *J Virol* 2000,74:6984-6991.**
39. **Goulder PJ, Lewin SR, Leitman EM. Paediatric HIV infection: the potential for cure. *Nat Rev Immunol* 2016,16:259-271.**
40. **Ahmed, R., Oldstone, M. B. & Palese, P. Protective immunity and susceptibility to infectious diseases: lessons from the 1918 influenza pandemic. Nat. Immunol. 8, 1188–1193 (2007).**
41. **Group IC, Brocklehurst P, Farrell B, King A, Juszczak E, Darlow B*, et al.* Treatment of neonatal sepsis with intravenous immune globulin. *N Engl J Med* 2011,365:1201-1211.**
42. **Mayer, K.H., et al., Clinical Safety and Pharmacokinetics of IV and SC VRC01, a Broadly Neutralizing mAb, in Conference on Retroviruses and Opportunistic Infections (CROI). 2016: Boston, MA.**
43. **IMPAACT P1112. Open-Label, Dose-Escalating, Phase I Study to Determine Safety and Pharmacokinetic Parameters of Subcutaneous (SC) VRC01 and VRC01LS, Potent Anti-HIV Neutralizing Monoclonal Antibodies, in HIV-1-Exposed Infants. http://impaactnetwork.org/DocFiles/P1112/V3/P1112_Protocol_V3.0_07APR2017_with_PSP_LoA1_CM1_2OCT2017.pdf (Accessed 3Jan18).**
44. **Cunningham CK ME, Capparelli E, et al. Safety and Pharmacokinetics of the Monoclonal Antibody, VRC01, in HIV-Exposed Newborns. In: *24th Conference on Retroviruses and Opportunistic Infections*. Seattle, WA; 2017.**
45. **Gaudinski MR, Coates EE, Houser KV, Chen GL, Yamshchikov G, Saunders JG*, et al.* Safety and pharmacokinetics of the Fc-modified HIV-1 human monoclonal antibody VRC01LS: A Phase 1 open-label clinical trial in healthy adults. *PLoS Med* 2018,15:e1002493.**
46. **Investigator's Brochure: VRC01LS (VRC-HIVMAB080-00-AB) A Broadly Neutralizing HIV-1 Human Monoclonal Antibody. Version 3.0. Release Date August 21, 2017.**
47. **Investigator's Brochure: 10-1074 A Broadly Neutralizing Human Monoclonal Antibody Targeting HIV-1 gp120. Edition no. 3.0; Release date: August 10, 2017. The Rockefeller University.**
48. **Rockefeller University. 10-1074 Original IND 123713. Section 2.6.6 Toxicology Written Summary.**
49. **Nishimura Y, Martin MA. Of Mice, Macaques, and Men: Broadly Neutralizing Antibody Immunotherapy for HIV-1. *Cell Host Microbe* 2017,22:207-216.**
50. **Cohen YZ, Butler A, Levin R, Millard K, Pack M, Belblidia SA, Dizon JP, Shimeliovich I, Seaton K, Yates NL, Tomaras G, Zingg M, Seaman MS, Nussenzweig M, Caskey M. A Phase 1 Trial of the Combination of 3BNC117 and 10-1074 In HIV-Uninfected Adults. 25th Conference on Retroviruses and Opportunistic Infections. March 4–7, 2018, Boston, MA. Abstract Number: 1062.**
51. **Deng R, Iyer S, Theil FP, Mortensen DL, Fielder PJ, Prabhu S. Projecting human pharmacokinetics of therapeutic antibodies from nonclinical data What have we learned? mAbs 3:1, 61-66; January/February 2011.**
52. **Violari A, Chan M, Otwombe KN, Panchia R, Jean-Philippe P, Gibb D, Cotton M, Babiker A. Time to viral rebound after stopping ART in children treated from infancy in CHER. 25th Conference on Retroviruses and Opportunistic Infections, Boston, MA. March 4-7, 2018. Abstract No. 137. .**
53. **Henrich TJ, Gallien S, Li JZ, Pereyra F, Kuritzkes DR. Low-level detection and quantitation of cellular HIV-1 DNA and 2-LTR circles using droplet digital PCR. *J Virol Methods* 2012,186:68-72.**
54. **Mendoza P, Gruell H, Nogueira L, Pai JA, Butler AL, Millard K*, et al.* Combination therapy with anti-HIV-1 antibodies maintains viral suppression. *Nature* 2018,561:479-484.**
55. **Ibrahim MR, Maswabi K, Ajibola G, Moyo S, Hughes M, Auletta-Young C, Kuritzkes D, Lichterfeld M, Makhema J, Shapiro RL. Targeted HIV Screening at Birth Can Identify the Majority of In Utero Transmissions. 24th Conference on Retroviruses and Opportunistic Infections, Seattle, February 13–16, 2017. Abstract 790.**

56. Persaud D, Palumbo PE, Ziemniak C, Hughes MD, Alvero CG, Luzuriaga K*, et al.* Dynamics of the resting CD4(+) T-cell latent HIV reservoir in infants initiating HAART less than 6 months of age. *Aids* 2012,26:1483-1490.

57. Lee GQ, Orlova-Fink N, Einkauf K, Chowdhury FZ, Sun X, Harrington S*, et al.* Clonal expansion of genome-intact HIV-1 in functionally polarized Th1 CD4+ T cells. *J Clin Invest* 2017,127:2689-2696.

58. Wamalwa D, Benki-Nugent S, Langat A, Tapia K, Ngugi E, Moraa H*, et al.* Treatment interruption after 2-year antiretroviral treatment initiated during acute/early HIV in infancy. *Aids* 2016,30:2303-2313.

59. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 2004,32:1792-1797.

60. Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, et al. Clustal W and Clustal X version 2.0. Bioinformatics 2007,23:2947-2948.

61. Tobin J. Estimation of Relationships for Limited Dependent Variables. Econometrica 1958,1:24-36.

62. Huang Y, Wu H, Acosta EP. Hierarchical Bayesian inference for HIV dynamic differential equation models incorporating multiple treatment factors. Biom J 2010,52:470-486.

63. Luzuriaga K, Gay H, Ziemniak C, Sanborn KB, Somasundaran M, Rainwater-Lovett K, et al. Viremic relapse after HIV-1 remission in a perinatally infected child. N Engl J Med 2015,372:786-788.

64. Persaud D, Luzuriaga K. Absence of HIV-1 after treatment cessation in an infant. N Engl J Med 2014,370:678.

65. Henrich TJ, Hanhauser E, Marty FM, Sirignano MN, Keating S, Lee TH, et al. Antiretroviral-free HIV-1 remission and viral rebound after allogeneic stem cell transplantation: report of 2 cases. Ann Intern Med 2014,161:319-327.

66. Bendall SC, Simonds EF, Qiu P, Amir el AD, Krutzik PO, Finck R*, et al.* Single-cell mass cytometry of differential immune and drug responses across a human hematopoietic continuum. *Science* 2011,332:687-696.

67. Lichterfeld M, Yu XG, Waring MT, Mui SK, Johnston MN, Cohen D, et al. HIV-1-specific cytotoxicity is preferentially mediated by a subset of CD8(+) T cells producing both interferon-gamma and tumor necrosis factor-alpha. Blood 2004,104:487-494.

68. Lichterfeld M, Mou D, Cung TD, Williams KL, Waring MT, Huang J, et al. Telomerase activity of HIV-1-specific CD8+ T cells: constitutive up-regulation in controllers and selective increase by blockade of PD ligand 1 in progressors. Blood 2008,112:3679-3687.

69. Lichterfeld M, Kaufmann DE, Yu XG, Mui SK, Addo MM, Johnston MN, et al. Loss of HIV-1-specific CD8+ T cell proliferation after acute HIV-1 infection and restoration by vaccine-induced HIV-1-specific CD4+ T cells. J Exp Med 2004,200:701-712.

70. Vigneault F, Woods M, Buzon MJ, Li C, Pereyra F, Crosby SD, et al. Transcriptional profiling of CD4 T cells identifies distinct subgroups of HIV-1 elite controllers. J Virol 2011,85:3015-3019.

71. Howie S. Blood sample volumes in child health research: review of safe limits. Bulletin of the World Health Organization 2011;89:46-53.

72. El-Sadr WM, Lundgren JD, Neaton JD, Gordin F, Abrams D, Arduino RC*, et al.* CD4+ count-guided interruption of antiretroviral treatment. *N Engl J Med* 2006,355:2283-2296.

73. Danel C, Moh R, Minga A, Anzian A, Ba-Gomis O, Kanga C*, et al.* CD4-guided structured antiretroviral treatment interruption strategy in HIV-infected adults in west Africa (Trivacan ANRS 1269 trial): a randomised trial. *Lancet* 2006,367:1981-1989.

74. Bunupuradah T, Duong T, Compagnucci A, McMaster P, Bernardi S, Kanjanavanit S*, et al.* Outcomes after reinitiating antiretroviral therapy in children randomized to planned treatment interruptions. *Aids* 2013,27:579-589.

75. Green H, Gibb DM. Treatment interruption in children with HIV infection. *Curr Opin HIV AIDS* 2007,2:62-68.

76. Palacios GC, Sanchez LM, Briones E, Ramirez TJ, Castillo H, Rivera LG*, et al.* Structured interruptions of highly active antiretroviral therapy in cycles of 4 weeks off/12 weeks on therapy in children having a chronically undetectable viral load cause progressively smaller viral rebounds. *Int J Infect Dis* 2010,14:e34-40.

77. Souda S, Gaseitsiwe S, Georgette N, Powis K, Moremedi D, Iketleng T*, et al.* No Clinically Significant Drug Resistance Mutations In HIV-1 subtype C Infected Women After Discontinuation Of NRTI-Based Or PI-Based HAART For PMTCT In Botswana. *J Acquir Immune Defic Syndr* 2013.

78. Fox Z, Phillips A, Cohen C, Neuhaus J, Baxter J, Emery S*, et al.* Viral resuppression and detection of drug resistance following interruption of a suppressive non-nucleoside reverse transcriptase inhibitor-based regimen. *Aids* 2008,22:2279-2289.