A phase 1 first-in-human study of the safety and pharmacokinetics of 3BNC117-LS in HIV-infected and HIV-uninfected individuals

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Statement of Compliance

The clinical trial will be conducted in compliance with the protocol, with the International Conference on Harmonization Good Clinical Practice E6 (ICH-GCP), and with 45 CFR 46 and 21 CFR 50, 56 and 312. All protocol investigators have completed Protection of Human Subjects Training.

Signature Page 1

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable U.S. federal regulations and ICH guidelines.

The Lead Principal Investigator (Protocol Chair) should sign Signature Page 1. A copy of this Signature Page 1 should be filed with the holder of the Regulatory documents and a copy should be maintained at the site.

Principal Investigator: _____
Print/Type

Signed: _____ Date: _____
Name/Title

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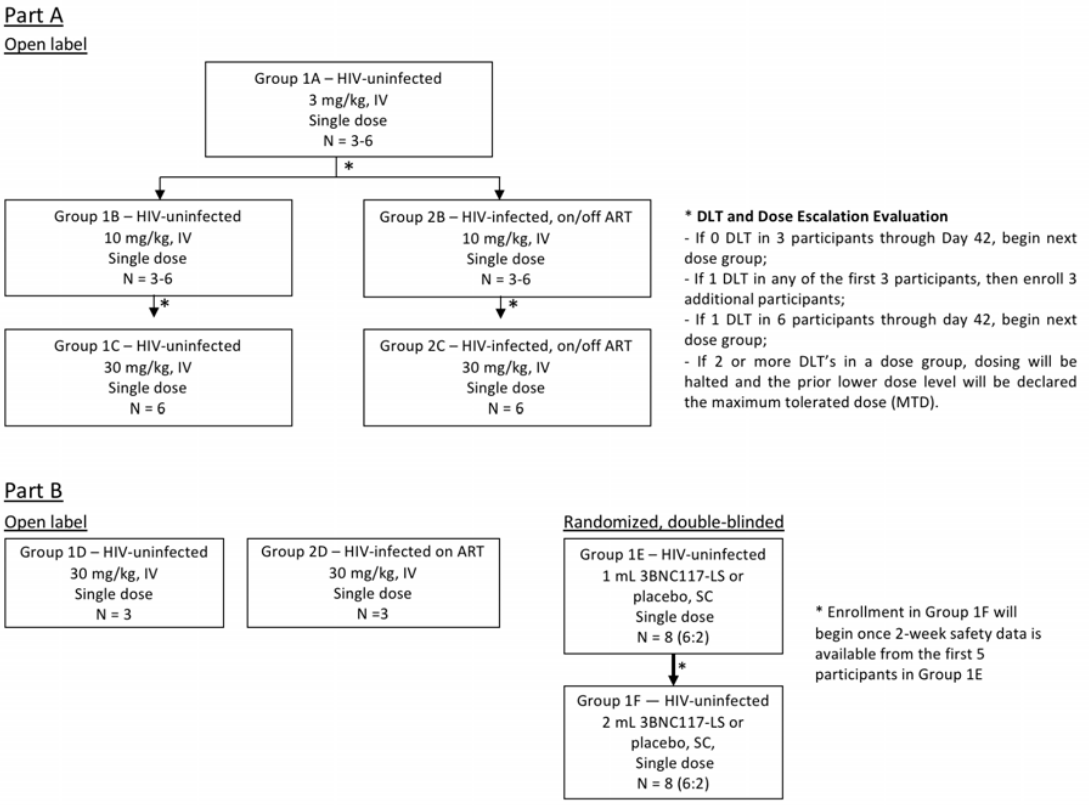
List of Abbreviations

10-1074	Anti-HIV-1 bNAb targeting the V3 loop of gp120
10-1074-LS	10-1074 with mutations in the Fc domain to extend half-life
3BNC117	Anti-HIV-1 bNAb targeting the CD4 binding site of gp120
3BNC117-LS	3BNC117 with mutations in the Fc domain to extend half-life
Ab	Antibody
AE	Adverse Event/Adverse Experience
ART	Antiretroviral Therapy
ATI	Analytic Treatment Interruption
bNAbs	Broadly Neutralizing Antibodies
CD4	T-cell Surface Glycoprotein CD4
CFR	Code of Federal Regulations
cGMP	Current Good Manufacturing Practices
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
CRSO	Clinical Research Support Office
CTSA	Clinical and Translational Science Award
CCTS	Center for Clinical and Translational Science
DNA	Deoxyribonucleic Acid
DSMB	Data and Safety Monitoring Board
FDA	Food and Drug Administration
FWA	Federal-Wide Assurance
GCP	Good Clinical Practice
gp120	HIV-1 Envelope Glycoprotein 120
HIPAA	Health Insurance Portability and Accountability Act
HIV-1	Human immunodeficiency virus
hu-mice	Humanized Mice
ICF	Informed Consent Form
ICH	International Conference on Harmonization
I.M.	Intramuscularly
IND	Investigational New Drug
IRB	Institutional Review Board
I.V.	Intravenously
mAb	Monoclonal antibody
MTD	Maximum tolerated dose
N	Number (typically refers to participants)
NHP	Non-human primate
NIAID	National Institute of Allergy and Infectious Diseases, NIH
NIH	National Institutes of Health
OHRP	Office for Human Research Protections
OHSR	Office for Human Subjects Research
PBMC	Peripheral Blood Mononuclear Cell
PI	Principal Investigator
RU	The Rockefeller University
RUH	The Rockefeller University Hospital
QA	Quality Assurance
QC	Quality Control
RNA	Ribonucleic Acid
SAE	Serious Adverse Event/Serious Adverse Experience
S.C.	Subcutaneously
SHIV	Chimeric Simian/Human Immunodeficiency Virus
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
T cell	T lymphocyte
V3 loop	Third Variable Loop of HIV-1 Envelope gp 120

Protocol Synopsis

Title	A phase 1 first-in-human study of the safety and pharmacokinetics of 3BNC117-LS in HIV-infected and HIV-uninfected individuals
Short Title	3BNC117-LS First-in-Human Phase 1 Study
Protocol Number	YCO-0946
Phase	Phase 1
Study Summary	The proposed study is a phase 1 study of the mAb 3BNC117-LS administered intravenously in HIV-uninfected individuals and HIV-infected individuals, and subcutaneously in HIV-uninfected individuals. The objectives of the study are to evaluate the safety, tolerability and pharmacokinetics of a single administration of 3BNC117-LS.
Study Design	The proposed study is a phase 1 study of 3BNC117-LS administered intravenously in HIV-uninfected and HIV-1 infected participants, and subcutaneously in HIV-uninfected participants. This study consists of two parts. In part A, study participants will be enrolled in an open label manner to receive a single intravenous infusion of 3BNC117-LS at one of three increasing dose levels (3 mg/kg, 10 mg/kg and 30 mg/kg). Participants in Part B will also receive a single administration of 3BNC117-LS, however, the product administered in Part B of the study derives from a new manufacturing lot. The manufacturing lot used in Part A had incomplete glycosylation of the 3BNC117-LS light chain, which has been corrected in the new lot. Participants in Part B will receive 3BNC117-LS intravenously at 30 mg/kg in an open label manner (HIV-uninfected and HIV-infected) or will be randomized to receive a subcutaneous injection of 3BNC117-LS or placebo in a double-blinded fashion (HIV-uninfected only). Part A has already been enrolled with 21 participants. Part B has a planned enrollment of 22 participants. (**Study Design**, below). **Part A** - **Group 1A** (n=3-6) – HIV-uninfected individuals will be administered one infusion of 3BNC117-LS dosed at 3 mg/kg. - **Group 1B** (n=3-6) – HIV-uninfected individuals will be administered one infusion of 3BNC117-LS dosed at 10 mg/kg. - **Group 1C** (n=3-6) – HIV-uninfected individuals will be administered one infusion of 3BNC117-LS dosed at 30 mg/kg. - **Group 2B** (n=6) – HIV-infected individuals **on ART** with HIV-1 plasma RNA levels < 20 copies/ml or **off ART** for at least 8 weeks with HIV-1 plasma RNA levels < 100,000 copies/ml, will be administered one infusion of 3BNC117-LS dosed at 10 mg/kg. - **Group 2C** (n=6) – HIV-infected individuals **on ART** with HIV-1 plasma RNA levels < 20 copies/ml, or **off ART** for at least 8 weeks with HIV-1 plasma RNA levels < 100,000 copies/ml, will be administered one infusion of 3BNC117-LS dosed at 30 mg/kg. **Part B** - **Group 1D** (n=3) – HIV-uninfected individuals will be administered one infusion of 3BNC117-LS dosed at 30 mg/kg. - **Group 2D** (n=3) – HIV-infected individuals **on ART** with HIV-1 plasma RNA levels < 20 copies/ml will be administered one infusion of 3BNC117-LS dosed at 30 mg/kg. - **Group 1E** (n=8) – HIV-uninfected individuals will be administered a single 1 mL (approximately 150 mg) subcutaneous injection of 3BNC117-LS or placebo in a 3:1 ratio. - **Group 1F** (n=8) – HIV-uninfected individuals will be administered a single 2 mL (approximately 300 mg) subcutaneous injection of 3BNC117-LS or placebo in a 3:1 ratio.

Study Design	**Study Design**  **Part A – Dose escalation** Eligible participants will be enrolled in **Group 1A** (HIV-uninfected, 3 mg/kg) and participants will be administered 3BNC117-LS at least one day apart. No more than 2 participants in Group 1A will be administered 3BNC117-LS in a given week. Enrollment in **Group 1B** (HIV-uninfected, 10 mg/kg) and **Group 2B** (HIV-infected on or off ART, 10 mg/kg) will begin after participants enrolled in **Group 1A** reach day 42 with ≤ 1 dose limiting toxicity (DLT: any adverse event of grade 3 or greater toxicity, if the study investigators recognize a probable or definite attribution to 3BNC117-LS). Participants will be administered 3BNC117-LS at least one day apart. Enrollment in **Group 1C** (HIV-uninfected, 30 mg/kg) will begin after participants enrolled in **Group 1B** reach day 42 with ≤ 1 DLT. The first 3 participants enrolled in **Group 1C** will be administered 3BNC117-LS at least one day apart. After the first 3 participants enrolled in **Group 1C** reach day 42 with ≤ 1 DLT, the remaining 3 participants will be enrolled, and administered 3BNC117-LS at least one day apart. Enrollment in **Group 2C** (HIV-infected on or off ART, 30 mg/kg) will begin after participants enrolled in **Group 2B** reach day 42 with ≤ 1 DLT. The first 3 participants enrolled in **Group 2C** will be administered 3BNC117-LS at least one day apart. After the first 3 participants enrolled in **Group 2C** reach day 42 with ≤ 1 DLT, the remaining 3 participants will be enrolled, and administered 3BNC117-LS at least one day apart. **Part B** Participants will be enrolled concurrently into **Groups 1D, 2D, and 1E**. The first 5 participants enrolled across the 3 groups will be administered 3BNC117-LS at least one day apart. Enrollment in **Group 1F** will begin once 2-week safety data is available from the first 5 participants in Group 1E. Following 3BNC117-LS administration, study participants will return for safety assessments and PK measurements as outlined in the Time of Events Schedule (Appendix A**)**. All participants will be followed for 48 weeks after 3BNC117-LS infusion.

Study Duration	24 months
Study Center(s)	Single-center – The Rockefeller University
Objectives	The primary objectives of the this study are to evaluate the safety and tolerability profile of a single intravenous infusion of 3BNC117-LS at 3 dose levels in HIV-infected and HIV-uninfected individuals, and a single subcutaneous injection of 3BNC117-LS at 2 dose levels in HIV-uninfected participants, and to determine the pharmacokinetic profile of a single administration of 3BNC117-LS. The secondary objective is to assess the frequency and magnitude of induced anti-3BNC117-LS antibodies. Exploratory objectives include to determine the effect of 3BNC117-LS on plasma HIV-1 RNA levels and on $CD4^+$ T cell counts, to measure 3BNC117-LS levels in cervicovaginal and rectal fluids, genotyping of escape variants that might arise after administration of 3BNC117-LS, measurement of cell-associated HIV-1 RNA and integrated DNA levels, as well as evaluation of HIV-1 specific T and B cell immune responses following 3BNC117-LS administration.
Number of Participants	43

Inclusion Criteria	**Inclusion Criteria**: ***Groups 1A-1F (HIV-uninfected):*** 1. Males and females, age 18 to 65 2. Amenable to HIV risk reduction counseling and agrees to maintain behavior consistent with low risk of HIV exposure. 3. If sexually active male or female, and participating in sexual activity that could lead to pregnancy, agrees to use two effective methods of contraception (i.e. condom with spermicide, diaphragm with spermicide, hormone-eluting IUD, hormone-based contraceptive with condom) from 10 days prior to and until seven months after 3BNC117-LS infusion or injection, and agrees to safer sex counseling at each visit. - Female study participants of reproductive potential are defined as pre-menopausal women who have not had a sterilization procedure (e.g. hysterectomy, bilateral oophorectomy, tubal ligation or salpingectomy). Women are considered menopausal if they have not had a menses for at least 12 months and have a FSH of greater than 40 IU/L or if FSH testing is not available, they have had amenorrhea for 24 consecutive months. ***Groups 2B-2D (HIV-infected):*** 1. Males and females, age 18 to 65. 2. HIV-1 infection confirmed by two laboratory assays. 3. HIV-infected individuals off ART for at least 8 weeks with HIV-1 plasma RNA levels < 100,000 copies/ml by standard assays (ART-naïve or off ART due to intolerance or by choice), or on ART with HIV-1 plasma RNA levels < 20 copies/ml. HIV-1 RNA levels should be measured on 2 occasions, at least 1 week apart. At least one measurement must be performed within 49 days prior to enrollment (day 0). Group 2D will only enroll HIV-infected individuals on ART. 4. Current CD4+ T cell count > 300 cells/μl. 5. If sexually active male or female, and participating in sexual activity that could lead to pregnancy or transmission of HIV, agrees to use two effective methods of contraception (i.e. condom with spermicide, diaphragm with spermicide, hormone-eluting IUD, hormone-based contraceptive with condom) from 10 days prior to and until seven months after 3BNC117-LS infusion or injection, and agrees to safer sex counseling at each visit. **Exclusion Criteria:** ***Groups 1A-1F (HIV-uninfected):*** 1. Confirmed HIV-1 or HIV-2 infection. 2. Weight > 110 kg (subcutaneous groups only: 1E and 1F) 3. History of immunodeficiency or autoimmune disease; use of systemic corticosteroids, immunosuppressive anti-cancer, or other medications considered significant by the trial physician within the last 6 months. 4. Any clinically significant acute or chronic medical condition (such as autoimmune diseases) that in the opinion of the investigator would preclude participation. 5. Within the 12 months prior to enrollment, the participant has a history of sexually transmitted infection. 6. Hepatitis B or C infection as indicated by the presence of Hepatitis B surface antigen (HBsAg) or hepatitis C virus RNA (HCV-RNA) in blood. 7. Laboratory abnormalities in the parameters listed: - Absolute neutrophil count ≤ 1,500 cells/μl; - Hemoglobin ≤ 11 gm/dL if female; ≤ 12.5 gm/dL if male; - Platelet count ≤ 125,000/μl; - ALT ≥ 1.25 x ULN; - AST ≥ 1.25 x ULN; - Alkaline phosphatase ≥ 1.5 x ULN - Total bilirubin > 1 x ULN; - eGFR < 60 mL/min/1.73m^2.

Exclusion Criteria	8. Pregnancy or lactation. 9. Any vaccination within 14 days prior to 3BNC117-LS administration. 10. Receipt of any experimental HIV vaccine or monoclonal antibody therapy of any kind in the past. 11. History of severe reaction to a vaccine or drug infusion, or history of severe allergic reactions. 12. Individuals with known hypersensitivity to any constituent of the investigational product. 13. Receipt of another investigational product currently or within past 12 weeks, or expected concurrent participation in another study in which investigational products will be administered. ***Groups 2B-2D (HIV-infected):*** 1. Have a history of AIDS-defining illness within 3 years prior to enrollment. 2. History of systemic corticosteroids, immunosuppressive anti-cancer, or other medications considered significant by the trial physician within the last 6 months. 3. Any clinically significant acute or chronic medical condition (such as autoimmune diseases), other than HIV infection, that in the opinion of the investigator would preclude participation. 4. Hepatitis B or C infection as indicated by the presence of Hepatitis B surface antigen (HBsAg) or hepatitis C virus RNA (HCV-RNA) in blood. 5. Laboratory abnormalities in the parameters listed below: - Absolute neutrophil count ≤ 1,000 cells/μl; - Hemoglobin ≤ 10 gm/dL; - Platelet count ≤ 100,000/μl; - ALT ≥ 1.5 x ULN; - AST ≥ 1.5 x ULN; - Alkaline phosphatase ≥ 1.5 x ULN; - Total bilirubin > 1 x ULN; - eGFR < 60 mL/min/1.73m$^{2.}$ 6. Pregnancy or lactation. 7. Any vaccination within 14 days prior to 3BNC117-LS administration. 8. Receipt of any experimental HIV vaccine or monoclonal antibody therapy of any kind in the past. 9. History of severe reaction to a vaccine or drug infusion or history of severe allergic reactions. 10. Individuals with known hypersensitivity to any constituent of the investigational product. 11. Receipt of another investigational product currently or within past 12 weeks, or expected concurrent participation in another study in which investigational products will be administered.
Study Product, Dose, Route, Regimen	3BNC117-LS is a recombinant, fully human monoclonal antibody (mAb) of the IgG1κ isotype that specifically binds to the CD4 binding site (CD4bs) within HIV-1 envelope gp-120. It contains two amino acid modifications in the Fc region to extend its biological half-life. The product used in Part A is concentrated at 20 mg/ml, while the product used in Part B is concentrated at 150 mg/ml. Single infusion at 3, 10 or 30 mg/kg, administered intravenously, or single subcutaneous injection at 1 or 2 mL.
Statistical Methodology	A standard "3+3" trial design will be used in the dose-escalation phase (Part A) to assess safety; stopping rules (as defined above) will be based on the occurrence of dose-limiting toxicity. For safety, the number and percentage of participants experiencing one or more AEs will be summarized by study group, relationship to study drug, and severity. Pharmacokinetic parameters will be calculated using standard non-compartmental analysis methods. Descriptive results will be presented for the pharmacokinetic parameters by dose group. Continuous data will be summarized by descriptive statistics, including sample size, mean, standard deviation, median and range. Categorical data will be summarized by the number and percentage of participants with an outcome.

1 Key Roles

1.1 Study site and associated institutions

The Rockefeller University Hospital
1230 York Ave.
New York, NY 10065

Clinical Laboratories:
Memorial Sloan Kettering Cancer Center
1275 York Avenue
NY, NY 10065

LabCorp
330 W 58th St
New York, NY, 10019

Funder: Bill and Melinda Gates Foundation

Data Management:
EMMES Corporation
401 N. Washington St. Suite 700
Rockville, MD 20850

Independent Safety Monitoring:
International AIDS Vaccine Initiative (IAVI)
125 Broad Street, 9th Floor
New York, NY 10004

1.2 Individuals

Rockefeller Site	
Principal Investigator: Marina CaskeyAssociate Professor of Clinical Investigation The Rockefeller University 1230 York Avenue Box 220 New York, NY 10065 Phone Number: 212 327-7396 Fax Number: 212 327-7234 E-mail: mcaskey@rockefeller.edu	Co-Investigators:Michel Nussenzweig, MD, PhD Irina Shimeliovich, MD, PhD Julio Lorenzi, PhD Allison Butler, FNP Katrina Millard, ANP Arlene Hurley, ANP Maggi Pack, PhD
Collaborators: Michael Seaman, PhD Beth Israel Deaconess Medical Center 330 Brookline Ave E/CLS-1001 Boston, MA 02215 Margaret Ackerman, PhD Dartmouth College 14 Engineering Dr | Hanover, NH 03755 Georgia Tomaras, PhD Duke University Rm 4079 MSRB II, 106 Research Drive Durham, NC 27710	

2 Lay Summary

Broadly neutralizing antibodies against HIV arise in a small fraction of HIV-infected individuals. These antibodies might be able to play an important role in protection from acquisition of HIV infection, and also have the potential to alter the course of HIV infection. 3BNC117 is an anti-HIV neutralizing antibody identified and cloned from an HIV-infected individual. It was chosen for clinical development for its neutralizing breadth and potency, and has been shown to have antiretroviral activity in clinical trials. 3BNC117-LS is a modified version of 3BNC117 that extends the duration of action and the time between administrations. This first clinical study of 3BNC117-LS will evaluate the safety, tolerability and pharmacokinetics profile of 3BNC117-LS administered intravenously in HIV-uninfected individuals and HIV-infected individuals, and subcutaneously in HIV-uninfected individuals.

3 Objectives and Rationale

3.1 Background

It is estimated that over 36 million people were living with HIV in 2015 (UNAIDS, 2016). Despite the success of combination antiretroviral therapy (ART) in suppressing viral replication and preventing disease progression, HIV-1 infection persists in a long-lived reservoir of latently infected cells (Chun et al., 1997). When combination ART is discontinued, plasma HIV-1 RNA levels rebound within 4 weeks in most individuals (Li et al., 2016), and intensified ART regimens do not result in lower levels of HIV-1 persistence (Markowitz et al., 2014). Moreover, the burden of daily medication regimens, toxicity, development of resistance and cost underscore the need for a continued search for additional complementary therapeutic modalities.

Additional modalities for the prevention of HIV infection are also needed. The use of antiretroviral agents by HIV-uninfected individuals before potential sexual exposure to HIV-infected partners, known as pre-exposure prophylaxis (PrEP), is a new highly effective preventive approach against HIV-1. Multiple randomized placebo-controlled clinical trials tested the efficacy of daily oral tenofovir-based PrEP against HIV infection, with PrEP efficacy up to 86% (Baeten et al., 2012; Grant et al., 2010; Marrazzo et al., 2015; Thigpen et al., 2012). However, an important challenge to the success of such strategy is that its efficacy is highly dependent on adherence to a daily oral drug regimen (Hanscom et al., 2016). Motivation and ability to adhere to a daily behavior, such as pill-taking, remains a major challenge for oral PrEP. As a result, alternative long-acting prophylactic modalities that are safe and have effective antiretroviral activity, and that allow for less frequent dosing are being investigated.

Broadly neutralizing antibodies are potential alternatives to standard antiretroviral drugs as they are likely to be safe and well tolerated, and to persist at neutralizing levels for longer periods of time.

A fraction of HIV-infected individuals (10 – 30%) mount a serologic response that can neutralize a broad spectrum of HIV-1 isolates (Walker et al., 2009). Although broadly neutralizing antibodies (bNAbs) that arise during HIV infection fail to resolve established infection, the selection of resistant strains indicates that bNAbs exert selective pressure on the virus. Passive transfer of bNAbs can prevent simian-human immunodeficiency virus (SHIV) infection after high-dose intravenous challenge in non-human primates (Mascola et al., 1999; Shibata et al., 1999; Shingai et al., 2014; Shingai et al., 2013). Neutralizing antibodies can also protect from multiple low dose mucosal challenges (Gautam et al., 2016; Hessell et al., 2009; Moldt et al., 2012).

Passive administration of anti-HIV-1 bNAbs has been evaluated in humans. Earlier antibodies (such as 2G12, 4E10, 2F5) were administered intravenously at doses ranging from 0.5 to 2 gm and were generally found to be safe and well tolerated. However, these early studies showed that the selected HIV-1 bNAbs passively transferred to HIV-infected individuals had rather limited effects on delaying viral rebound during interruption of antiretroviral treatment (Mehandru et al., 2007; Trkola et al., 2005).

Single cell antibody cloning techniques have allowed a better understanding of the molecular composition of the human anti-HIV-1 antibody response (Klein et al., 2013; West et al., 2014). These techniques allowed the identification of antibodies with broadly neutralizing activity that target different epitopes on the HIV-1 envelope protein, including the CD4 binding site (Scheid et al., 2011; Wu et al., 2010). Individual antibodies can neutralize up to 90% of viruses tested *in vitro* and combinations of antibodies achieve greater than 99% coverage of > 100 strains tested, with a geometric mean of the IC_{80} concentrations below 50 ng/ml (Klein et al., 2012; Kong et al., 2015; Wagh et al., 2016).

3BNC117 is a fully human anti-HIV-1 neutralizing antibody that targets the CD4 binding site of HIV-1 gp120 and shows neutralizing activity against > 80% of viral strains from multiple clades. In humans, 3BNC117 has been administered in doses up to 30 mg/kg to HIV-uninfected and HIV-infected individuals. It has been generally safe and well tolerated, and shows a half-life of approximately 10-14 days. A single infusion of 3BNC117 at 30 mg/kg led to decline in plasma viremia of approximately 1.5 log_{10} copies/ml in viremic individuals (Caskey et al., 2015). 3BNC117-LS is the same molecule as the original 3BNC117 drug product, with the exception of two amino acid substitutions of Met to Leu at Fc position 428 (M428L), and Asp to Ser at Fc position 434 (N434S). These substitutions enhance the antibody binding affinity to the neonatal Fc receptor (FcRn), prolonging its half-life *in vivo.*

The proposed study aims to evaluate the safety, tolerability and pharmacokinetics profile of 3BNC117-LS at three increasing dose levels (3, 10 and 30 mg/kg) in both HIV-infected and HIV-uninfected individuals.

3.2 Preclinical characterization of 3BNC117-LS

3.2.1 Fc modification to extend half-life

FcRn is an MHC class I like molecule associated with beta-2-microglobulin (β2m), known to play a role in IgG transport and homeostasis. It functions to protect IgG and albumin from catabolism, which explains the prolonged half-life of these two proteins compared with other classes of immunoglobulins and liver synthesized proteins. FcRn binds to the Fc portion of IgG with high affinity at an acidic pH (6.0 – 6.5) and protects the bound Ig from degradation in lysosomes. FcRn is a recycling receptor and it releases bound IgG into the extracellular space when it returns to the cell surface, thus prolonging the half-life of IgG (Roopenian and Akilesh, 2007; Ward and Ober, 2009).

Mutations in the CH2-CH3 domain of the IgG Fc region can enhance binding affinity to FcRn at low pH, and in turn extend antibody serum half-life. Two sets of mutations in the Fc domain, YTE (M252Y, S254T, T256E) and LS (M428L/N434S), are being evaluated in antibodies currently undergoing clinical testing (i.e. MEDI-557, VRC01-LS, VRC07-523LS).

The LS mutation was identified through rational design methods and high-throughput protein screening. The M428L/N434S variant of an anti-vascular endothelial growth factor IgG (Xtend-VEGF) provided 11-fold improvement in human and non-human primate FcRn binding affinity at pH 6.0 (Zalevsky et al., 2010). Half-lives of two LS-variant antibodies (anti-VEGF-LS and anti-EGFR-LS) in non-human primates were extended 3-3.2 fold in comparison to the native IgG1 antibodies. Moreover, tumor burden decreased at a faster rate with the mutated antibodies than with the unmutated antibodies, indicating a positive correlation between antibody half-life and antibody effector response (Zalevsky et al., 2010).

Ko *et al.* evaluated if modulation of FcRn binding affinity can enhance the protective efficacy of the anti-HIV-1 antibody, VRC01. Similar to 3BNC117, VRC01 targets the CD4 binding site of HIV-1 gp-120. The VRC01-LS-mutant bound to HIV-1 gp 120, similarly to the unmutated VRC01 antibody, and showed enhanced binding to FcRn at pH 6.0. Binding affinities of VRC01-LS for human FcRn and other Fc receptors were further analyzed by surface plasmon resonance (SPR) analysis. Consistent with the ELISA results, VRC01-LS exhibited a 12-fold higher human FcRn binding affinity thanVRC01, whereas both displayed similar binding to human FcγRIIIa, FcγRIIa and FcγRIIb. These data suggest that VRC01-LS does not differ from VRC01 with respect to ADCC effector function or immune suppression through FcγRIIa or FcγRIIb. Moreover, VRC01-LS showed comparable ADCC activity to VRC01 in an *in vitro* ADCC assay using human PBMCs as effector cells and HIV-infected CEM-NKR cells (a NK-cell-resistant humanT leukaemia cell line) as targets. In addition, VRC01-LS showed similar *in vitro* neutralization potency and breadth against HIV-1 strains than the unmutated antibody (Ko et al., 2014).

In vivo, the VRC01 LS-mutant had a 2.5-fold longer half-life in rhesus macaques

(VRC01, 4.65 days; VRC01-LS, 11.80 days) and a slower clearance rate. Low dose VRC01-LS (0.3 mg kg) protected 7 out of 12 rhesus macaques from intrarectal challenge with $SHIV_{BaLP4}$ (Ko et al., 2014). Another study evaluated the protective efficacy of a single intravenous infusion of VRC01 or VRC01-LS (20 mg/kg) against weekly low dose (10 $TCID_{50}$) $SHIV_{AD8-EO}$ intrarectal challenges (Gautam et al., 2016). VRC01-LS showed median protection time of 14.5 weeks, while VRC01 protected animals for a median of 8 weeks. All together, these results demonstrated that the introduction of the LS mutation extended the *in vivo* activity of the unmutated VRC01 antibody

3.2.2 *In vitro* characterization of 3BNC117-LS

Binding affinities of 3BNC117-LS and 3BNC117 for all human FcγRs and FcRn were analyzed by surface plasmon resonance (SPR) analysis (**Table 1**). In all cases, the presence of the LS mutations had no impact on FcγR binding, except for increasing binding to FcRn, as expected.

Table 1: FcγR affinity of 3BNC117 and 3BNC117-LS Fc variant

FC RECEPTOR	FC VARIANT	
	1.3 **3BNC117**	1.4 **3BNC117-LS**
FCΓRIIA H131	$3.14x10^{-6}$	$2.87x10^{-6}$
FCΓRIIA R131	$2.91x10^{-6}$	$3.30x10^{-6}$
FCΓRIIB	$6.21x10^{-6}$	$5.92x10^{-6}$
FCΓRIIIA F158	$1.43x10^{-6}$	$1.22x10^{-6}$
FCΓRIIIA V158	$5.21x10^{-7}$	$5.89x10^{-7}$
FCRN/B2M	**$2.20x10^{-8}$**	**$1.66x10^{-9}$**

*Values represent KD (M) determined by SPR using soluble human FcR ectodomains.

The *in vitro* neutralizing activities of the unmutated 3BNC117 drug product (Lot 201307-A) and 3BNC117-LS were measured by a GLP TZM.bl luciferase-based neutralization assay performed in the laboratory of Dr. Michael Seaman (Beth Israel Deaconess Medical Center, Boston). In this assay, virus neutralization is detected as reduction in luciferase reporter gene expression after single round infection in TZM.bl cells. MuLV (murine leukemia virus) is used as a negative control.

3BNC117 and 3BNC117-LS were tested at a primary concentration of 25 µg/ml and titrated 5-fold to a concentration of < 0.001 µg/ml. A panel of twelve tier 2 HIV-1 pseudoviruses from different HIV clades was used (deCamp, Hraber et al. 2014). The median and 80% inhibitory concentrations (IC_{50} and IC_{80}) were calculated and are displayed in **Table 2**.

As expected, 3BNC117-LS titers were highly concordant with the unmutated 3BNC117 reference standard (< 2-fold difference for all IC_{50}/IC_{80} measurements).

Table 2: *In Vitro* Neutralizing Activity of unmutated 3BNC117 and 3BNC117-LS Against Selected HIV-1 Pseudoviruses

		Titer in TZM.bl cells (µg/ml)					
		3BNC117 WT			3BNC117.LS		
Virus ID	Clade	IC_{50}	IC_{80}	MPI*	IC_{50}	IC_{80}	MPI
3301.v1.c24	AC	0.021	0.065	100	0.030	0.087	100
WITO4160.33	B	0.024	0.083	100	0.014	0.064	100
SC422661.8	B	0.028	0.083	100	0.018	0.074	100
Du156.12	C	0.053	0.129	100	0.047	0.143	100
X1193_c1	G	0.063	0.184	100	0.057	0.222	100
ZM135M.PL10a	C	0.076	0.201	100	0.075	0.260	100
235-47	CRF02_AG	0.078	0.796	94	0.065	0.747	93
CNE53	BC	0.125	1.101	94	0.125	0.829	93
CNE30	BC	0.284	0.997	100	0.423	1.527	100
CAAN5342.A2	B	0.421	1.522	100	0.336	1.338	100
Du172.17	C	0.434	3.873	93	0.812	5.178	90
CNE17	BC	4.327	>25	77	8.227	>25	69
MuLV (Neg. Control)		>50	>50	0	>50	>50	5

*Maximum Percent Inhibition

3.2.3 3BNC117-LS *in vivo* activity in non-human primates

The *in vivo* activity of 3BNC117-LS was evaluated in a non-GLP study in rhesus macaques conducted by Dr. Malcolm Martin's group at NIH/NCI. Six animals were administered a single intravenous infusion of 3BNC117-LS (20 mg/ml) or a single subcutaneous injection of 3BNC117-LS plus 10-1074-LS (each mAb dosed at 7.5 mg/kg) and were challenged weekly with low doses of $SHIV_{AD8-EO}$ (10 $TCID_{50}$ intrarectally). The median time to infection was 16.5 weeks after 3BNC117-LS alone. All animals remained free from infection for 16 to 23 weeks following a single 3BNC117-LS intravenous infusion, with the exception of 1 animal that became infected at week 11. The median time to infection after the 3BNC117-LS plus 10-1074-LS combination was 20 weeks (Figure 1**a**).

Plasma neutralizing activity (ID_{50}) against $SHIV_{AD8-EO}$ pseudovirus was monitored using a TZM.bl assay. The estimated average half-life of 3BNC117-LS, when administered intravenously, was 18 days as compared to 9 days for 3BNC117 ($p < 0.0001$) (Figure 1**b**).

Figure 1. 3BNC117-LS delays SHIV infection in non-human primates

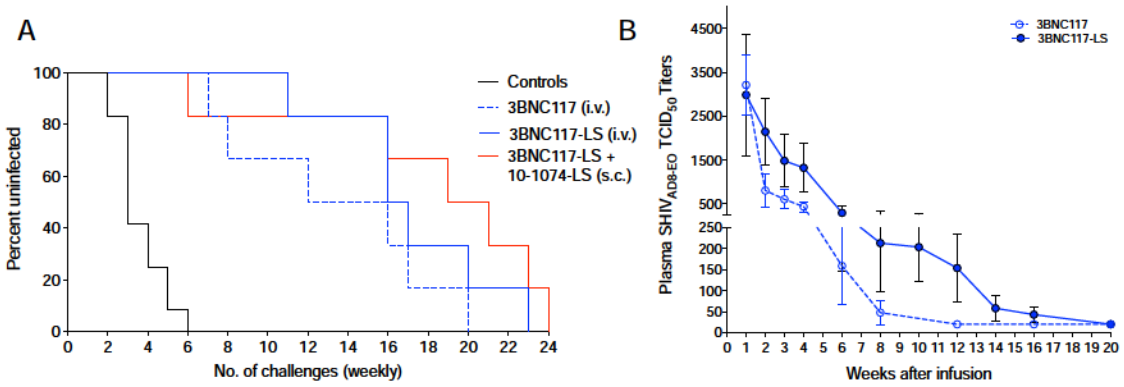


Figure 1. (**a**) Kaplan–Meier plot summarizing number of $SHIV_{AD8}$ intrarectal challenges in macaques receiving a single 20 mg/kg intravenous infusion of 3BNC117 (blue dotted, n=6) or 3BNC117-LS (blue solid, n=6), the combination 7.5 mg/kg of 3BNC117-LS plus 7.5 mg/kg 10-1074-LS (red, n=6) or no antibody (controls, n=12), 1 week before the first challenge on week 0. (**b**). Mean plasma neutralizing antibody titers (ID_{50}) over time after a single 20 mg/kg intravenous infusion of 3BNC117 (n=6) or 3BNC117-LS (n=6) in rhesus macaques.

Non-GLP safety assessments were performed during this study. Clinical observations included daily assessments of behavior, feeding patterns, and local and/or systemic reactogenicity as well as weekly assessments of body weights by senior veterinarians and veterinary technicians at NIH/Office of Research Services, Division of Veterinary Resources. No clinical adverse events were observed in these rhesus macaques following intravenous administration of 20 mg/kg of 3BNC117-LS over a 24-week observation period. Non-GLP blood hematologies included assessments of white blood cell counts, hematocrit, cellular differentials, and lymphocyte subsets, and no clear changes in hematologies were observed during follow up after administration.

3.2.4 3BNC117-LS tissue cross reactivity study

A tissue cross-reactivity study was performed on a full panel of human tissues. The objective of this study was to determine the potential cross reactivity of 3BNC117 and 3BNC117-LS with cryosections of human tissues. In order to detect binding, the test articles were applied to cryosections of normal human tissues (3 donors per tissue) at two concentrations (10 and 2 µg/mL). In addition, the test articles were substituted with a human IgG1κ antibody, which has a different antigenic specificity from that of the test articles, designated HuIgG1 (control article). Other controls were produced by omission of the test or control articles from the assay (assay control).

3BNC117 and 3BNC117-LS produced moderate to intense membrane and cytoplasmic staining of frequent positive control CEMenv-1 cells at the higher concentration with

reduced intensity and/or frequency of these cells at the lower concentration. 3BNC117 and 3BNC117-LS did not specifically react with the negative control CCRF-CEM cells (acute lymphoblastic leukemia cells) at either staining concentration. The control article, HuIgG1, did not specifically react with either the positive or negative control materials. There also was no staining of the assay control slides.

Staining with 3BNC117 and 3BNC117-LS was observed in the human tissue panel as summarized below:
• Plasma membrane only:
- squamous epithelium in the esophageal mucosa

• Plasma membrane and cytoplasm:
- Kupffer cells (specialized macrophages) in the liver
- macrophages in the lymph node

• Cytoplasm only:
- acinar epithelium of the pancreas, endocrine epithelium in the pituitary, squamous epithelium in the epidermis of the skin, germinal epithelium in the testis, and glandular epithelium in the uterus (endometrium)
- extracellular material in the placenta and spinal cord.

As the HIV-1 envelope glycoprotein is a viral protein not expressed in any normal human tissues, all staining observed with 3BNC117 and 3BNC117-LS represented unexpected tissue cross reactivity. Membrane staining with 3BNC117 and 3BNC117-LS was present in Kupffer cells of the liver, macrophages of the lymph node, and mucosal epithelium of the esophagus. All other cellular staining with 3BNC117 and 3BNC117-LS in the current study was cytoplasmic in nature and was considered of little to no toxicologic significance, as it is unlikely that the cytoplasm and cytoplasmic structures would be accessible to the test articles in vivo. The toxicologic relevance of the binding of 3BNC117 and 3BNC117-LS to extracellular material in the placenta and spinal cord is unknown.

In general, the observed staining was more intense and/or frequent with 3BNC117-LS compared to 3BNC117, however the staining pattern was similar between the two test articles.

3.2.5 3BNC117 *in vivo* toxicology study in rats

Since the 3BNC117-LS has the same antigen binding characteristics and showed comparable binding as 3BNC117 in the GLP-compliant human tissue crossreactivity study (above), the previously performed toxicology study of 3BNC117, supports the proposed phase 1 clinical study.

3BNC117 was evaluated for safety in a multidose study in rats to determine its toxicity and toxicokinetic profiles, following twice weekly intravenous/subcutaneous administrations to Sprague-Dawley rats over 25 days and to assess the reversibility of any

changes following a 47-day recovery period. Main animals were euthanized on Day 27; following the end of the 47-day recovery period, recovery animals were euthanized on Day 72.

Despite some animals producing anti-drug antibodies, the rats appeared to have maintained adequate drug exposure in the study, with twice per week dosing for four weeks. Aside from injection site findings, there were no 3BNC117 related effects, in the Main and Recovery group animals, on clinical observations, body weight, food consumption, body temperature, clinical pathology parameters, organ weights or macroscopic and microscopic observations, and the NOAEL (no-observed-adverse-effect level) was determined to be the high dose of 60 mg/kg twice a week for four weeks.

3.3 Clinical safety of monoclonal antibodies to treat or prevent infection

Passive administration of antibodies is successfully used to prevent or treat several viral diseases. For example, Palivizumab, a humanized monoclonal antibody (IgG) directed against the fusion protein of respiratory syncytial virus (RSV), was the first monoclonal antibody approved for clinical use and is indicated for the prevention of serious lower respiratory tract disease caused by RSV in infants at high risk of RSV disease. Pavilizumab, dosed at 15 mg/kg, is generally safe and well-tolerated. Rare cases of severe hypersensitivity reactions (<1 per 100,000 recipients) have been described after an initial dose, as well as after re-exposure.

Passive administration of anti-HIV-1 antibodies has also been evaluated in humans. HIVIG was evaluated in HIV-infected pregnant females and their newborns in a phase III trial to assess whether HIVIG plus single dose nevirapine given to mothers and infants would provide additional benefit over single dose nevirapine alone for prevention of peripartum HIV-1 transmission. Women received a single intravenous (IV) infusion of 240 ml (approximately 200 mg/kg) of HIVIG at 36-38 weeks gestation. Infants born to these mothers received a single IV infusion of 24 ml (approximately 400 mg/kg) of HIVIG, preferably within 18 hours of birth. Infusion related events occurred in both mothers and infants but all infusion related events resolved with no complications. While there was no demonstrable difference in treatment efficacy, the study showed that there were no significant differences in mortality or serious AEs between the two arms of the trial (Onyango-Makumbi et al., 2011).

Several monoclonal antibodies that target HIV-1 gp120 have been evaluated in clinical studies. 2F5 and 4E10 are IgG1 (kappa) monoclonal antibodies that target the membrane-proximal ectodomain of gp41, while 2G12 binds to a carbohydrate moiety on the silent face of gp41. These neutralizing antibodies were evaluated in combination in HIV-infected individuals. The first two studies included ART-naïve individuals with CD4 cell counts > 350 cells/mm^3, and plasma viral levels $\leq 10^4$ in one study (Armbruster et al., 2002), n=7) or $\leq 10^5$ in another study ((Armbruster et al., 2004)), n=8). The antibodies were administered intravenously at 0.5 to 1 gm doses; 4 to 8 weekly infusions were given. The antibodies were safe and well tolerated and no clinical or laboratory

abnormalities were observed throughout the studies. A low-level antibody response against 2G12 was found in two participants. Anti-4E10 and anti-2F5 IgM and IgG immune responses were not detected, even after repeated infusions of high doses of the mAbs.

Two other studies with 2F5, 4E10 and 2G12 included HIV-infected individuals on combination ART and plasma viral levels < 50 copies/ml ((Trkola et al., 2005) n = 14; (Mehandru et al., 2007), n =10). The antibodies were administered intravenously at doses ranging from 1 to 2 gm for each antibody; 13-16 weekly antibody infusions were given. ART was interrupted following 1 or 4 antibody infusions. Antibody infusions were well tolerated in most participants; mild and transient adverse events were reported only occasionally. No serious adverse events (SAEs) were recorded. Adverse events included body aches, fatigue, flushed sensation, joint soreness and redness at infusion site. These mAbs did not prevent viral rebound despite high doses at frequent intervals. However, the emergence of resistance to 2G12, demonstrated that it exerted selective pressure on the circulating viral strains.

10-1074, a broadly neutralizing anti-HIV antibody which targets a peptide and carbohydrate-dependent epitope on the V3 stem of the HIV-1 envelope protein (Mouquet et al., 2012), was evaluated in phase 1 dose escalation study (IND 123713, NCT02511990). Study participants were administered a single intravenous infusion of 10-1074 at doses ranging from 3 to 30 mg/kg. Thirty-three individuals (14 HIV-uninfected, 16 viremic HIV-infected, and 3 ART-HIV-infected individuals) received 10-1074. Of these, 23 received one infusion of 30 mg/kg. 10-1074 was generally safe and well-tolerated at all doses tested. The most commonly reported adverse event deemed possibly related to the study drug was headache of grade 1 severity. It showed a half live of approximately 2-3 weeks, and led to an average decline in HIV-1 viremia of 1.46 $\log_{10}$ copies/ml, when dosed at 30 mg/kg. Rebound viral strains carried mutations at known antibody contact sites and plasma HIV-1 RNA levels returned to baseline over time.

VRC01 is another human anti-HIV-1 broadly neutralizing antibody, targeting the CD4 biding site of HIV-1 gp120. Published results are available for 28 HIV-uninfected and 47 HIV-infected adults who received one or repeated doses of VRC01. HIV-uninfected individuals received up to 2 intravenous infusions at a dose of 5, 20, or 40 mg/kg administered 28 days apart, or 2 subcutaneous infusions at a dose of 5 mg/kg administered 28 days apart. There were no serious adverse events or dose-limiting toxicities. Four adverse events assessed as possibly related to VRC01 were mild in severity and resolved with no residual effects. The half-life of VRC01 in HIV-uninfected individuals was 15 days and no anti-VRC01 antibody responses were detected (Ledgerwood et al., 2015). Of the 47 HIV-infected individuals who received VRC01, 39 were virologically suppressed on ART and 8 were viremic (Bar et al., 2016; Lynch et al., 2015). Twelve virologically suppressed individuals received up to 2 infusions of VRC01, 4 weeks apart, at doses of 1 mg/kg, 5 mg/kg, 20 mg/kg, or 40/mg/kg, and three received 1 or 2 subcutaneous injections at 5 mg/kg. All viremic individuals received a single intravenous infusion of 40 mg/kg. Twenty-four virologically suppressed individuals were enrolled in an analytic treatment interruption study in which they received 3 to 6

intravenous infusions of 40 mg/kg, 3 weeks apart. The half-life of VRC01 in HIV-infected individuals was 12 days for intravenous infusion and 11 days for subcutaneous infusion. No anti-VRC01 antibodies were detected and no grade 2 or higher adverse events considered possibly related to the antibody were reported. In viremic individuals with virus sensitive to VRC01, a reduction in viremia of 1.1 to 1.8 $\log_{10}$ copies/ml was observed. In the analytic treatment interruption study, which included individuals harboring VRC01-resistant viruses, VRC01 failed to delay rebound in the majority of participants.

VRC01 is currently being tested in two multi-center phase 2b studies to evaluate its safety and efficacy to reduce acquisition of HIV-1 infection in women, MSM or transgender persons who have sex with men. Participants are administered up to 10 intravenous VRC01 infusions of 20 or 40 mg/kg every 8 weeks. Over 1,000 participants have been recruited in the Americas and over 300 in several African countries.

3.3.1 Clinical experience with Fc-modified antibodies to enhance binding to the FcRN receptor

Motavizumab is a humanized anti-RSV IgG1 monoclonal antibody, designed to have higher affinity for RSV than palivizumab, from which it was derived. Three amino acid substitutions were introduced to the parental antibody at positions M252Y, S254T, T256E. This combination of amino acid substitutions is referred to as YTE, and the modified antibody is motavizumab-YTE (MEDI-557). A phase 1 study evaluated the pharmacokinetics (PK), antidrug antibody responses (ADA) and safety of motavizumab-YTE in healthy adults. A total of 31 participants were randomized in a 1:1 ratio to receive a single intravenous dose of motavizumab-YTE or motavizumab at 0.3, 3, 15, or 30 mg/kg dose levels. Motavizumab-YTE's half-life was 2- to 4-fold longer than with motavizumab, and serum clearance was decreased 71 to 86% (43 to 68 ml/day) with mota-YTE compared with motavizumab (165 to 326 ml/day). In contrast, similar peak concentrations and volume-of-distribution values, indicative of similar distribution properties, were seen at all dose levels. Serum RSV neutralizing activity persisted for 240 days after motavizumab-YTE versus 90 days after for motavizumab infusion. Safety and frequency of ADA were comparable between groups. The frequencies of AEs were generally similar in both treatment groups. The most common AEs for motavizumab-YTE and motavizumab, respectively, were increased respiratory rate (50% versus 46.7%), headache (25% versus 33.3%), decreased hemoglobin (18.8% versus 20%), proteinuria (12.5% versus 26.7%), and upper respiratory tract infection (0% versus 20%). All AEs in the mota-YTE groups and all except one AE in the motavizumab treatment arms were mild or moderate in severity. In this study the Fc-modified monoclonal antibody was well tolerated and exhibited an extended half-life of up to 100 days (Robbie et al., 2013).

VRC01-LS is another human anti-HIV-1 CD4 binding site antibody that has been modified in the Fc region to include the LS substitutions (M428L/N434S). Like 3BNC117-LS, it is being evaluated for HIV-1 therapy and prevention. VRC01-LS is

being evaluated in four ongoing clinical studies in HIV-infected (NCT02840474) and HIV-uninfected adults (NCT02599896, NCT02797171), and in infants (NCT02256631). The studies are testing single or repeated doses of VRC01-LS at 5, 20 or 40 mk/kg administered intravenously, or 5 mg/kg administered subcutaneously. VRC0-LS has shown good safety profile in HIV-uninfected participants, according to presentations at scientific meetings.

3.4 Clinical experience with 3BNC117 (IND 118225)

3BNC117 is being investigated under IND 118225. To date, it has been administered to 123 participants at doses ranging from 1 mg/kg to 30 mg/kg. (40 HIV-uninfected and 83 HIV-infected). Of these, 73 participants received the 30 mg/kg dose. Dosing is ongoing in 4 protocols: MCA-866, MCA-906, YCO-899 and ROADMAP (MCA-896).

In protocol MCA-835, study participants were administered one or two intravenous infusions of 3BNC117 at increasing dose levels (1 mg/kg, 3 mg/kg, 10 mg/kg or 30 mg/kg), and were followed for 24 weeks after last infusion. In total, 55 participants (22 HIV-uninfected, 17 viremic HIV-infected and 16 ART-treated HIV-infected individuals) were enrolled in the study. Five HIV-uninfected individuals received two infusions of 3BNC117 at 30 mg/kg, 12 weeks apart. Twenty-two participants (3 HIV-uninfected and 19 HIV-infected) were administered one dose of 30 mg/kg.

Preliminary PK data show that 3BNC117's half-life is 17.6 days in HIV-uninfected and 9.6 days in viremic HIV-infected individuals. 3BNC117 decay rates following first and second infusions appear to be similar.

When administered at 30 mg/kg, 3BNC117 induced rapid decreases in plasma HIV-1 RNA levels, with mean VL decline of 1.48 log10 copies/ml at nadir (range 0.8 – 2.5 log10 copies/ml). The median time to reach nadir in viremia was 7 days. Emergence of resistant viral strains was variable, with some individuals remaining sensitive to 3BNC117 for a period of 28 days after infusion (Caskey et al., 2015).

Anti-drug antibody (ADA) responses were assessed at up to 6 time points per participant. First, a "Tier 1" screening assay was performed. A total of 230 serum samples from 55 participants were tested, and 29 samples (12.6%) were positive by the screening assay, representing 15 of the 55 participants tested (23.6%). Of these 15 participants, 2 only had positive results at baseline, prior to 3BNC117 administration.

Next, a more specific "Tier 2" assay was performed on the 29 serum samples found to be positive in the Tier 1 assay. Of the 29 samples, 13 were confirmed to be positive by the Tier 2 assay. Of these, 2 of 13 were only detected at day 0 prior to 3BCN117 administration. In total, 7 of the 55 participants enrolled in the study (12.7%) had detectable post-3BNC117 specific anti-3BNC117 antibody response by the Tier 2 assay. Of note, only 2 participants had positive ADA at more than a single time point, and in both cases the day 0 pre-infusion samples from these participants also tested positive.

Importantly, the antibody responses seen in the 7 participants were not associated with adverse events suggestive of an immune-mediated process or with more rapid clearance of 3BNC117. ADA testing for participants who have received 3BNC117-LS has yet to be performed.

In protocol MCA-867, study participants were administered two 30 mg/kg intravenous infusions of 3BNC117 at weeks 0 and 3 (group A), or four infusions at weeks 0, 2, 4 and 6 (group B). ART was discontinued 2 days after the first 3BNC117 infusion. Participants were followed weekly and ART was resumed when viral rebound occurred (HIV-1 RNA > 200 copies/ml in 2 consecutive measurements). 3BNC117 infusions were associated with delay in viral rebound of 5-9 weeks (2 infusions) or up to 19 weeks (4 infusions) after ATI. The median time to rebound was 6.6 and 9.9 weeks in the 2- and 4-infusion groups, respectively, and in contrast to an average rebound of 2-3 weeks in previous ATI studies. In most individuals, emerging viruses showed increased IC_{50} values against 3BNC117, indicating escape. However, 30% of participants maintained viral suppression until antibody concentrations waned below 20 μg/ml, and the viruses emerging in all but one of these individuals showed no apparent resistance to 3BCN117, suggesting failure to escape over a period of 9-19 weeks (Scheid et al., 2016).

In protocol MCA-866, HIV-infected participants on ART are administered four 3BNC117 infusions at 30 mg/kg, at weeks 0, 12, 24 and 27. ART is discontinued at week 24, and participants are followed weekly to monitor plasma HIV-1 RNA levels. ART is reinitiated upon viral rebound, as in protocol MCA-867 (see above). A total of 17 participants enrolled in this study and are in follow up, 13 have completed four 3BNC117 infusions.

Overall, 3BNC117 was generally safe and well-tolerated in these three studies. The most commonly reported adverse events (AEs) were mild headache (18%), malaise/fatigue (15%), nausea (11%) and flu-like symptoms (10%). Approximately 6% of participants reported mild ophthalmologic complaints (such as pruritus, conjunctival erythema, increased lacrimation, blurry vision) during study follow up of 6 to 9 months, but a causal relationship with 3BNC117 was not established. No SAEs or grade 3 or 4 AEs deemed related to 3BNC117 occurred.

Two phase 1 clinical trials of the combination of 3BNC117 plus 10-1074, administered intravenously, are currently underway. In one study (protocol YCO-0899) HIV-uninfected individuals will receive 1-3 doses of the antibody combination at 3 or 10 mg/kg or placebo. A total of 24 participants have enrolled to date, and 18 have received the 3BNC117 and 10-1074 combination (the study is placebo-controlled). In the second study (protocol MCA-0906), HIV-infected individuals on or off ART will receive 1 to 3 doses of 3BNC117 at 10 or 30 mg/kg each or placebo. To date, 20 individuals have enrolled, 4 of them received a single intravenous infusion of 30mg/kg of each of the 2 antibodies, 5 have received 3 infusions of 30 mg/kg, administered 3 weeks apart. To date, there have been no SAEs, and the safety profile of the 3BNC117 plus 10-1074 combination is similar to what we observed with either antibody alone. The analyses of pharmacokinetics and antiviral activity are underway.

3.5 3BNC117-LS drug product manufacture

The manufacture of the recombinant human monoclonal 3BNC117-LS was carried out by *in vitro* serum-free CHO cell culture. 3BNC117-LS was manufactured by Celldex therapeutics as a sterile solution intended for parenteral use, in compliance with Good Manufacturing Practices (GMP). No animal-derived raw materials were used during the cell culture, purification, and formulation of the drug substance. The drug substance was manufactured in a dedicated suite utilizing single-use equipment (e.g., WAVE bioreactor) to minimize potential for product cross contamination. A low pH step and a nanofiltration step were used for virus inactivation and reduction. Viral clearance studies used the model viruses PPV and A-MuLV. Testing for adventitious agents was performed in accordance to FDA Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use (1997). The clinical trial product was formulated as a single-use sterile solution intended for parenteral use.

A drug product stability-testing program is established to monitor the quality of 3BNC117-LS over the duration of the clinical dosing period as well as to gain predictive information regarding the product's stability characteristics. It is based on 21 CFR 211.166 and ICH Q1A (R2) and ICH Q5C.

The new lot of 3BNC117-LS to be used in Part B of this study uses the same master cell bank and overall manufacturing process, however it utilizes a different feed (Glycan Tune B) during production in the bioreactor. Key parameters, conditions, and media used in the original process were maintained in this modified Glycan Feed process development. This modification led to higher overall production of antibodies with sialic acid glycosylation.

3.6 Hypotheses

The administration of 3BNC117-LS will be safe and well tolerated at the tested doses in both HIV-infected and HIV-uninfected individuals.

3BNC117-LS's estimated half-life will be approximately 6 weeks.

3.7 Aims

Primary Aims*:*

- To evaluate the safety and tolerability profile of a single intravenous infusion of 3BNC117-LS at 3 dose levels in HIV-infected and HIV-uninfected individuals.

- To evaluate the safety and tolerability profile of a single subcutaneous injection of 3BNC117-LS at two fixed dose levels: 1 and 2 mL (approximately 150 and 300 mg, respectively) in HIV-uninfected individuals.
- To determine the pharmacokinetic profile of a single administration of 3BNC117-LS in HIV-infected and HIV-uninfected individuals.

Secondary Aims:
- To assess the frequency and magnitude of induced anti-3BNC117-LS antibodies in all groups.

Exploratory Aims:
- To evaluate the effect of 3BNC117-LS on plasma HIV-1 RNA levels.
- To measure 3BNC117-LS levels in cervicovaginal and rectal fluids.
- To characterize escape variants that might arise after administration of 3BNC117-LS.
- To measure cell-associated HIV-1 RNA and DNA levels in CD4+ T cells.
- To evaluate HIV-1 specific T and B cell immune responses following 3BNC117-LS administration.

3.8 Outcomes

Primary Outcomes:
- The rate of signs, symptoms and laboratory abnormalities, in addition to local and systemic adverse events 2 weeks after 3BNC117-LS infusion or injection in all study groups.
- The pharmacokinetic profile of 3BNC117-LS, including elimination half-life ($t_{1/2}$), clearance (CL/F), volume of distribution (Vz/F), AUC, and decay curve in all study groups.

Secondary Outcomes:
- Frequency and levels of induced anti-3BNC117-LS antibodies in all study groups.
- The rate of signs, symptoms and laboratory abnormalities that occur during study follow up after 3BNC117-LS infusion or injection in all study groups.

Other evaluations:
- 3BNC117-LS levels in cervicovaginal and rectal fluids. The decline in plasma HIV-1 RNA level after 3BNC117-LS infusion in viremic HIV-infected individuals.
- Phenotypic and genotypic analysis of escape viruses in individuals not on ART.
- Levels of cell-associated HIV-1 RNA and DNA before and after 3BNC117-LS infusion.
- Serum neutralizing activity against a panel of HIV-1 isolates before and after 3BNC117-LS infusion.
- HIV-specific T and B cell immune responses following 3BNC117-LS infusion.
- Absolute and relative CD4 + and CD8+ T cell counts after 3BNC117-LS infusion.

4 Study Design

This study is a phase 1 clinical trial to evaluate the safety and pharmacokinetics of the fully human IgG1κ isotype monoclonal antibody 3BNC117-LS in HIV-infected and HIV-uninfected individuals.

The study has 2 parts. In Part A, study participants will be administered one intravenous infusion of 3BNC117-LS at one of three increasing dose levels (3 mg/kg, 10 mg/kg and 30 mg/kg) in an open-label manner. In Part B, participants will also receive a single administration of 3BNC117-LS, however, the product administered in Part B of the study derives from a new manufacturing lot. The manufacturing lot used in Part A had incomplete glycosylation of the 3BNC117-LS light chain, which has been corrected in the new lot. Participants in Part B will receive 3BNC117-LS intravenously at 30 mg/kg in an open label manner (HIV-uninfected and HIV-infected) or will be randomized to receive a subcutaneous injection of 1 or 2 mL (approximately 150 or 300 mg, respectively) of 3BNC117-LS or placebo in a double-blinded fashion (HIV-uninfected only). (
Figure 2. Study Design; **Appendix A**, Time of Events Schedule).

The proposed study doses were chosen based on available human data with the original 3BNC117 antibody, which has an average half-life of 17.6 days in HIV-uninfected individuals and 9.6 days in viremic HIV-infected individuals. The average decline in viremia after a single dose of 3BNC117 at 30 mg/kg was 1.48 $\log_{10}$ copiesl/ml (range 0.8 to 2.5) in HIV-infected participants off ART (Caskey et al., 2015).

Based on the location of the amino acid substitutions as well as on experimental observations in non-human primates, we expect that 3BNC117-LS will demonstrate similar safety profile and antiviral activity as the original 3BNC117 antibody. Experiments in hFcRn-Tg mice, NHP primate models (Ko et al., 2014) and preliminary data from an ongoing phase 1 study in humans (NCT02599896) with another anti-HIV-1 antibody VRC01-LS, showed that the LS mutation prolongs half-life by 3-4 fold. The half-life of 3BNC117-LS in NHP appears to be at least 2-fold longer than that of the original 3BNC117. Therefore, we expect that 3BNC117-LS will have a $t_{1/2}$ in humans of 42-56 days, and that serum concentrations will be maintained above 10 μg/ml for approximately 36 weeks when administered at 30 mg/kg.

Part A has already been enrolled, with a total of 21 participants. Part B has a planned enrollment of 22 participants. (
Figure 2, Study Design**).**

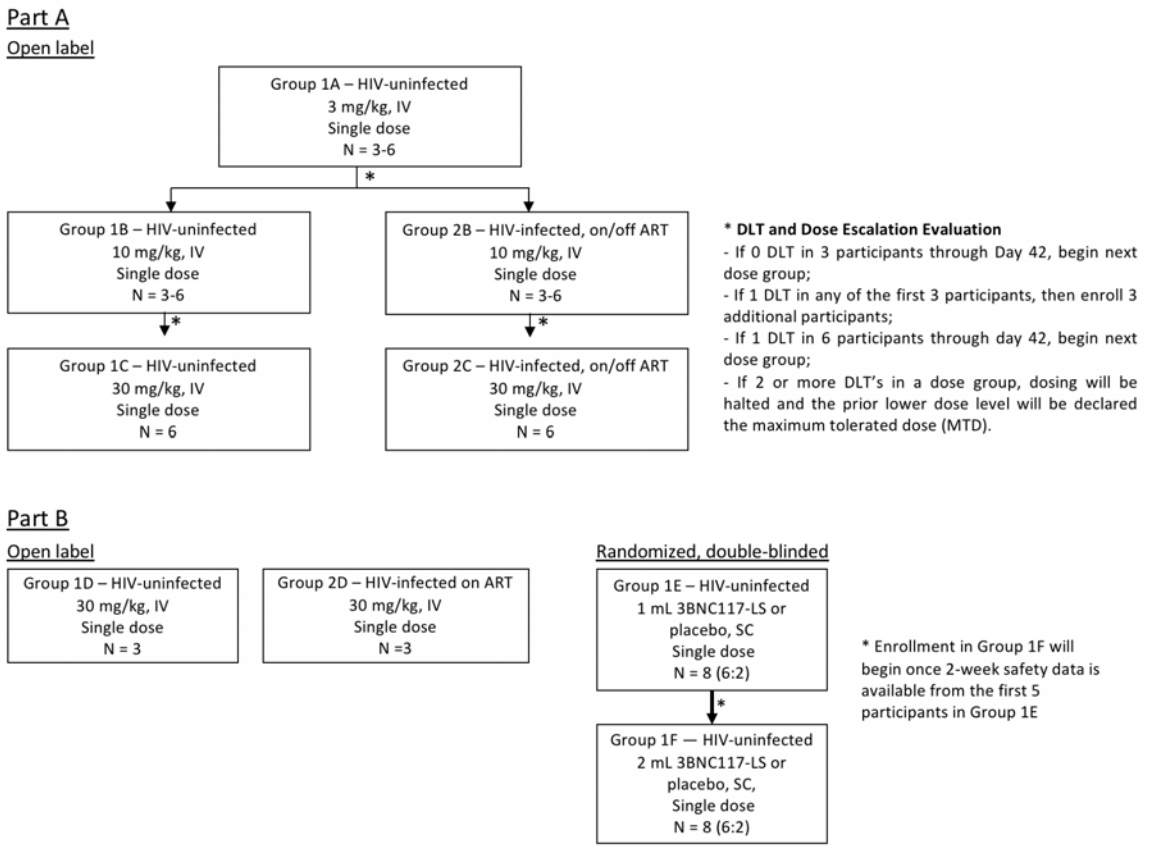
Part A
- **Group 1A** (n=3-6) – HIV-uninfected individuals will be administered one infusion of 3BNC117-LS dosed at 3 mg/kg.
- **Group 1B** (n=3-6) – HIV-uninfected individuals will be administered one infusion of 3BNC117-LS dosed at 10 mg/kg.

- **Group 1C** (n=3-6) – HIV-uninfected individuals will be administered one infusion of 3BNC117-LS dosed at 30 mg/kg.
- **Group 2B** (n= 3-6) – HIV-infected individuals **on ART** with HIV-1 plasma RNA levels < 20 copies/ml, or **off ART** for at least 8 weeks with HIV-1 plasma RNA levels < 100,000 copies/ml, will be administered one infusion of 3BNC117-LS dosed at 10 mg/kg .
- **Group 2C** (n=3-6) – HIV-infected individuals **on ART** with HIV-1 plasma RNA levels < 20 copies/ml, or **off ART** for at least 8 weeks with HIV-1 plasma RNA levels < 100,000 copies/ml, will be administered one infusion of 3BNC117-LS dosed at 30 mg/kg.

Part B

- **Group 1D** (n=3) – HIV-uninfected individuals will be administered one infusion of 3BNC117-LS dosed at 30 mg/kg.
- **Group 2D** (n=3) – HIV-infected individuals **on ART** with HIV-1 plasma RNA levels < 20 copies/ml will be administered one infusion of 3BNC117-LS dosed at 30 mg/kg.
- **Group 1E** (n=8) – HIV-uninfected individuals will be administered a single 1 mL (150 mg) subcutaneous injection of 3BNC117-LS or placebo in a 3:1 ratio.
- **Group 1F** (n=8) – HIV-uninfected individuals will be administered a single 2 mL (300 mg) subcutaneous injection of 3BNC117-LS or placebo in a 3:1 ratio.

Figure 2. Study Design



Part A - Dose Escalation Scheme
Dose escalation will proceed as outlined above (
Figure 2):

Eligible participants will be enrolled in **Group 1A** (HIV-uninfected, 3 mg/kg) and participants will be administered 3BNC117-LS at least one day apart. No more than 2 participants in Group 1A will be administered 3BNC117-LS in a given week.

Enrollment in **Group 1B** (HIV-uninfected, 10 mg/kg) and **Group 2B** (HIV-infected on or off ART, 10 mg/kg) will begin after participants enrolled in **Group 1A** reach day 42 with ≤ 1 dose limiting toxicity (DLT: any adverse event of grade 3 or greater toxicity, if the study investigators recognize a probable or definite attribution to 3BNC117-LS). Participants will be administered 3BNC117-LS at least one day apart.

Enrollment in **Group 1C** (HIV-uninfected, 30 mg/kg) will begin after participants enrolled in **Group 1B** reach day 42 with ≤ 1 DLT. The first 3 participants enrolled in **Group 1C** will be administered 3BNC117-LS at least one day apart. After the first 3 participants enrolled in **Group 1C** reach day 42 with ≤ 1 DLT, the remaining 3 participants will be enrolled, and administered 3BNC117-LS at least one day apart.

Enrollment in **Group 2C** (HIV-infected on or off ART, 30 mg/kg) will begin after participants enrolled in **Group 2B** reach day 42 with ≤ 1 DLT. The first 3 participants enrolled in **Group 2C** will be administered 3BNC117-LS at least one day apart. After the first 3 participants enrolled in **Group 2C** reach day 42 with ≤ 1 DLT, the remaining 3 participants will be enrolled, and administered 3BNC117-LS at least one day apart.

If no DLT occurs within 42 days from 3BNC117-LS infusion in 3 participants in a dose group, dose escalation to next dose group will proceed (
Figure 2). If 1 DLT occurs, 3 additional participants will be enrolled. If 1 DLT occurs within 42 days from 3BNC117-LS infusion in the 6 enrolled participants, study can proceed with enrollment of next dose group. If 2 or more DLTs occur, dosing will be halted and the prior lower dose level will be declared the maximum tolerated dose (MTD).

Part B
Participants will be enrolled concurrently into **Groups 1D, 2D, and 1E**. The first 5 participants enrolled across the 3 groups will be administered 3BNC117-LS at least one day apart.

Enrollment in **Group 1F** will begin once 2-week safety data is available from the first 5 participants in Group 1E.

Following 3BNC117-LS administration, study participants will return for safety assessments and PK measurements as outlined in the Time of Events Schedule

(**Appendix A)**. All participants will be followed for 48 weeks after 3BNC117-LS infusion.

The HIV-uninfected and HIV-infected groups will be assessed both independently and combined for safety and tolerability, as there might be differences in safety and/or tolerability between the two study populations.

Serum 3BNC117-LS levels will be assessed at baseline (before 3BNC117-LS administration), at the end of 3BNC117-LS infusion, 1, 3, 7 days post administration and on weeks 2, 4, 6, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44 and 48. Pharmacokinetic analyses will be performed independently for participants in Part A and Part B considering the expected differences in peak levels between the 2 lots of 3BNC117-LS.

5 Study Population

5.1 Inclusion

Groups 1A-1F (HIV-uninfected):

1. Males and females, age 18 to 65
2. Amenable to HIV risk reduction counseling and agrees to maintain behavior consistent with low risk of HIV exposure.
3. If sexually active male or female, and participating in sexual activity that could lead to pregnancy, agrees to use two effective methods of contraception (i.e. condom with spermicide, diaphragm with spermicide, hormone-eluting IUD, hormone-based contraceptive with condom) from 10 days prior to and until seven months after 3BNC117-LS infusion or injection, and agrees to safer sex counseling at each visit.
   - Female study participants of reproductive potential are defined as pre-menopausal women who have not had a sterilization procedure (e.g. hysterectomy, bilateral oophorectomy, tubal ligation or salpingectomy). Women are considered menopausal if they have not had a menses for at least 12 months and have a FSH of greater than 40 IU/L or if FSH testing is not available, they have had amenorrhea for 24 consecutive months.

Groups 2B-2D (HIV-infected):

1. Males and females, age 18 to 65.
2. HIV-1 infection confirmed by two laboratory assays.
3. HIV-infected individuals off ART for at least 8 weeks with HIV-1 plasma RNA levels < 100,000 copies/ml by standard assays (ART-naïve or off ART due to intolerance or by choice), or on ART with HIV-1 plasma RNA levels < 20 copies/ml. HIV-1 RNA levels should be measured on 2 occasions, at least 1 week apart. At least one measurement must be performed within 49 days prior to enrollment (day 0). Group 2D will only enroll HIV-infected individuals on ART.
4. Current CD4+ T cell count > 300 cells/μl.
5. If sexually active male or female, and participating in sexual activity that could lead to pregnancy or transmission of HIV, agrees to use two effective methods of contraception (i.e. condom with spermicide, diaphragm with spermicide, hormone-

eluting IUD, hormone-based contraceptive with condom) from 10 days prior to and until seven months after 3BNC117-LS infusion, and agrees to safer sex counseling at each visit.

5.2 Exclusion Criteria

Groups 1A-1F (HIV-uninfected):

1. Confirmed HIV-1 or HIV-2 infection.
2. Weight > 110 kg (subcutaneous groups only: 1E and 1F)
3. History of immunodeficiency or autoimmune disease; use of systemic corticosteroids, immunosuppressive anti-cancer, or other medications considered significant by the trial physician within the last 6 months.
4. Any clinically significant acute or chronic medical condition (such as autoimmune diseases) that in the opinion of the investigator would preclude participation.
5. Within the 12 months prior to enrollment, the participant has a history of sexually transmitted infection.
6. Hepatitis B or C infection as indicated by the presence of Hepatitis B surface antigen (HBsAg) or hepatitis C virus RNA (HCV-RNA) in blood.
7. Laboratory abnormalities in the parameters listed:
   - Absolute neutrophil count ≤ 1,500 cells/μL;
   - Hemoglobin ≤ 11 gm/dL if female; ≤ 12.5 gm/dL if male;
   - Platelet count ≤ 125,000 cells/μL;
   - ALT ≥ 1.25 x ULN;
   - AST ≥ 1.25 x ULN;
   - Alkaline phosphatase ≥ 1.5 x ULN;
   - Total bilirubin > 1 x ULN;
   - eGFR < 60 mL/min/1.73m^2.
8. Pregnancy or lactation.
9. Any vaccination within 14 days prior to 3BNC117-LS administration.
10. Receipt of any experimental HIV vaccine or monoclonal antibody therapy of any kind in the past.
11. History of severe reaction to a vaccine or drug infusion or history of severe allergic reactions.
12. Individuals with known hypersensitivity to any constituent of the investigational product.
13. Receipt of another investigational product currently or within the past 12 weeks, or expected concurrent participation in another study in which investigational products will be administered.

Groups 2B-2D (HIV-infected):

1. Have a history of AIDS-defining illness within 3 years prior to enrollment.
2. History of systemic corticosteroids, immunosuppressive anti-cancer, or other medications considered significant by the trial physician within the last 6 months.
3. Any clinically significant acute or chronic medical condition (such as autoimmune diseases), other than HIV infection, that in the opinion of the investigator would preclude participation.

4. Hepatitis B or C infection as indicated by the presence of Hepatitis B surface antigen (HBsAg) or hepatitis C virus RNA (HCV-RNA) in blood.
5. Laboratory abnormalities in the parameters listed below:
   - Absolute neutrophil count ≤ 1,000 cells/μl;
   - Hemoglobin ≤ 10 gm/dL;
   - Platelet count ≤ 100,000 cells/μl;
   - ALT ≥ 1.5 x ULN;
   - AST ≥ 1.5 x ULN;
   - Alkaline phosphatase ≥ 1.5 x ULN;
   - Total bilirubin > 1 x ULN;
   - eGFR < 60 mL/min/1.73m^2.
6. Pregnancy or lactation.
7. Any vaccination within 14 days prior to 3BNC117-LS infusion.
8. Receipt of any experimental HIV vaccine or monoclonal antibody therapy of any kind in the past.
9. History of severe reaction to a vaccine or drug infusion or history of severe allergic reactions.
10. Individuals with known hypersensitivity to any constituent of the investigational product.
11. Receipt of another investigational product currently or within the past 12 weeks, or expected concurrent participation in another study in which investigational products will be administered.

6 Methods and Procedures

6.1 Screening Procedure and Study Visits

The Time of Events Schedule (for Groups 1 and 2) summarizes the frequency and timing of various study assessments. See **Appendix A**.

6.1.1 Pre-Screening Questionnaire

Potential participants will first undergo pre-screening by telephone to assess medical history, preliminary HIV risk assessment, and qualification for the study. Potential participants will have the opportunity to discuss the study and ask questions of the study recruiter at this time. Those who are eligible and interested in participation will attend a screening visit at the Rockefeller University Hospital (RUH) Outpatient Clinic.

6.1.2 Screening Visit

Initial Screening Visit:

Study personnel will answer any questions about the study. Written informed consent will be obtained prior to conducting any study procedures. To ensure informed consent, the principal investigator or designee will discuss the following processes individually with each potential participant:

1. HIV-test counseling (for HIV-uninfected individuals screening for Group 1);
2. Risk-reduction counseling including safe-sex and pregnancy avoidance counseling;
3. One must assume that no protection or improvement in control of HIV infection will occur given the experimental nature of this monoclonal antibody;
4. That sexually active males and females, participating in sexual activity that could lead to pregnancy, should use two reliable forms of contraception for seven months following 3BNC117-LS infusion or injection.
5. MSM will at this visit and subsequent ones be asked to use condoms for safer sex practices in order to avoid transmission of HIV infection.

If the potential participant consents to participate, site personnel will:

- Perform detailed HIV risk assessment (Group 1);
- Perform complete medical history (including review of concomitant medication);
- Perform a general physical examination including height, weight, vital signs (pulse, respiratory rate, blood pressure and temperature), examination of skin, respiratory, cardiovascular and abdominal systems;
- Collect blood and urine specimens for all tests as indicated in the Time of Events Schedule (**Appendix A**);
- Perform a pregnancy test for female volunteers of child-bearing potential.

If the initial screening visit occurs more than 49 days prior to the date of the 3BNC117-LS administration, laboratory tests (blood and urine specimens) will be repeated and a review of the medical history will be performed.

HIV-1 infected participants that do not meet eligibility criteria due HIV viral load levels outside of the range specified in the protocol will be independently counseled regarding their HIV care. Participants who are not on ART will be counseled and encouraged to initiate therapy and referrals to HIV providers will be provided, if needed. Participants who are on ART but do not have undetectable HIV viral load at screening will be counseled regarding their ongoing therapy and encouraged to return to their primary care providers as soon as possible. Referral to other HIV providers will be provided, if needed.

6.1.3 Pre-Infusion Visit

- Collect blood samples for repeat plasma HIV-1 RNA level (Groups 2B and 2C) and baseline research assays, as indicated in the Time of Events Schedule (**Appendix A**).
- Ophthalmologic assessment (including a slit lamp exam) by an ophthalmologist, at no cost to the participant.
- Perform a pregnancy test for female volunteers of child-bearing potential.

- Perform pregnancy counseling.

6.1.4 3BNC117-LS administration visit

Prior to drug administration, site personnel will:

- Review the informed consent form administered at the screening visit with the participant;
- Answer any questions about the study;
- Review interim medical history (including concomitant medications);
- Review safety laboratory data;
- Perform a directed physical examination including weight, vital signs (pulse, respiratory rate, blood pressure and temperature) and any further examination indicated by history or observation;
- Collect blood and urine specimens for all tests as indicated in the Time of Events Schedule (**Appendix A**);
- Perform pregnancy counseling;
- Perform a serum pregnancy test for female volunteers of child-beating potential and obtain results prior to study drug administration. Results must be available prior to start of infusion or injection;
- Cervicovaginal fluid collection (Groups 1A, 1B and 2B, according to section 6.2.6);
- Rectal fluid collection (Groups 1A, 1B and 2B, according to section 6.2.6);
- Perform baseline assessment and record any systemic symptoms;

<u>Intravenous administration</u>

- 3BNC117-LS will be prepared for administration according to the RUH Pharmacy Standard Operating Procedures;
- 3BNC117-LS mAb will be administered via a peripheral vein over 60 minutes. The IV line will be flushed with normal saline after all the contents of the infusion bag are administered;
- Participants in all groups will be observed for adverse reactions at the study site for 1 hour after the end of 3BNC117-LS infusion. Presence or absence of adverse events will be recorded at 1 hour post infusion.
- Vital signs (pulse, respiratory rate, blood pressure and temperature) will be monitored at the end of 3BNC117-LS infusion and at 1 hour (+/- 10 min) post infusion;
- If volunteers develop acute infusion reaction during study drug administration, the infusion will be discontinued and will not be reinitiated. Rescue medications, including acetaminophen, diphenhydramine (or an alternative antihistaminic) and glucocorticoids will be available in the RUH infusion unit for use if clinically indicated.

Subcutaneous Administration:

- 3BNC117-LS will be administered subcutaneously, as a single injection of 1 mL (approximately 150 mg) or 2 mL (approximately 300 mg) in the abdomen, upper arms, or thighs.
- Participants receiving a subcutaneous injection will be observed for adverse reactions at the study site for 1 hour following subcutaneous administration. Presence or absence of adverse events will be recorded at 1 hour post administration.
- Vital signs (pulse, respiratory rate, blood pressure and temperature) will be monitored before administration, and at 1 hour (+/- 10 min) post administration;
- Rescue medications, including acetaminophen, diphenhydramine (or an alternative antihistaminic) and glucocorticoids will be available in the RUH infusion unit for use if clinically indicated.

6.1.5 Post-3BNC117-LS Administration Visit

Participants will be followed through study week 48.

At these follow up visits the following will be conducted:

- Review of interim medical history and use of concomitant medications;
- Examine the infusion or injection site on day 1 visit;
- During other visits, perform a symptom-directed physical examination if symptoms are present;
- Local and systemic solicited and unsolicited adverse events will be assessed;
- Pregnancy counseling;
- Vital Signs;
- Collect blood and urine specimens for all tests as indicated in the Time of Events Schedule (**Appendix A**);
- In case of adverse event(s), the participant will be assessed and followed up by the clinical team. Supplemental visit(s) for further investigation can be planned at the discretion of the principal investigator or designee. Supplemental visit(s) may be recommended if clinically indicated or to clarify observations.

Specific procedures to be performed at each follow up visit for Groups 1 and 2 are illustrated in the Time of Events Schedules (**Appendix A**).

Any abnormalities (adverse events) including laboratory abnormalities, should be subsequently followed until the event or its sequelae resolve or stabilize.

6.1.6 Final Visit/Early termination Visit

Assessments will be undertaken according to the Time of Events Schedule (**Appendix A**).

6.1.7 Discontinuation of 3BNC117-LS infusion and/or participant withdrawal from study

6.1.7.1 Discontinuation of 3BNC117-LS infusion

3BNC117-LS infusion will be discontinued for any of the following reasons:

1. Any immediate hypersensitivity reaction (such as urticarial rash; bronchospasm; laryngeal edema; anaphylaxis; syncope).
2. Life threatening medical event during 3BNC117-LS infusion.

6.1.7.2 Withdrawal from the study (Early Termination)

Participants may be withdrawn from the study permanently for the following reasons:

1. Participants may withdraw from the study at any time if they wish to do so, for any reason.
2. Following an adverse event at the discretion of the investigator (or designee).
3. Intercurrent HIV infection (in Group 1).
4. Request of the primary care provider if s/he thinks the study is no longer in the best interest of the participant.
5. Participant judged by the investigator to be at significant risk of failing to comply with the protocol in a manner that might lead to harm to self or seriously interfere with the validity of the study results.
6. At the discretion of the FDA or investigator.

6.1.7.3 Follow up after withdrawal from study (Early Termination)

Any adverse event resulting in withdrawal of a participant will be followed up until resolution or until the adverse event is judged by the principal investigator or designee to have stabilized where possible.

At the time of withdrawal, provided the participant is willing, all the requested termination visit procedures will be performed according to the Time of Events Schedule (**Appendix A**).

The date and reason for withdrawal from the study (early termination) should be collected and reported to the Safety Monitoring Committee (SMC), the Clinical Research Support Office (CRSO) at the Rockefeller University Hospital and the RUH-IRB.

A pregnant participant will not receive the 3BNC117-LS infusion or injection. If pregnancy occurs after 3BNC117-LS administration, the participant will be followed until the end of the study and until delivery, if delivery occurs after the study has ended. Approximately 2-4 weeks after delivery, the baby will be examined by a pediatrician to assess his/her health status. The outcome of the pregnancy and the health status of the baby will be reported to the RUH-IRB, Clinical Research Office at the RUH (CRSO) and the SMC.

6.2 Study Procedures

6.2.1 Consent Procedure

Prior to the initiation of any study related procedures, the potential participants will be given a copy of the most recent IRB stamped and approved informed consent to read. Additionally, the PI or study staff member who has been designated to consent will discuss the specifics of the study including but not limited to the purpose of the research, procedures, time commitment, required tasks, test article, alternative treatments, benefits, risks, confidentiality etc. in a comprehensible (non-scientific) manner, using language readily understandable by the participant. Participants will be told that participation is voluntary and that, if they do not consent, they will not be penalized. The person consenting will assure the voluntariness of the participant.

A private, confidential setting will be provided for the potential participant to read and discuss the informed consent free from coercion, undue influence or constraints of time. All participants will be given a chance to ask questions and express concerns. They will be given the option to take the consent home and discuss it with family, friends, and /or health care providers. After a participant and the person conducting the consenting process sign and date the consent, the participant will be given a copy of the signed informed consent form.

An enrollment note will be written in the source document as to who obtained consent, how, when, were questions asked and answered, and that a copy of the informed consent was given to the participant.

The "Teach Back" method will be used in the clinical research setting to ask research participants to repeat or “teach back” the information, concepts and directions that the staff member has attempted to convey to the participant. This method is used to assess comprehension and retention of protocol requirements, adverse event information, risks and benefits, and the participant's rights described in the Informed Consent process.

6.2.2 Study Assignment and Randomization

Intravenous Groups: Enrollment in the intravenous groups will be open-label, and participants will be enrolled sequentially as they meet enrollment criteria. The RUH pharmacist will dispense 3BNC117-LS according to the study dose group.

Subcutaneous Groups: HIV-uninfected participants will be given the option to enroll in the subcutaneous or intravenous groups as long as enrollment in a particular group is not complete.

In the subcutaneous groups, participants will be randomized in a 3:1 ratio to receive the study drugs or placebo. Participants will be enrolled sequentially as they meet enrollment criteria, according to the randomization schedule.

Randomized treatment assignments will be generated by the Rockefeller University Hospital pharmacy. The 8 participants in Groups 1E and 1F will be randomized in a ratio of 6 study drug recipients to 2 placebo recipients. Randomization will be generated using SAS 9.4. Blinding will not apply to the assignment of dose level (1 mL vs. 2 mL). The study preparation will be provided to the study nurses for injection under a coded, masked identification. The placebo and mAb preparations will be indistinguishable in the preparation provided for participants. The nurses, study staff, investigators, and participants will be blinded as to the identity of the study preparation. Participants will be unblinded at the end of the study. To accomplish this, study nurses will be provided with the randomization code after the study has been completed, and they will notify each participant. In the event of a medical emergency wherein knowledge of the treatment assignment will influence the participant's care, the Principal Investigator may unblind the treatment assignment.

6.2.3 3BNC117-LS Administration Procedure

Intravenous administration

In Part A, 3BNC117-LS will be provided in single-use vials containing 10 ml of the product at a concentration of 20 mg/ml. The appropriate volume of 3BNC117-LS will be diluted in sterile normal saline to a total volume of 250 ml, and will be administered as an intravenous infusion over 60 minutes.

In Part B, 3BNC117-LS will be provided in single-use vials containing 1 ml of the product at a concentration of 150 mg/ml. The volume of 3BNC117-LS to be administered will be calculated by the RUH research pharmacist, according to study assignment. The appropriate volume of 3BNC117-LS will be diluted in sterile normal saline to a total volume of 100 ml, and will be administered as an intravenous infusion over 60 minutes.

3BNC117-LS will be administered via a peripheral vein in one of the upper extremities. The administration site should be free of potentially complicating dermatologic conditions. The entire contents of the infusion bag must be administered. At the end of infusion, the IV line will be flushed with Normal Saline to ensure all the medication has been delivered.

Subcutaneous administration

The RUH research pharmacist will provide the study drug or placebo in a syringe ready for administration. The volume of 3BNC117-LS will follow study assignment: Group 1E, 1 mL of 3BNC117-LS; Group 1F, 2 mL of 3BNC117-LS. The product or placebo will be administered subcutaneously in the abdomen, upper arms or thighs.

6.2.4 Medical History and Physical Examination

At the time of screening, participant's past medical history will be collected and will include details of any previous reaction to vaccination, and contraceptive practices. Interim medical histories will be collected at time-points according to the Time of Events Schedule (**Appendix A**).

A general physical examination will be conducted at screen including weight, height, vital signs, and examination of skin, respiratory, cardiovascular and abdominal systems. At the time of 3BNC117-LS administration and follow up visits directed physical examinations will be performed according to the Time of Events Schedule (**Appendix A**). A directed physical examination will include vital signs, examination of infusion or injection site, and any further examination indicated by history or observation.

6.2.5 Blood Collection, Storage and Shipment

Venous blood will be collected at every study visit according to the Time of Events Schedule (**Appendix A**). Up to 120 ml will be collected at day 0. Up to 100 ml will be collected at other visits. At no time will the total volume of blood collected exceed 550 ml over an 8-week period. All specimens will be handled according to SOPs that were developed in the GLP Processing Lab within the Laboratory of Molecular Immunology.

In all groups, the total volume of blood samples that will be collected during the entire study will be < 1,500 ml.

All specimens will be handled according to SOPs that were developed in the Processing Lab within the Laboratory of Molecular Immunology at the Rockefeller University.

PBMCs, plasma and serum samples will be processed and stored at the study site. Long-term storage of samples will be located at the Rockefeller University, Laboratory of Molecular Immunology.

6.2.6 Collection of Cervicovaginal and Rectal Fluids (Part A only)

Cervicovaginal and rectal fluids will be collected on day 0 and week 2 from participants that are enrolled in Groups 1A, 1B and 2B. Samples will be processed and stored in the Laboratory of Molecular Immunology. 3BNC117-LS levels will be determined by sandwich ELISA. This evaluation will generate information regarding the biodistribution of the antibody and its availability at potential sites of HIV transmission.

Cervicovaginal fluids will be collected with the use of an Instead Softcup®. The participant must refrain from receptive sexual intercourse, douching, and inserting any intravaginal products prior to the collection of cervical specimens for at least 72 hours. Ideally, the participant should have completed menses 72 hours prior to specimen

collection. If not, she should contact a study staff to re-schedule the visit. The participant should not be pregnant during the cervicovaginal fluid collection. The participant should not have abnormal vaginal discharge or symptoms. In that case, she needs to contact or inform the study investigators for further management before cervical fluid collection and reschedule after treatment is completed. Participants who have an intrauterine device in place will not be asked to collect cervicovaginal fluids given the risk of displacement of the IUD upon removal of the Instead Softcup®.

The study participants will be given the Instead Softcup® for placement at home. They will be instructed on how to insert the cup. Once inserted, the cup should be retained in the vagina for at least 4 hours but no more than 12 hours. Participants will remove the cup at the inpatient or outpatient clinic during a scheduled study visit. Study investigators may offer to assist with removal if the participant is not comfortable with self-removal.

Rectal secretions will be collected with a Weck-cel sponge, inserted through an anoscope. The study participant must refrain from receptive anal intercourse, enema, or inserting any intrarectal products prior to the collection of rectal specimens for at least 72 hours. The study participant should not have abnormal rectal discharge or symptoms. In that case, the participant should contact or inform the study investigators for further management before rectal secretion collection and reschedule after treatment is completed.

Briefly, the anoscope will be minimally lubricated with a small amount of lubricant on the side edges. The anoscope will be gently inserted approximately 3-4 cm into the anal canal. The collection sponge will be placed 1-2 cm above the dentate line and will rest on the rectal mucosa to absorb the secretions for approximately 5 minutes. The sponge will be removed and placed inside a 15 ml Falcon tube and transported to the Laboratory of Molecular Immunology at 2°C to 8°C within 8 hours.

Participant will be screened for gonorrhea, chlamydia and syphilis at the time of collection of genital secretions, as active infection with these pathogens might interfere with the levels of 3BNC117-LS in those body fluids.

6.2.7 HIV testing and Counseling (Group 1)

Study personnel will assess Group 1 participants for past and current risk of HIV infection and counsel them prior to collecting blood for an HIV test. Study personnel will perform post HIV-test counseling as indicated in the Time of Events Schedule (**Appendix A**). The counseling process will include information on HIV, safe sex practices and risk reduction. The objective of counseling is to ensure that participants have sufficient knowledge about HIV infection to understand the purpose of the test, the implications of a positive or negative result and the standard of care available locally for HIV infection. Additionally, risk reduction counseling, including safe-sex counseling, will be provided during the study to reinforce low-risk behavior.

6.2.8 HIV Infection and HIV Serology Testing (Group 1)

Though not considered an SAE, intercurrent HIV infection in study participants must be reported promptly using the same procedures for reporting SAEs.

3BNC117-LS mAb cannot cause HIV infection. Study participants in all groups will be tested for HIV-1 and HIV-2 antibodies during screening and on the day of 3BNC117- LS administration, prior to administration.

Participants in group 1A-1D will be tested for HIV-1 and HIV-2 antibodies 2 weeks after administration and also at the end of the study. If HIV serology is indeterminate or positive, HIV-1 viral load will be performed to distinguish new HIV-1 infection from a false-positive serology result due to 3BNC117-LS administration. If HIV serology is indeterminate or positive at 2 weeks after administration, it will be repeated every 3 months until negative and at the end of the study.

To prevent possible unblinding in groups 1E and 1F as a result of false-positive or indeterminate HIV serologies due to the administration of 3BNC117-LS, participants in these groups will be tested for HIV-1 and HIV-2 antibodies and HIV-1 viral load 2 weeks after administration and every 3 months until the end of the study. The study team will remain blinded to the HIV serology results. HIV serologies and viral loads will be reviewed by a physician at The Rockefeller University Hospital who is trained in infectious diseases and not involved in the study. The physician will notify the study team if any participant is found to have true HIV infection.

Only individuals found to be HIV-negative at screening will be enrolled in Group 1. HIV testing at additional time points may be performed at the request of the participant or at the discretion of study investigators as medical or social circumstances arise.

Participants who are found to be HIV-infected at screening and participants who acquire HIV infection during the trial will be withdrawn from the study and managed in the following way:

a) The participant will be counseled by the study investigators. The counseling process will assist the participant with issues such as: psychological and social implications of HIV infection; whom to inform and what to say; implications for sexual partners and avoidance of transmission to others in the future.

b) Participants will then be referred to a patient support center or institution of his/her choice for a full discussion of the clinical aspects of HIV infection. Referral will be made to a designated physician or center for discussion of options of treatment of HIV infection.

6.2.9 Ophthalmologic evaluations

Participants will be evaluated by an ophthalmologist (including slit lamp exam) prior to (pre-infusion visit) and 6 weeks after 3BNC117-LS administration. In addition, participants will be monitored for the occurrence of ocular symptoms. If symptoms of ocular disease develop after study drug administration, participants will be promptly referred to an ophthalmologist for diagnosis and management.

6.2.10 Diagnostic work up in the event of grade 3 or 4 elevations in total bilirubin

Any participant with a grade 3 or 4 elevation in total bilirubin will undergo a diagnostic work up that includes right upper quadrant ultrasound, testing for gamma glutamyl transferase (GGT) HAV (IgM and IgG), HBV (hepatitis B surface antigen and IgM anti-hepatitis B core antigen), HCV (anti-hepatitis C antibody and HCV PCR), EBV (IgM and IgG VCA and EBNA antibodies), CMV (IgM and IgG), VZV (IgM and IgG) and HSV (IgM and IgG), and any other relevant laboratory tests as determined by the investigators. These evaluations will be of no cost to the participant.

6.2.11 Monitoring for cytokine release associated adverse events and treatment of cytokine release syndrome

Based on previous clinical experience with similar monoclonal antibodies, it is unlikely that administration of 3BNC117-LS would lead to cytokine release syndrome. However, a potential side effect of a monoclonal antibody can be the stimulation of a massive release of cellular cytokines, which can have profound effects on blood pressure, vascular integrity, and myocardial, lung, liver, and kidney functions. If cytokine release syndrome occurs, the participant may need to be treated with intravenous fluids, vasopressors, and high-dose corticosteroids and may require ventilatory support.

Study participants will be observed at the Rockefeller University Hospital inpatient unit on the day of 3BNC117-LS administration. Participants will remain in the unit for at least 60 minutes after administration. Access to a twenty-four hour on-call physician is available. The RUH outpatient and inpatient units are equipped with crash carts for immediate medical care, should the need arise. In case of an emergency, after stabilization of the volunteer, he/she will be transferred to the neighboring tertiary care center, New York Presbyterian Hospital (Cornell) for specialized medical care.

6.2.12 Family Planning Counseling

During screening and subsequent study visits, study personnel will counsel participants about the importance of prevention of pregnancies and the use of condoms, as well as other effective family planning methods. Condoms will be provided.

Female study participants of reproductive potential are defined as pre-menopausal women who have not had a sterilization procedure (e.g. hysterectomy, bilateral oophorectomy, tubal ligation or salpingectomy). Women are considered menopausal if they have not had a menses for at least 12 months and have a FSH of greater than 40 IU/L or if FSH testing is not available, they have had amenorrhea for 24 consecutive months.

Should pregnancy occur, a pregnant participant will not receive 3BNC117-LS. If pregnancy occurs after 3BNC117-LS administration, the participant will be followed until the end of the study and until delivery, if it occurs after the study has ended. Approximately 2-4 weeks after delivery, a pediatrician will examine and assess the health status of the baby. The baby's health status will be reported to the principal investigator, the CRSO, RUH-IRB and SMC.

6.2.13 Reimbursement

Participants will be compensated $25 for the initial screening visit.

They will be compensated:

- $25 for the pre-infusion visit
- $50 for each ophthalmologic assessment
- $50 for each collection of cervicovaginal and rectal fluids
- $200 for the infusion/injection visit
- $80 for each post infusion follow up visit
- $100 for the final study visit

Payment will be made to participants who fill out a form from The Rockefeller University Finance Office and are eligible for and want to receive payment.

Participants who complete all study visits will receive a total of $1,730 throughout the study. For groups in which cervicovaginal and rectal fluid are collected, up to an additional $200 may be received.

If a member of the study team asks a participant to return for an unscheduled visit, the participant will be compensated $25 each time.

Compensation is provided to help cover their travel expenses, as well as child care and time lost from gainful employment. Participants will be compensated only for the visits they complete

6.2.14 Safety Assessments

6.2.14.1 Solicited Adverse Events

Solicited adverse events in this study include presence of feverishness, chills, headache, nausea, vomiting, malaise, myalgia and arthralgia occurring in the two weeks following

3BNC117-LS administration, as well as injection or infusion site reaction or extravasation changes.

Solicited adverse events will be collected prospectively by structured interviews on infusion/injection and post-infusion/injection follow up visits; recorded and graded according to pre-established criteria (see **Appendix B**). The Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials will be used to grade adverse events in HIV-uninfected participants. The DAIDS AE Grading Table v2.1 will be used to grade adverse events in HIV-infected participants. In addition, the Common Terminology Criteria for Adverse Events (CTCAE) v4.03 grading scale will be used for reporting and grading adverse events in all groups occurring within 24 hours of the start of 3BNC117-LS administration that are considered infusion reactions or cytokine release syndromes. Symptoms that may constitute an infusion reaction or cytokine release syndrome include: fever and/or shaking chills, flushing and/or pruritus, alterations in heart rate and blood pressure, dyspnea or chest discomfort, back or abdominal pain, nausea, vomiting, and/or diarrhea, skin rash.

Vital signs (pulse, respiratory rate, blood pressure and temperature) will be measured prior to antibody infusion, at the end, and 1 hour post-infusion, and prior to and 1 hour after subcutaneous injection. Vital signs will be graded according to **Appendix B** and recorded. All medications required for treatment of adverse events will be recorded.

6.2.14.2 Unsolicited Adverse Events

During all follow up visits, the occurrence of unsolicited adverse events will be assessed following an open question to participants, with the dates of commencement and resolution and any medication required. Adverse events will be followed to resolution or stabilization. They will be graded as indicated in **Appendix B**.

Laboratory abnormalities that are considered clinically significant and are grade 3 or higher will be reported as adverse events.

6.2.14.3 Routine Laboratory Parameters

Laboratory parameters will routinely include hematology (WBC and differential, hemoglobin/hematocrit, platelets), clinical chemistry (Creatinine, Total and Direct bilirubin, AST and ALT, alkaline phosphatase), and urinalysis (protein, WBCs, RBCs and casts). Female participants will have serum beta-HCG measured on the day of 3BNC117-LS infusion, and urine beta-HCG checked at screening and follow up visits. The laboratory samples for these tests will be collected at the time points indicated in the Time of Events Schedule (**Appendix A**).

In the event of an abnormal laboratory value, participants may be asked to have additional sample(s) collected at the discretion of the principal investigator or designee.

HIV-1 viral load and CD4+ T cell counts will be closely monitored for Group 2 participants, as outlined in the Time of Events Schedule (**Appendix A**).

Participants will be screened for syphilis (RPR) and viral hepatitis (HBsAg and HCV viral load) at the Screening Visit.

6.2.14.4 Antiretroviral and Immunogenicity Assessments

1. Standard HIV-1 viral load assay (CLEP-certified) will be performed at a contracted laboratory, LabCorp. The detection range of the assay is 20-10x10^6 copies/ml. HIV-1 viral load will be determined at multiple time points before and after 3BNC117-LS infusion.

2. Levels of circulating CD4+ and CD8+ T cell counts will be determined by a CLEP-certified assay, performed at LabCorp.

3. Measurement of 3BNC117-LS levels by sandwich ELISA will be performed in the laboratory of Dr. Georgia Tomaras at Duke University (a Core Laboratory sponsored by the Collaboration for AIDS Vaccine Discovery, CAVD). 3BNC117-LS levels will be measured in serum at multiple time points during the study. 3BNC117-LS levels will also be measured in cervicovaginal and rectal fluids before and 2 weeks after 3BNC117-LS.

4. TZM-bl neutralization assay will be performed in the laboratory of Dr. Michael Seaman of the Beth Israel Deaconess Hospital in Boston (a CAVD Core Laboratory). This assay will be performed for all study groups, with samples collected before and after 3BNC117-LS infusion, to determine neutralizing activity against a panel of pseudoviruses representative of multiple clades.

5. Anti-drug (3BNC117-LS) antibody responses in serum. Assays will be performed in the Laboratory of Dr. Margaret Ackerman at Dartmouth College (a CAVD Core Laboratory).

6. T cell assays - HIV-1 Env and Gag specific responses will be evaluated in PBMC's by IFNγ-ELISpot and by multiparametric cytokine flow cytometry. Phenotypic analysis, specifically the expression of immune activation/exhaustion markers on CD8+ T cells will also be evaluated. These assays will be performed in the Laboratory of Molecular Immunology.

7. Genotyping – Sequencing of HIV-1 env viral isolates will be performed in plasma samples or culture supernatants collected before and after the 3BNC117-LS infusions. Genotyping will be performed in the RU Laboratory of Molecular Immunology.

8. Phenotyping of viral escape variants - HIV-1 pseudoviruses expressing selected envelope sequences will be generated for subsequent characterization by *in vitro*

neutralization assays. Representative sequences, including sequences with substitutions not known to confer reduced susceptibility to 3BNC117, will be selected for phenotypic analysis. Pseudoviruses will be generated in the RU Laboratory of Molecular Immunology and the neutralization assays will be performed in Dr. Michael Seaman's Laboratory at the Beth Israel Deaconess Hospital in Boston.

All immune responses and antiretroviral activity will be evaluated for proportion of responders and the mean responses will be compared. Optimal sample collection, processing, cryopreservation, archiving and storage will be maintained. Additional studies will be performed as warranted at the discretion of the investigators.

6.2.14.5 Pharmacokinetic evaluations

3BNC117-LS serum levels will be measured by validated sandwich ELISA methods performed at Duke University. Serum samples will be collected before the 3BNC117-LS administration, at the end of the infusion or one hour following injection, and at all follow up visits.

Briefly, serum samples, positive and negative controls, and multiple dilutions of a 3BNC117-LS reference standard will be incubated in plates coated with a murine anti-idiotype antibody to 3BNC117-LS. Immobilized 3BNC117-LS will be detected with a murine anti-human IgG kappa chain mAb-HRP conjugate. Colorimetric detection will be afforded with HRP substrate, tetra-methylbenzidine.

The concentration of 3BNC117-LS in the samples will be interpolated from a standard curve of 3BNC117-LS, using a logistic curve-fitting algorithm. The reference standard and positive controls will be created from the drug product lot of 3BNC117-LS.

Pharmacokinetic parameters, including AUC, Cmax, $T_{1/2}$, Tmax, clearance rate and others will be estimated by performing a non-compartmental analysis (NCA) using WinNonlin 6.3. ANOVA model will be used to compare cohort differences for AUC and Cmax. Log-transformation will be applied if exploratory analysis indicates departures from the Normal assumption. The dose proportionality for the 2 parameters (AUC and Cmax) will be examined by regression analysis. Pharmacokinetic parameters will be examined to correlate exposure with safety and pharmacodynamic parameters (decline in plasma HIV-1 RNA), and variance, based on population intrinsic factors such as weight and gender, will be explored.

7 Investigational Product

Investigational Drug Name:	3BNC117-LS
Manufacturer name of drug:	Celldex Therapeutics, Inc.
IND Number:	131872
IND Sponsor:	Rockefeller University

Investigational Drug Name:	Placebo
Manufacturer Name of Drug:	Celldex Therapeutics, Inc.
IND Number:	136387
IND Sponsor:	Rockefeller University

7.1 Regimen

3BNC117-LS will be administered intravenously at 3, 10 or 30 mg/kg, or subcutaneously at 150 mg (1 mL) or 300 mg (2 mL) on day 0.

7.2 Study Product Formulation and Preparation

Part A
3BNC117-LS will be provided by Celldex Therapeutics in single-use vials containing 10 ml of 3BNC117-LS at a 20 mg/ml concentration.

3BNC117-LS will be diluted in normal saline (NaCl 0.9%) to a volume of 250 ml.

Part B
3BNC117-LS will be provided by Celldex Therapeutics in single-use vials containing 1 ml of 3BNC117-LS at a 150 mg/ml concentration.

3BNC117-LS will be diluted in normal saline (NaCl 0.9%) to a volume of 100 ml.

Placebo will be provided by Celldex Therapeutics in single-use vials containing 1 mL of buffered solution composed of 5 mM Histidine, 250 mM Trehalose, 10 mM Methionine, 5 mM Sodium Acetate, 0.05% Polysorbate 20, pH 5.5.

7.3 Dispensing and Handling of Investigational Product

Part A
3BNC117-LS will be shipped from Celldex Therapeutics and will be stored in the RUH Pharmacy at < - 65 °C.

Part B
The new lot of 3BNC117-LS and Placebo will be shipped from Celldex Therapeutics and will be stored in the RUH Pharmacy at 5 ± 3°C.

Study group assignment will be determined by the RU study team and 3BNC117-LS will be dispensed by the RUH pharmacist according to group assignment. Trial personnel will ensure that the study ID number on the infusion bag or syringe matches the study ID assigned to the participant prior to administration.

For intravenous administration, the appropriate dose for infusion will be calculated by the RU pharmacist according to study allocation and participant's weight. 3BNC117-LS will be dispensed in a piggy-back, diluted in normal saline (NaCl 0.9%) ready for administration by the study investigators. 3BNC117-LS should be administered through a 0.2 or 0.22 micron in-line filter. For subcutaneous administration, syringes will be prefilled by the pharmacy and ready for administration.

7.4 Accountability and Disposal of Used and Unused Investigational Product

The date, allocation number and location of storage of the vials will be recorded in a log. During the trial, the product accountability form, and the dispensing log will be monitored. At the end of the trial, unused vials will be returned to Celldex Therapeutics or destroyed.

8 Data Analysis

8.1 Analysis of Safety, PK and Antiretroviral effects

Primary Outcomes

- Safety: The safety population will include all participants who receive 3BNC117-LS. A baseline measurement and at least one laboratory, vital sign, or other safety-related measurement obtained after 3BNC117-LS infusion may be required for inclusion in the analysis of a specific safety parameter.

The number and percentage of participants experiencing one or more AEs will be summarized by study group, relationship to study drug, and severity. AEs will be summarized by the number and percentage of participants who experienced the event, according to system organ class (SOC) and preferred term. AEs will also be summarized by severity grade and by relationship to study drug according to the DAIDS AE Grading Table v2.1 (HIV-infected groups), the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (HIV-uninfected groups). The CTCAE v4.03 grading scale will be used for reporting and grading adverse events related to infusion reactions and cytokine release syndromes in all groups (**Appendix B**). Changes in hematology, chemistry, and other laboratory values will be summarized descriptively. Changes will be calculated relative to the values collected at baseline.

- Pharmacokinetic parameters: will be calculated using standard non-compartmental analysis methods. Descriptive results will be presented for the pharmacokinetic parameters by dose group. Pharmacokinetic parameters, including AUC, Cmax, $T_{1/2}$, Tmax, clearance and others will be summarized. ANOVA model will be used to compare group differences for AUC and Cmax. The dose proportionality for the 2 parameters (AUC and Cmax) will be examined by regression analysis. Pharmacokinetic parameters will be examined to correlate exposure with safety and pharmacokinetics parameters, and

variance based on population intrinsic factors such as weight and gender will be explored. Pharmacokinetic parameters will be calculated separately for participants in Part A and Part B considering the expected differences in peak concentrations between the 2 lots of 3BNC117-LS.

Secondary outcomes

- Anti-3BNC117-LS antibodies: The frequency of induced anti-3BNC117-LS antibodies will be reported for all groups and the proportion will be compared using Fisher's Exact test. The levels of the anti-antibodies will be compared using ANOVA or Kruskal-Wallis test.

Other exploratory measurements

- Antiretroviral activity: The magnitude of change in plasma HIV-1 RNA levels from baseline will be evaluated in viremic individuals enrolled in groups 2B and/or 2C. Plasma HIV-1 RNA levels will be evaluated prior to and at multiple time points following 3BNC117-LS infusion and the log copies/ml reduction in HIV-1 RNA level from baseline will be calculated for each dose group.

- *In vitro* neutralization assays will be performed with serum from study participants in Groups 2B and 2C to evaluate antiretroviral activity against Tier 1 and Tier 2 HIV strains before and after 3BNC117-LS administration. Results will be descriptive.

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- Genotyping of HIV isolates will be performed to analyze the induction of escape mutations following 3BNC117-LS infusion in viremic individuals enrolled in groups 2B and/or 2C. Results will also be descriptive.

Continuous data will be summarized by descriptive statistics, including the sample size, mean, standard deviation, median and range. Categorical data will be summarized by the number and percentage of participants.

The analysis of study data will be primarily descriptive, with emphasis on tabular and graphical displays. Summary statistics will be calculated, along with point and interval estimates of solicited and unsolicited adverse events. The pharmacokinetic profile of a single infusion of 3BNC117-LS will also be determined. This study is exploratory, and any statistical inferences will be hypothesis generating and not confirmatory.

8.2 Sample Size Considerations

The sample size will be of 15 to 43 participants according to the dose escalation design used to characterize the safety and the pharmacokinetics profile of one 3BNC117-LS infusion, administered at one of three increasing dose levels to HIV-uninfected and two dose levels to HIV-infected individuals. An over-enrollment of up to 2 participants will be allowed in the groups that receive 30 mg/kg (or the maximum tolerated dose) of 3BNC117-LS, if participants are lost to follow up.

- Pharmacokinetics:

The pharmacokinetic profile of 3BNC117-LS will be evaluated in this study. Based on the preliminary PK profile of VRC01-LS in humans, another anti-HIV-1 antibody targeting the same gp120 site as 3BNC117-LS, and 3BNC117-LS in non-human primates, it is expected that the half-life of 3BNC117-LS will be 3 to 4-fold higher than the unmodified 3BNC117, or 42-56 days.

With the proposed expected sample sizes: Group 1A-F HIV-uninfected (n=27 who receive product) and Group 2B-D HIV-infected (n=12), there is a 95% probability that the error margin for estimating the mean elimination half-life of 3BNC117-LS will be less than 1.7 days (n=39, combined groups), 2.1 days (n=27, Group 1A-F) or 3.1 days (n=12, Group 2B-D), assuming a standard deviation of +/- 5.5 days, as demonstrated with 3BNC117, which has a half-life of approximately 17.6 days in HIV-uninfected and 9.6 days in viremic HIV-infected individuals (Caskey et al., 2015).

- Safety:

If none of the HIV-infected participants experiences a grade 3 adverse event related to 3BNC117-LS (n=12), the 95% upper confidence bound for the rate of adverse events in the population is 26.5%.

If none of the HIV-uninfected participants experiences a grade 3 adverse event related to 3BNC117-LS (n=27), the 95% upper confidence bound for the rate of adverse events in the population is 12.8%.

If none of the participants (HIV-infected and HIV-uninfected combined) experiences a grade 3 adverse event related to 3BNC117-LS (n=39), the 95% upper confidence bound for the rate of adverse events in the population is 9.0%.

9 Data and Sample Storage

The Principal Investigator will oversee how the data are collected, entered, and protected. All study data will be collected by the clinical study staff using designated source documents and entered onto the appropriate electronic case report forms (eCRFs). Data collection forms (DCFs) will be provided by Emmes Corporation for use as source documents as appropriate. All study data must be verifiable to the source documentation. All source documents will be kept in a locked facility at the clinical site and remain separate from participant identification information (name, address, etc.) to ensure confidentiality. All medical records (when not being reviewed by the research team) will be kept under lock and key in the Medical Record Department of the hospital with access limited to the appropriate RUH personnel, members of the RUH-IRB and the FDA. Source documentation will be available for review to ensure that the collected data are consistent with the eCRFs.

All eCRFs and laboratory reports will be reviewed by the clinical team, who will ensure that they are accurate and complete.

All research samples will have a unique identifier. The PI will be responsible for ensuring project compliance, data analysis and entry, regulatory monitoring, and coordination of the activities of the entire study team. Standard GCP practices will be followed to ensure accurate, reliable and consistent data collection.

Source documents include, but are not limited to:
- Signed Informed Consent Documents
- Dates of visits including date of 3BNC117-LS infusion
- Documentation of any existing conditions or past conditions relevant to eligibility
- Reported laboratory results
- All adverse events
- Concomitant medications
- Reported unsolicited and solicited adverse events

10 Recruitment and Advertising

Both men and women ages 18 through 65 will be recruited for the study from the community at large and will be referred by physicians in the community. We will make every effort to recruit minorities and women.

Advertisements – The RUH Clinical Research Support Office (CRSO) will utilize the Volunteer Repository. Advertisements will also be placed: online (e.g. Craiglist, Centerwatch, etc), in newspapers (Metro, AMNY) and on campus.

Centralized Call Management – The RUH CRSO will conduct telephone screenings of selected Volunteer Repository members, and of volunteers who call 1800-RUCARES, to facilitate screening efficiently. Based on IRB approved eligibility criteria, potentially eligible candidates pre-screened by CRSO staff will be referred to the study coordinator/investigator for further evaluation. The research team and CRSO will work together on a protocol-specific pre-screening script to optimize the process.

11 Potential Benefits to Participants

There are no direct benefits to HIV-uninfected participants that enroll in this study. This is the first-in-human study of this monoclonal antibody; it is unlikely that HIV-infected participants will benefit from participating in this study.

12 Potential risks to the participant including to the fetus

This study entails moderate risk to participants since 3BNC117-LS mAb has not been tested in humans yet. Potential study participants will be informed about the possible risks associated with 3BNC117-LS mAb administration and that there may be unknown risks.

While each mAb product has unique safety issues related to its mechanism of action, the major safety concern related to mAbs in general is an infusion/hypersensitivity reaction. These types of reactions are more common for mAbs that contain murine elements compared to human mAbs, such as 3BNC117-LS. Passive administration of anti-HIV-1 antibodies has been evaluated in humans in the past, including 3BNC117, which is identical to 3BNC117-LS except for two amino acid mutations in the Fc region. As observed with other monoclonal antibodies, anti-HIV-1 antibodies were generally safe and well tolerated and most adverse events observed were infusion-related events.

- Immunologic symptoms such as listed below are possible with administration of a monoclonal antibody and will be considered adverse events of interest. Potential allergic-type reactions during and immediately following the administration of 3BNC117-LS will be carefully monitored.
    - Constitutional symptoms, such as fever, rigors/chills;
    - Infusion site reaction/extravasation changes, pruritus, urticaria;
    - Serum sickness like syndromes as evidenced by fever, rash, arthralgia, arthritis, nephritis;
    - Deposition of immune complexes in the kidneys leading to renal insufficiency;
    - Adult Respiratory Distress Syndrome, bronchospasm/wheezing, anaphylaxis;
    - Cytokine release syndrome/ acute infusion reaction.

- In HIV-infected participants who are off ART, 3BNC117 or 3BNC117-LS-resistant viral strains might be selected following 3BNC117-LS infusion. Development of 3BNC117-resistance might limit the future use of 3BNC117 and 3BNC117-LS by the study participant, if 3BNC117 or 3BNC117-LS are licensed for clinical use.

- Two FDA-approved drugs target early steps in viral replication: maraviroc (a CCR5 inhibitor) and enfuvirtide (an entry inhibitor that binds to HIV-1 Env gp-41), while 3BNC117-LS targets different sites on HIV-1 Env gp120. Cross-resistance was not observed between 3BNC117 and maraviroc or enfuvirtide when HIV-1 pseudoviruses were tested in a TZM-bl neutralization assay. Therefore, it is unlikely that development of 3BNC117 or 3BNC117-LS-resistance would interfere with the antiretroviral activity of maraviroc or enfuvirtide.

- In a GLP-cross-reactivity study in human tissues, 3BNC117 stained rare cells in the conjunctival recesses. In a repeat GLP-cross-reactivity study in human tissues, 3BNC117 and 3BNC117-LS did not stain these cells. When rats were administered 3BNC117, ocular toxicity was not observed. 3BNC117 has been administered to 123 participants to date, approximately 6% of participants enrolled in 3BNC117

trials reported mild ophthalmic complaints (such as pruritus, conjunctival erythema, increased lacrimation, blurry vision) during study follow up of 6 months. In all instances symptoms resolved without specific treatment and ophthalmologic evaluations 1 or 5 months after 3BNC117 administration did not show changes from baseline. It remains unclear if these symptoms were related to 3BNC117 infusion. In this study, participants will be monitored closely and evaluated by an ophthalmologist before and after 3BNC117-LS administration and if ocular complaints occur.

- HIV-uninfected participants might have false positive HIV serology results after 3BNC117-LS administration. If this happens, it will be temporary, while 3BNC117-LS levels are detectable in blood.

- Blood drawing and phlebotomy can be associated with pain, bruising, anemia or infection at the site of venipuncture. Rarely, fainting may follow phlebotomy.

- Subcutaneous administration may be associated with local symptoms including pain, redness, and swelling.

- The adverse effects 3BNC117-LS administration would have in a fetus or unborn child are unknown.

- Participants may engage in increased risk taking after receiving anti-HIV-1 mAb administration.

13 Procedures to minimize risk

- As outlined above, this study will be an exploratory phase 1 trial of 3BNC117-LS in humans. Potential study participants will be informed about possible risks of the monoclonal antibody and that there may be unknown risks.

- Medical records and routine laboratory data will be handled with HIPAA compliance and protected by the rules and regulations of the RUH, a JCAHO approved institution.

- With any new medicine or monoclonal antibody, there is a possibility of totally unexpected side effects. On the day of 3BNC117-LS administration participants will be observed for one hour after administration. The RUH inpatient unit is equipped for providing emergency medical interventions in the unlikely event of acute allergic or other reactions. In case of an emergency, after stabilization of the participant, he/she will be transferred to the neighboring tertiary care center, New York Presbyterian Hospital (Cornell) for specialized medical care.

- In Part A, enrollment will be staggered by 1 day for the first 3 participants of each dose group, and a maximum of 2 participants will be administered 3BNC117-LS in a given week for Group 1A. If no DLT occurs within 42 days from 3BNC117-LS infusion of the 3 participants in a dose group, dose escalation to next dose group will proceed. If DLT occurs, 3 additional participants will be enrolled. If no additional DLTs occur within 42 days of 3BNC117-LS infusion in all 6 participants, study can proceed with enrollment of next dose group. If 2 or more DLTs occur, dosing will be halted and the prior lower dose level will be declared the maximum tolerated dose. Study participants will be first enrolled in Group 1A (HIV-uninfected, 3mg/kg), enrollment in additional groups will follow the dose escalation schema outlined in
- **Figure** 2.

- In Part B, participants will be enrolled concurrently into **Groups 1D, 2D, and 1E**. However, the first 5 participants enrolled across these 3 groups will be administered 3BNC117-LS at least one day apart. Enrollment in **Group 1F** will begin once 2-week safety data is available from the first 5 participants in Group 1E.

- In the HIV-infected groups, HIV-1 RNA levels and CD4+ T cell counts will be monitored during the study. HIV-infected participants will be advised to start combination ART 6 weeks after 3BNC117-LS infusion. ART may be initiated sooner if clinically indicated. ART will be initiated in collaboration with the participant's HIV primary care physician. ART will not be provided by the study.

- To minimize risks associated with phlebotomy, blood drawing will be performed by experienced phlebotomists. Should discomfort occur, they will provide appropriate treatment.

- To minimize risks associated with blood drawing, participants will be closely monitored for signs and symptoms of anemia.

- Females of childbearing potential and who participate in sexual activity that might lead to pregnancy will be advised to use two reliable forms of contraception from 10 days prior to 3BNC117-LS administration and for seven months following 3BNC117-LS administration. In addition, a pregnancy test will be performed at screening, pre-infusion visit, on the day of 3BNC117-LS administration, and throughout the course of the study. Males who are not anatomically sterile and who participate in sexual activity that might lead to pregnancy will be advised to use condoms from 10 days prior to and for seven months following 3BNC117-LS administration, to avoid pregnancy in a spouse or partner.

- Safer sex counseling will be provided to all participants, including MSM. Condoms will be provided.

- Participants will have regularly scheduled visits to the outpatient clinic and routine safety laboratories will be checked according to the Time of Events Schedule (**Appendix A**). HIV-infected individuals will have close monitoring of HIV-1 viral

load and CD4/CD8 T cell counts according to the Time of Events Schedule (**Appendix A**).

- Adverse events will be monitored and graded using the DAIDS AE Grading Table v2.1 (HIV-infected groups), the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (HIV-uninfected). The CTCAE v4.03 grading scale will be used for reporting and grading adverse events related to infusion reactions and cytokine release syndromes in all groups (**Appendix B**).

- Adverse events will be managed by the clinical trial team who will assess and treat the event as appropriate, including referral to an independent physician and/or department.

- Safety monitoring will be conducted by the study investigators, by an external Study Monitoring Committee (SMC), and by the International AIDS Vaccine Initiative (IAVI). The RUH-IRB will conduct the initial review of the proposed study and will follow progress through annual reports and by immediate notification of serious adverse events. Any serious and unanticipated adverse events will be immediately reviewed by the study investigators. Investigators will notify the RUH-IRB and the sponsor at the RU within 2 working days from the investigators being made aware of the event. The RU sponsor will notify the FDA, per 21 CFR 312. The SMC will be available to the investigators for consultation and review of severe adverse events if needed.

14 Alternative methods or treatments

HIV-infected individuals who are off ART at the time of screening may choose to start ART immediately, instead of participating in the study.

15 Data and Safety Monitoring Plan

This is a first-in-human phase 1 study, which exposes study participants to "moderate risk". A Study Monitoring Committee (SMC) will be established to monitor the study.

15.1 Safety Monitoring Committee

The purpose of the Safety Monitoring Committee (SMC) is to provide an ongoing assessment of participant safety during the conduct of the study. The SMC will consist of three independent individuals who have no relationship to the Principal Investigator and Co-Investigators involved in the trial. No member of the SMC will have any direct responsibility for the clinical care of study participants. No representative of Celldex Therapeutics, the Rockefeller University, or their designees may be a member of the

SMC. However, the SMC may invite the principal investigator (PI) or designee and a Celldex Therapeutics, and/or Rockefeller University representative to an open session of a SMC meeting to provide information on study conduct, present data, or to respond to the members' questions. Dr. Pat Fast, from the International AIDS Vaccine Initiative (IAVI) can be invited to participate in SMC meetings and to comment on study occurrences, as the medical monitor for the study.

The names, university affiliation and title, area of expertise, and contact information of each of the SMC are provided below:

Raphael Dolin, MD
Maxwell Finland Professor of Medicine
Beth Israel Deaconess Medical Center
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Areas of expertise: HIV vaccine clinical trials

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Areas of expertise: HIV vaccine clinical trials

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University of Rochester Medical Center
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Rochester, NY 14642
Email: Michael_keefer@urmc.rochester.edu
Areas of expertise: HIV vaccine clinical trials

At least two members of the SMC must be in attendance (phone, video, or in-person meetings) to constitute a quorum for an SMC meeting. SMC members may also review and comment by email, if scheduling cannot be worked out in a timely manner. One member of the SMC will be appointed as chair of the committee. The SMC chair (or his/her alternate) will be responsible for summarizing and communicating in writing SMC acknowledgments and recommendations to the PI within 5 business days following each SMC meeting and/or review.

The SMC will be asked to review study safety data on an interim basis:

1. Grade 3 solicited and unsolicited adverse events judged by the principal investigator or designee to be possibly, probably or definitely related to 3BNC117-LS.

2. Grade 3 laboratory adverse events confirmed on retest and judged by the principal investigator or designee to be possibly, probably or definitely related to 3BNC117-LS.

3. The investigator will report any "late occurring" DLT (i.e., a DLT occurring after dose-escalation has proceeded to the next cohort) to the SMC. The investigators and SMC will mutually assess this information, along with safety from subsequent study groups, to determine whether a change to study conduct is warranted.

4. If two or more grade 3 adverse events, deemed probably or definitely related to the study drugs occur, no additional administration of the investigational product will take place pending a Safety Monitoring Committee (SMC) review. The SMC will provide a recommendation regarding subsequent enrollment in the study.

5. If a grade 2 ophthalmic AE, judged to be at least possibly related to 3BNC117-LS occurs, or any grade 3 or 4 ophthalmic AE occur, regardless of causality assessment, no additional administration of the investigational product will take place pending a Safety Monitoring Committee (SMC) review. The SMC will provide a recommendation regarding subsequent enrollment in the study.

6. SAEs, which are deemed possibly, probably or definitely related to 3BNC117-LS by the principal investigator or designee, and unanticipated adverse events will be reported to the SMC within 2 working days of the site becoming aware of the event.

If there is one SAE, grade 3 or higher, and judged as possibly, probably or definitely related to the administration of 3BNC117-LS by the principal investigator or designee, no additional administration of the investigational product will take place pending a review by at least two members of the SMC. Following this review, the SMC will make a recommendation to the principal investigator regarding the continuation of investigational product administration.

The occurrence of such adverse events will not result in a study pause, unless it is judged by the principal investigator or designee that the risk/benefit ratio of the study has changed such that risk of currently enrolled or future participants has increased; or unless recommended by the IRB, SMC, or FDA.

7. If, at any time, a fatal, life-threatening or permanently disabling SAE with a suspected causal relationship to 3BNC117-LS occurs, no further administration of the investigational product will occur until a consensus plan forward has been approved by investigators, SMC, the IRB and the FDA.

All updated versions of the protocol will be provided to the SMC. The review of trial data by the SMC will take place at least annually. Prior to data review, the study team will provide the SMC with updated records of all adverse events (AEs) of a grade 2 or higher.

The SMC will acknowledge receipt of annual reports and will indicate if there are concerns with the continuation of the study.

15.2 Monitoring

Safety monitoring will be conducted by the study investigators, by the external Study Monitoring Committee (SMC) and by the International AIDS Vaccine Initiative (IAVI). IAVI. The RUH-IRB will conduct the initial review of the proposed study and will follow progress through annual reports and by immediate notification of serious adverse events. External monitoring will occur at least quarterly, and will be conducted by IAVI.

15.3 Adverse Event Classification

Scales to be used: the DAIDS AE Grading Table v2.1 (HIV-infected groups), the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (HIV-uninfected). The CTCAE v4.03 grading scale will be used for reporting and grading adverse events related to infusion reactions and cytokine release syndromes in all groups (**Appendix B**).

15.4 Reporting Adverse Events

All adverse events will be reported to the IRB and the SMC at least annually. Serious Adverse Events, (SAEs) will be reported to the IRB, SMC, and an external monitor within two working days of identification of the SAE. SAEs will be reported directly to the FDA, per 21 CFR 312.

15.5 Reporting Unanticipated AEs

Unanticipated Adverse Events (UAEs) will be reported to the IRB and SMC. UAEs that are related and greater than moderate severity must be reported to the IRB and SMC, within two working days of identification of the UAE. UAEs will be reported to the FDA, per 21 CRF 312.

15.6 Clinical Laboratory Improvement Amendment/Clinical Laboratory Evaluation Program (CLIA/CLEP)

This study includes tests that are not CLIA/CLEP certified. The results of such tests will not be used in clinical decision-making or shared with participants or their health care providers.

15.7 Toxicity Management and Stopping Rules

A dose limiting toxicity (DLT) will be defined as any adverse event of Grade 3 or greater toxicity, if the study investigators recognize a probable or definite attribution to 3BNC117-LS mAb. Grade 3 laboratory abnormalities must be confirmed by a repeat test, obtained as soon as possible following the initial result.

Study participants will be first enrolled in Group 1A (HIV-uninfected, 3mg/kg). During the dose escalation phase of the study, enrollment will be staggered by 1 day for the first 3 volunteers of each dose group (a maximum of 2 participants will be enrolled in any given week).

If no DLT occurs within 42 days from 3BNC117-LS infusion of 3 participants in Group 1A, enrollment in Groups 1B (HIV-uninfected, 10 mg/kg) and 2B (HIV-infected, 10 mg/kg) will begin. If no DLT occurs within 42 days from 3BNC117-LS infusion of 3 participants in Groups 1B and 2B, enrollment in Groups 1C (HIV-uninfected, 30 mg/kg) and 2C (HIV-infected, 30 mg/kg) will begin respectively, as outlined in
Figure 2. If 0 or 1 DLT occurs within 42 days from 3BNC117-LS infusion of the first 3 participants in Groups 1C and 2C, the final 3 participants in each of these groups will be enrolled.

In general, the following rules in dose escalation will be followed:
- If no DLT occurs within 42 days from 3BNC117-LS infusion of 3 participants in a dose group, dose escalation to next dose group will proceed.

- If 1 DLT occurs, 3 additional participants will be enrolled. If no additional DLTs occur within 42 days of 3BNC117-LS infusion in all 6 participants, the study can proceed with enrollment of next dose group.

- If 2 or more DLTs occur, dosing will be halted and the prior lower dose level will be declared the maximum tolerated dose (MTD).

Enrollment in Group 1F will begin once 2-week safety data is available from the first 5 participants in Group 1E.

16 Clinical Trial Registration

The proposed study involves testing of FDA regulated drugs or biologics is registered at www.ClinicalTrials.gov (NCT03254277).

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