PROTOCOL RV 398

SAFETY AND VIROLOGIC EFFECT OF A HUMAN MONOCLONAL ANTIBODY, VRC-HIVMAB060-00-AB (VRC01), WITH BROAD HIV-1 NEUTRALIZING ACTIVITY, ADMINISTERED INTRAVENOUSLY TO ADULTS DURING EARLY ACUTE HIV INFECTION

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SCHEMA

Title

Safety and Virologic Effect of a Human Monoclonal Antibody, VRC-HIVMAB060-00-AB (VRC01), With Broad HIV-1 Neutralizing Activity, Administered Intravenously to Adults During Early Acute HIV Infection

Clinical Phase

Phase I

Study Duration

Study duration is 25 weeks.

Study Design

This is a placebo controlled study of the safety and impact of broadly neutralizing monoclonal antibody (mAb) therapy with VRC01 on viral dynamics in acute HIV infection, alone or in combination with antiretroviral therapy (ART). Twenty-four subjects will be enrolled during early acute HIV infection (AHI): defined either as two positive nucleic acid amplification tests (NAATs) for HIV-1 RNA within 21 days of a prior negative NAAT OR as a positive NAAT or positive 4th generation HIV EIA in the context of a negative 2nd or negative 3rd generation EIA. They will be subsequently randomized to one of three groups:

- Group 1, Immediate ART and placebo infusion (n=8): start ART with a single infusion of placebo on day 0 during AHI
- Group 2, Immediate ART and mAb Therapy (n=8): start ART with a single infusion of 40 milligrams/kilograms (mg/kg) VRC01 on day 0 during AHI
- Group 3, Immediate mAb Therapy and Subsequent ART (n=8): a single infusion of 40 mg/kg VRC01 on day 0 during AHI followed by ART initiation on day 7

Group	Number of Subjects	Day 0	Day 7
1	**8**	ART initiation and single placebo infusion	(continued ART)
2	**8**	ART initiation and single infusion 40mg/kg VRC01	(continued ART)
3	**8**	Single infusion 40mg/kg VRC01	ART initiation

For groups 1 and 2, participants and study team will be blinded to active mAb vs. placebo infusion. After day 7, all groups will be on ART and be followed for an additional 24 weeks.

Sample Size

Sample size is 24 participants; 8 per group

Participants

Adults aged 18-50 years enrolled at the time of early acute HIV infection in Thailand, Kenya,Tanzania, and Uganda.

Intervention

VRC-HIVMAB060-00-AB (VRC01) is a broadly neutralizing human IgG1 mAb targeted against the HIV-1 CD4 binding site. It was developed by the Vaccine Research Center (VRC)/National Institutes of Allergy and Infectious Diseases (NIAID)/ National Institutes of Health (NIH) and manufactured under cGMP for the VRC by the Vaccine Pilot Plant operated by Leidos Biomedical Research, Inc, formerly Science Applications International Corporation **(**SAIC)-Frederick, Inc., Frederick, Maryland, under contract to VRC. Vials are provided at 100 ± 10 mg/mL. The mAb will be administered intravenously at a dose of 40 mg/kg.

Antiretroviral therapy will consist of once daily oral combination antiretroviral therapy.

Study Objectives

Primary Objectives

1. Safety of VRC01 in acutely HIV-infected individuals
2. Impact of VRC01 on plasma viremia in each mAb arm compared to the ART plus placebo control at day 7 (+/- 1 day)

Secondary Objectives

1. Impact of VRC01 on viral dynamics in plasma over the course of 24 weeks
2. Impact of VRC01 on reservoir measures in peripheral blood mononuclear cells and plasma
3. Impact of VRC01 on clinical outcomes/characteristics of AHI
4. Impact of VRC01 on CD4 + T cell count
5. Pharmacokinetics of VRC01 in AHI in peripheral blood

Exploratory Objectives

1. Impact of VRC01 on reservoir measures in CSF, mucosal, and tissue compartments
2. Comparison of peripheral blood reservoir measures to those in the CSF, mucosal, and tissue compartments
3. Impact of VRC01 on reservoir measures in T cell subsets
4. Pharmacokinetics of VRC01 in CSF and mucosal compartments

5. Anti-VRC01 antibody detection
6. Impact of VRC01 on immune responses and activation
7. Effect of VRC01 on viral sequence, phenotype, and evolution
8. Predictors of favorable viral dynamics, immune responses, and reservoir seeding
9. Impact of VRC01 on neuropsychological testing outcomes

NOTE: Exploratory objectives involving additional specimen collections will only be performed on specimens of those participants who consent to these optional procedures.

LIST OF ABBREVIATIONS

TERM	DEFINITION
ADCC	Antibody-dependent cell-mediated cytotoxicity
ADCP	Antibody-dependent cell-mediated phagocytosis
ADCVI	Antibody-dependent cell-mediated viral inhibition
AE	Adverse experience
AFRIMS	Armed Forces Research Institute of Medical Sciences
AHI	acute HIV infection
AI	Associate Investigator
AIDS	Acquired immunodeficiency syndrome
ALP	Alkaline phosphatase
ALT/AST	Alanine aminotranferase and aspartate aminotransferase liver enzymes
ANOVA	Analysis of Variance
APGAR	American Pediatric Gross Assessment Record
ART	Antiretroviral therapy
ARV	Antiretroviral
bnMAb	Broadly neutralizing monoclonal antibody
CBER	Center for Biologics Evaluation and Research (FDA)
CCR5	chemokine receptor 5
CD4+	A functional subclass of T cells, helper T lymphocytes (Th) that are necessary for augmentation and coordination of innate and adaptive effector responses, humoral and cellular
CD8+	Cytotoxic T-Cells that destroy host cells, which have become infected by viruses or other intracellular pathogens
CDR	complementarity-determining regions
CFR	Code of Federal Regulations
cGMP	Current Good Manufacturing Practices
CHMP	Committee for Medicinal Products for Human Use
CHO	Chinese Hamster Ovary
CIOMS	Council of International Organization of Medical Sciences
Cl	Clearance
Cmax	The maximum concentration of a drug observed after its administration
Cmin	The minimum concentration of a drug observed after its administration and just prior to the administration of a subsequent dose.
CRF	Case report form
CRPMC	Clinical Research Products Management, NIAID, DAIDS
CRR	Continuing Review Report
CRS	Cytokine release syndrome
CSF	Cerebrospinal fluid

DAERS	DAIDS Adverse Experience Reporting System
DAIDS	U.S. Division of AIDS, NIAID, NIH
DAIDS CSRC	DAIDS Clinical Sciences Review Committee
DAIDS PRO	Protocol Registration Office, DAIDS
DCAC	Data Coordinating and Analysis Center
dL	Deciliter
DNA	Deoxyribonucleic acid
DoD	United States Department of Defense
E01	End of Infusion
EAE	Expedited Adverse Events
ECHO	Early Capture HIV Cohort (RV 217)
ELISA	Enzyme linked immunosorbent assay
ELISPOT	Enzyme-Linked Immunospot
ERC	Ethical Review Committee
FDA	U.S. Food and Drug Administration
GCP	Good Clinical Practices
GLP	Good Laboratory Practices
gp120	glycoprotein 120
H1	hypervariable loop 1
H3	hypervariable loop 3
HADS	Hospital Anxiety and Depression Scale
HIV and HIV-1	Human immunodeficiency virus, type 1
HRPO	Human Research Protection Office, Office of Research Protection, USAMRMC
HSPB	Human Subjects Protection Branch, WRAIR
IB	Investigator's Brochure
IC50	inhibitory concentration (IC_{50}) of <50 micrograms/milliliter (mcg/mL)
ICH	International Conference on Harmonization
ICS	Intracellular cytokine staining
ID	Identification
IgG	Immunoglobulin G
IND	Investigational New Drug
IQR	interquartile range
IRB	Institutional Review Board
IRB	Institutional Review Board
IV	Intravenous(ly)
kg	kilogram
LTR	long terminal repeat
mAb	Monoclonal antibody
mcg	microgram
mg	Milligram

MHRP	US Military HIV Research Program
MHRP COO	U.S. Military HIV Research Program-Clinical Operations Office
mL	Milliliter
mm	Millimeter
MO	Medical Officer
MOP	Manual of Operations
MUWRP	Makerere University Walter Reed Project
NAAT	Nucleic acid amplification tests
NAb	Neutralizing antibody
NHP	Non-human primate
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NK	Natural killer
NOAEL	“no observed adverse effect level”
NOEL	“no observed effect level”
NP	Neuropsychological Testing
NSAID	Non-steroidal anti-inflammatory drug
OHRP	Office for Human Research Protection
ORP	Office of Research Protections, USAMRMC
ORTA	WRAIR Office of Research Technology and Applications
PBMC	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
PHQ-9	Patient Health Questionnaire-9
PI	Principal investigator
PIR	post-injection reactogenicity
PK	pharmacokinetics
PSRT	Protocol Safety Review Team
QA	Quality assurance
RAB	DAIDS Regulatory Affairs Branch
RBC	Red blood cell count
RNA	Ribonucleic acid
RSC	DAIDS Regulatory Support Center
SAE	Serious adverse experience
SAIC-Frederick	Science Applications International Corporation-Frederick
SBV	Small blood volume
SC	Subcutaneous injection
SHIV	Simian-human immunodeficiency virus
SIV	Simian immunodeficiency virus
SMC	Safety Monitoring Committee
SOE	Schedule of Evaluations
SSN	Social security number

TILDA	Tat/Rev Induced Limiting Dilution Assay
Tmax	The time after administration of a drug when the maximum plasma concentration is reached; when the rate of absorption equals the rate of elimination
U.S.	United States
ul	microliter
UNAIDS	The Joint United Nations Programme on HIV/AIDS
UPIRTSOs	Unanticipated problems related to the study and involving risk to subjects or others
USA	United States of America
USAMRMC	U.S. Army Medical Research and Materiel Command
V	variable
Vd	volume of distribution
VRC	Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Maryland
WBC	White blood cell count
WHO-QOL	World Health Organization-Quality of Life
WRAIR	Walter Reed Army Institute of Research
B-HCG	B-human chorionic gonadotropin

1.0 INTRODUCTION

1.1 BACKGROUND

The global incidence of new human immunodeficiency virus (HIV) infections peaked in the mid-1990s. The incidence of new infections in 2012 is reported by the Joint United Nations Programme on HIV/AIDS (UNAIDS) as 2.3 million new cases, down from 3.4 million in 2001, with an estimated global total of 35.3 million people living with HIV. The reduction of HIV incidence is due to multiple factors that include prevention and treatment programs. The decrease in incidence is an encouraging trend, but the scope and cost of the epidemic remain of great global concern. The wider availability of antiretroviral therapy (ART), prevention of mother-to-child transmission programs, and a diverse array of other interventions have all contributed to turning the tide of the epidemic [1].

The United States (US) Military HIV Research Program (MHRP) and The National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH) are committed to the development of safe, effective methods to prevent and treat HIV infection and acquired immunodeficiency syndrome (AIDS) worldwide.

Long-term use of antiretroviral therapy (ART) in HIV-positive persons may be challenged by the need for high-level life long adherence to a daily regimen, development of drug resistance and cross-resistance, short and long-term toxicities, and cost [2-5]. Even with complete and durable viral suppression, standard antiretroviral therapies do not fully restore health, as some degree of immunodeficiency and/or chronic immune activation and inflammation persists. Furthermore, the large and growing global population of HIV infected individuals and the costs of ART present a significant challenge for providing treatment to those in need, particularly through public health systems where resources are already constrained. There is, therefore, a growing interest and need for the development of curative approaches for HIV that include treatment strategies that confer durable virologic control with less frequent dosing or even sustained remission in the absence of ART [6].

The MHRP has pioneered clinical research methods to establish cohorts of individuals acutely infected with HIV, including the RV 217 Early Capture HIV Cohort (ECHO) study and the RV 254/SEARCH10 study. These cohorts are ideal for evaluating curative strategies, given their successes in early diagnosis and treatment of early AHI and the limited establishment of a latent reservoir during the time period of very early infection [7-10]. Intervening with single or combination treatment modalities during this time period very early in AHI may decrease the magnitude of early viremia and profoundly limit establishment of the latent reservoir that gives rise to rebound viremia upon ART cessation in chronically treated HIV infection.

The Vaccine Research Center (VRC)/NIAID, Division of AIDS (DAIDS)/NIAID, and MHRP are collaborating to evaluate the clinical uses of VRC01 in these acutely diagnosed populations, This broadly neutralizing human mAb has thus far been demonstrated to be safe and generally well tolerated in initial Phase 1 studies [11]. It has also been demonstrated to decrease viremia in a rhesus macaque model [11] and in viremic HIV-infected individuals [12]. The current study

aims to determine the safety and impact of mAb therapy on AHI in humans. It will evaluate the effect of VRC01, with and without ART, on viremia and the establishment of an HIV-1 reservoir during early acute infection.

1.2 RATIONALE FOR VRC01 AS A STUDY AGENT

Administration of broadly neutralizing monoclonal antibodies has the potential to treat HIV infection by preventing viral spread (as with ART), by facilitating clearance of virus particles and mediating destruction of virus-producing cells. This may decrease the number of cells with HIV DNA incorporated and limit chronic immune activation. Indeed, recent data from nonhuman primate (NHP) and humanized mice models demonstrate that broadly neutralizing mAbs can control viremia and reduce the persistent cellular HIV DNA in infected animals in the absence of ART [13-15].

This proof of concept study will evaluate the impact of VRC01 on viremia in acute HIV infection and explore its impact on the establishment of the latent reservoir, informing considerations for the development of the VRC01 mAb for use in therapeutic settings, including administration in early and chronic HIV infection.

The VRC01 mAb was originally isolated from an adult who had been infected with HIV-1 for more than 15 years and had maintained viral control without ART [16]. Using novel methods to isolate and screen memory B lymphocytes from the peripheral blood mononuclear cells (PBMC) of HIV infected donors, investigators at the VRC were able to clone out this antibody that neutralized more than 90% of genetically diverse heterologous strains of HIV-1 at a concentration of 50 mcg/mL[17]. Since the initial isolation and characterization of the VRC01 mAb, subsequent investigations on longitudinal serum samples from HIV-1 infected individuals have shown that VRC01-like epitopes are induced during HIV-1 infection but only in a subset of HIV-infected individuals and may take years to develop [18].

The structure of VRC01 in complex with the HIV glycoprotein (gp)120 core glycoprotein has also been determined. It is highly affinity-matured, has a disulfide link between complementarity-determining regions (CDR) hypervariable loop 1 (H1) and hypervariable loop 3 (H3) and has a glycan in the variable (V) region of the light chain. However, none of these features appears to affect its binding affinity or neutralization activity [19]. VRC01 does not have an unusually long CDR-H3 region like some other HIV-1 neutralizing antibodies. It is not self- or poly-reactive, lacks anti-phospholipid antibody activity [13], and it does not bind to human adult or fetal tissue. These features suggest that mAb administration will not precipitate adverse immune phenomena. The VRC01 mAb has been safe in multiple NHP studies, and first-in-human studies (VRC 601 and 602) conducted in HIV-infected and -uninfected individuals, respectively, have demonstrated the product to be safe and well tolerated across a range of doses (1 to 40 mg/kg) and delivery methods (IV and SC). These studies have observed no drug-related serious adverse events, discontinuations due to drug-related adverse events, nor dose-related toxicities.

From *in vitro* testing, it has been observed that VRC01 has a half-maximal inhibitory concentration (IC_{50}) of <50 micrograms/milliliter (mcg/mL) against 91% of primary isolates of various HIV-1 clades and <1 mcg/mL against 72% of primary isolates [17]. Several proof-of

concept studies have been conducted to determine whether the *in vitro* neutralization capabilities of VRC01 translate into protecting NHPs from challenge with virulent chimeric simian-human immunodeficiency virus (SHIV), which contains the HIV envelope in an simian immunodeficiency virus (SIV) background. Rhesus macaques that received a single dose of IV VRC01 at 20mg/kg or 5mg/kg were fully or partially protected, respectively, against a single high-dose rectal challenge of SHIV-SF162P3 (a chemokine receptor 5 (CCR5) tropic strain of HIV). Rhesus macaques were protected against vaginal challenge if they had received 20mg/kg IV the mAb. VRC01 at the same dose also protected infant macaques from oral SHIV challenge.

Additional NHP work with VRC01 is exploring the impact of the mAb on viremia and the latent reservoir in acute SHIV infection. Rhesus macaques were challenged intravenously with SHIV SF162P3 and subsequently administered VRC01 (40 mg/kg IV, VRC01 group) or started on a daily antiretroviral drug regimen (ARV group) at day ten following challenge. A control group was left untreated. Both VRC01 and antiretroviral (ARV) groups demonstrated a substantial antiviral effect compared to the control group, with reduced peak viremia [11].

2.0 STUDY AGENT

2.1 BACKGROUND

The study agent, VRC-HIVMAB060-00-AB, was produced under current Good Manufacturing Practice (cGMP) by SAIC-Frederick, Inc., Frederick, Maryland, under contract to VRC. Specific manufacturing information is included on the product vial labels and Certificates of Analysis and can be found in the Investigator's Brochure (IB). Quality Assurance (QA) lot release testing by the manufacturer and ongoing stability programs verify conformance to product specifications prior to use in clinical trials.

2.2 DESCRIPTION OF STUDY AGENT VRC-HIVMAB060-00-AB (VRC01)

VRC-HIVMAB060-00-AB (VRC01) is a broadly neutralizing human mAb targeted against the HIV-1 CD4 binding site. It was developed by the VRC/NIAID/NIH. VRC01 is of the Immunoglobulin G1 (IgG1) subtype and is highly somatically mutated from its germ-line precursor. The heavy chain CDR3 region is 14 amino acids long, which is an average length relative to natural antibodies, and the glycosylation pattern is derived from its production in a Chinese Hamster Ovary (CHO) mammalian cell line.

The study agent was produced using recombinant DNA technology. The mammalian Glutamine Synthetase Gene Expression System in the CHO cell line developed by Lonza Biologics (Slough, UK) was used to produce the VRC01 mAb. The methods by which VRC01 was isolated and produced have been described previously in detail [20]. Briefly, PBMC from HIV-1 infected adults stained with specific antigenic probes and passed through a flourescence activated cell sorter (FACS) through which single B lymphoctyes were isolated. Heavy and light chain variable regions of single B cell receptors were amplified by polymerase chain reaction (PCR) and sequenced. Heavy and light chains were intially expressed in an HEK 293 suspension cell culture.

The bulk lot of the mAb was manufactured under cGMP using a stably transfected CHO cell line. The product was then purified and labeled at the VRC Vaccine Pilot Plant (Frederick, Maryland), operated by Leidos Biomedical Research, Inc., Frederick, Maryland. There are two fill volumes available-- a 3 ml glass product vial contains 2.25 mL ± 0.10 mL volume and a 10 ml glass product viral containing 6.25 ± 0.10 mL fill volume at a concentration of 100 ± 10 mg/mL VRC01 in an isotonic, sterile solution. Vials contain a clear, colorless to yellow liquid, essentially free of visible particles; some opaque or translucent particles may be present. The formulation buffer is composed of 25 mM Sodium Citrate, 50 mM Sodium Chloride, and 150 mM L-Arginine Hydrochloride at pH 5.8.

More details on the VRC-HIVMAB060-00-AB composition and manufacturing can be found in the IB.

2.3 PRECLINICAL GLP TOXICOLOGY STUDY

A single-dose pharmacokinetics (PK) study and repeat-dose toxicity study of IV and

subcutaneous (SC) administration of VRC-HIVMAB060-00-AB (VRC01) was performed by SRI International (Menlo Park, CA) in male and female Sprague-Dawley rats according to U.S. Food and Drug Administration (FDA) "Good Laboratory Practice (GLP) for Nonclinical Laboratory Studies." This study was conducted with a pre-GMP pilot lot of VRC01 manufactured at a smaller scale using a purification process similar to that of the GMP clinical-grade drug product.

For the safety assessment, vehicle control, 4 mg/kg VRC01, 40 mg/kg VRC01, and 400 mg/kg of VRC01 were administered by tail vein injection on Days 1 and 8 to Groups 1 through 4, respectively. The vehicle consisted of the VRC01 formulation buffer containing 25mM sodium citrate, 50mM sodium chloride and 150mM L-arginine-HCl at pH 5.8. An additional group (Group 5) received 40 mg/kg VRC01 via SC administration to the dorsal scapular region on Days 1 and 8. Each group contained 10 male and 10 female rats. Five of each sex were sacrificed on Day 9, one day after the second administration; the remaining animals were sacrificed on Day 30, 22 days after the second administration.

Results obtained showed that both routes of administration were well tolerated in the rats. All animals survived until their scheduled necropsy. No findings or changes were seen in general clinical condition, body weight, food consumption, body temperature, injection site irritation, organ weight or in hematologic or coagulation factor measurements that could be attributed to VRC01. However, VRC01 administration resulted in small, transient, dose-dependent increases in aspartate aminotransferase (AST) and alkaline phosphatase (ALP) on Day 9. By Day 30, AST values had returned to normal, and ALP values were returning to normal.

Other than red discoloration of the administration site in one male in the SC group on Day 9, there were no other gross necropsy observations attributable to VRC01 administration. There were no histopathology findings that were considered related to IV administration of VRC01. However, histopathology evaluation revealed subacute inflammation at the SC injection site on Day 9, one day after injection, in all 10 SC administered rats; dermal inflammation was usually minimal or mild while SC inflammation was usually mild, moderate, or marked. By Day 30, this inflammation had completely resolved, and the SC dose site was normal in all rats.

A "no observed effect level" (NOEL) was not determined in this study because transient elevations of AST and ALP were observed on Day 9 after IV administration and transient inflammation at the dose site was observed on Day 9 after SC administration. Because the elevated AST and ALP levels were transient and minor and did not correlate with histopathology findings, the "no observed adverse effect level" (NOAEL) for VRC01 by the IV route of administration in rats was 400 mg/kg, the highest dose used in this study. The systemic NOAEL for the SC route of administration of VRC01 in rats was 40 mg/kg, the only SC dose level examined in this study.

For the PK analysis, a separate cohort of rats received VRC01 on Day 1 at 4 mg/kg and 40 mg/kg by the IV route of administration and at 40 mg/kg by the SC route of administration. VRC01 levels in serum were determined by enzyme-linked immunosorbent assay (ELISA) on pre-dose samples collected from each animal. Untreated control samples were collected from and tested on an additional 3 males and 3 females. Blood was collected from 3 rats/sex/PK group for a total of 4–5 collections per PK animal at each of the following post-dose time points: 1, 4, 8,

24, 48, and 72 hours and 7, 14, 21, and 29 days.

VRC01 administration by the IV route resulted in dose-proportional exposure. The terminal elimination phase half-life ($t_{1/2}$) was about 10 days, with clearance (Cl) of approximately 20 ml/day/kg and volume of distribution (Vd) that was about 0.28 l/kg, indicating that the drug was distributed primarily in the serum and eliminated slowly.

2.4 TISSUE CROSS-REACTIVITY GLP STUDY OF VRC01 WITH HUMAN TISSUES *IN VITRO*

A tissue cross-reactivity study of VRC-HIVMAB060-00-AB (VRC01) using normal adult and neonatal human tissues *in vitro* (Testing Facility Study No. A255-12) was performed by Charles River Laboratories (Reno, NV) in accordance with U.S. FDA "Good Laboratory Practice (GLP) for Nonclinical Laboratory Studies." The tissue panels used as the test system for this *in vitro* cross-reactivity study included all of the tissues on the "Suggested list of human tissues to be used for immunohistochemical or cytochemical investigations of cross reactivity of monoclonal antibodies" in Annex I of the "European Medicines Agency Guideline on Development, Production, Characterisation and Specifications for Monoclonal Antibodies and Related Product, Adopted by the Committee for Medicinal Products for Human Use (CHMP) on December 18, 2008" and all of the tissues recommended in the FDA/Center for Biologics Evaluation and Research (CBER) "Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use (February 28, 1997)."

To determine the cross-reactivity of VRC01 binding, VRC01 was applied to cryosections from a full panel of tissues from normal human adults and a limited panel of human neonatal tissues, immunohistochemically detected using a biotinylated rabbit anti-human immunoglobulin G (IgG) secondary antibody and binding visualized with a streptavidin-horseradish peroxidase complex and a diaminobenzidine chromogen substrate. VRC01 binding was evaluated at concentrations of 5 and 50 mcg/mL.

Specific VRC01 staining was not observed in any normal adult human or neonatal human tissues evaluated. Therefore, *in vitro* evaluation of cross-reactivity in tissue specimens did not identify potential tissue sites or organ systems to more thoroughly evaluate in subsequent preclinical studies, thus supporting the use of VRC01 in humans.

2.5 NONHUMAN PRIMATE (NHP) STUDIES OF VRC01

Several non-GLP studies of VRC01 have been completed in NHPs to assess for preclinical evidence of potential efficacy for prevention of HIV infection. Table 1 is a brief summary of the studies performed [11].

Table 1. Preclinical proof-of-concept studies performed with VRC01 in NHP

Study Purpose	Study Outcome
Demonstration of plasma and secretion concentrations of VRC01 given by IV or SC routes in female rhesus macaques	Kinetics of decay of 40 mg/kg of VRC01 given IV or SC in plasma, rectal, vaginal and nasal secretions established

Demonstration of challenge-protection against intrarectal high-dose SHIV SF162P3 in male rhesus macaques	100% protection from challenge demonstrated at 20 mg/kg dose administered IV
Demonstration of challenge-protection against intravaginal high-dose SHIV SF162P3 in female rhesus macaques	100% protection from challenge demonstrated at 20 mg/kg dose administered IV
Demonstration of challenge-protection against intrarectal high-dose SHIV-BaL in male rhesus macaques	100% protection from challenge demonstrated at 20, 5, and 1.25 mg/kg dose administered IV
Demonstration of effect of VRC01 during the acute and chronic phases of SHIV SF162P3 infection in rhesus macaques	The administration of VRC01 (40 mg/kg dose administered IV) during the acute phase of infection led to a reduction of peak viremia during the chronic phase of infection

2.6 PHASE 1 AND PHASE 2 CLINICAL TRIALS OF VRC01

Evaluation of VRC01 as an investigational drug began in humans with the initiation of the VRC 601 study in September 2013. Initial development of VRC01 was based on an intended indication for the prevention of HIV-1 infection in infants at risk for HIV-1 infection through maternal transmission at birth or during breastfeeding. The safety, tolerability, PK and antiviral effect of VRC01 are assessed in HIV-1 infected adults, healthy adults and infants in the United States (U.S.) and internationally. Additional studies in HIV-unifected adults and HIV-infected adults are on going to evaluate potential HIV prevention and therapeutic indications, respectively.

Cumulatively, across all studies as of January 2017, VRC01 has been administered either IV or SC to over 840 HIV-uninfected and HIV-infected adults and 33 HIV-uninfected infants. There have been no SAE related to VRC01 requiring expedited safety reporting to the FDA or other regulatory authorities. There were no study safety pauses for adverse events. Although experience in HIV-infected, viremic adults is limited (n=8), there is evidence of a statistically significant antiviral effect of VRC01.

Protocols VRC 601, VRC 602, HVTN 104, P1112, HVTN 704/HVTN 085, HVTN 703/HVTN 081, HVTN 116, and VRC 606 - Prevention Indication: VRC 601 [12] and VRC 602 [21] were the first Phase 1 studies of VRC-HIVMAB060-00-AB (VRC01) administered to HIV-infected and HIV-uninfected adults, respectively. Both the IV or SC routes of administration were evaluated on schedules with 1 or 2 administrations.

HVTN 104 is a Phase 1 clinical trial of VRC01 administered IV or SC on schedules with multiple administrations to HIV-uninfected adults.

P 1112 is a Phase 1 study of VRC01 administered SC one time or up to 19 times (maximum until 72 month of age) to HIV-1-exposed infants.

HVTN 704/HPTN 085 is a Phase 2b study to evaluate the safety and efficacy of VRC01 in reducing acquisition of HIV-1 infection among men and transgender persons who have sex with men.

HVTN 703/HPTN 081 is a Phase 2b study to evaluate the safety and efficacy of VRC01 in reducing acquisition of HIV-1 infection in women in sub-Saharan Africa.

HVTN 116 is a Phase 1 study to evaluate the safety, pharmacokinetics, and anti-viral activity of VRC-HIVMAB060-00-AB (VRC01) and VRC-HIVMAB080-00-AB (VRC01LS) in the serum and mucosa of healthy, HIV-uninfected adult participants.

VRC 606 is a Phase 1, dose-escalation study to evaluate the safety and pharmacokinetics of VRC-HIVMAB080-00-AB (VRC01LS), and VRC-HIVMAB060-00-AB (VRC01), administered IV or SC to healthy adults.

The doses, routes evaluated and protocol statuses for these studies are summarized in the Investigator's Brochure. Overall, as of January 10, 2017, VRC01 infusions in the dose range from 1 to 40 mg/kg IV and 5 to 40 mg/kg SC have been assessed as well-tolerated in adults and infants and safe for further evaluation. Cumulatively, across all studies, there have been no SAE related to VRC01 that required expedited reporting to the FDA or other regulatory authorities. A non-serious AE of urticaria was submitted to regulatory authorities as a safety report because urticaria, at the grade 3 severity, was not reported in the IB, Version 6.0, dated October 5, 2016. The HVTN 104 study has expanded experience with repetitive administration of VRC01 beyond the two doses per subject that was the maximum administered in the VRC 601 and VRC 602 protocols. HVTN 704/HPTN 085 and HVTN 703/HPTN 081 studies are further expanding on experience with infusion of VRC01 or placebo every 2 months for up to 72 weeks, with approximately 2,500 infusions administered and no related SAEs reported as of January 2, 2017.

Protocols RV 397, RV 398, A 5340, A 5342, 15-I-0140, and IMPAACT 2008 - THERAPEUTIC INDICATION: Protocols RV 397, RV 398 (this study) A 5340, A 5342 and 15-I-0140 evaluate the safety and virologic effect of VRC01 in HIV-infected adults. Protocol IMPAACT 2008 evaluate the safety and antiviral activity of VRC01 administered to HIV-1-infected infants. Each protocol was designed to address different aspects of the effect of VRC01, including virologic effect when administered during acute HIV infection, effect on the HIV reservoir and effect when administered during an analytical treatment interruption (ATI).

RV 397 is a Phase 1 placebo controlled study evaluating the safety and therapeutic efficacy of VRC01 during ATI in patients who initiated ARV during early HIV infection.

A 5340 is a Phase 1, open-label study of the safety, pharmacokinetics, and antiviral activity of VRC01 administered IV to HIV-infected adults undergoing a brief ATI.

A 5342 is a Phase 1 study evaluating the safety, tolerability**,** and effect of VRC01 on markers of HIV persistence in ART-treated, HIV-infected adults.

15-I-0140 is a Phase 1 study evaluating the safety, tolerability**,** and effect of VRC01 on markers of HIV persistence in ART-treated, HIV-infected adults undergoing ATI.

IMPAACT 2008 is a Phase 1/2, randomized, controlled study to evaluate the safety and antiviral activity of VRC01 administered in addition to combination antiretroviral therapy (cART) to HIV-1-infected infants to promote clearance of HIV-1-infected cells.

The dose, routes evaluated and protocol statuses these and other ongoing studies with therapeutic indications are shown in the Investigator's Brochure.

Overally, as of January 10, 2017, VRC01 infusions at 40 mg/kg IV have been safe and well-tolerated in these studies in about 80 HIV-infected adults. There have been no SAE related to VRC01 that required expedited reporting to the FDA or other regulatory authorities. RV 397 reported an infusion reaction consisting of Grade 3 urticaria and grade 1 pruritis and nausea that required early termination of study infusion and discontinuation from the study intervention. Emergence of VRC01-resistant HIV has been detected during plasma viral rebound in subjects undergoing analytic treatment interruption, however resumption of ART routinely results in re-suppression of the viral load. Additional detail on the safety profile of VRC01 administration is contained in the Investigator's Brochure.

2.6.1 INITIAL PHARMACOKINETIC DATA

The pharmacokinetic (PK) parameters of passively administered VRC01 have been evaluated in HIV-infected and healthy uninfected adults in the VRC 601 and VRC 602 studies, respectively. The Investigator's Brochure shows PK parameters by population, dose and administration route.

In these initial studies of VRC01 no more than two doses were administered and, by study design, many subjects received only one dose. At 28 days after second administration at 20 mg/kg and 40 mg/kg IV, mean VRC01 serum levels were 55.9±16.8 and 88.9±40.4 mcg/mL in uninfected adults, and were 46.0±26.7 and 64.9±56.7 mcg/mL in HIV-infected adults, respectively. The time to maximum concentration (T_{max}) is about 1-3 hours after IV administration and about 1-3 days after SC administration.

The PK of VRC01 by the IV and SC routes of administration after repeat dosing in adults is under investigation in ongoing clinical trials. Evaluation of the SC route of administration in adults has been limited to the 5 mg/kg dose due to limitation on the volume that can be administered to adults. This is expected to be the primary route of administration to infants, but there are not yet any PK data from pediatric studies.

2.6.2 VIRAL LOAD EFFECTS

Analysis of the VRC 601 viral load data obtained from 8 viremic adults shows that VRC01 has a statistically significant effect on HIV viral load when administered as a single 40 mg/kg IV dose. None of these adults had been taking antiretroviral therapy (ART) when enrolled into the study and had not started ART during the time period when the viral load data were collected. Six of the eight adult subjects had ≥1 log10 copies/mL decrease in viral load and two subjects had a viral load drop of 0.26 and 0.18 log10 copies/mL respectively [12].

These data indicate the following for a single dose of VRC01 at 40 mg/kg IV:

- A statistically significant change from baseline viral load post-infusion days 5 to 16;
- The median time to reach ≥0.5 log decrease in viral load is 5 days; and,

- The median time to greatest decrease in viral load is 7 days.

A 0.5 log10 copies/mL or greater decrease in viral load is considered to be a positive response to ART [22] - the data from 8 viremic adults who received VRC01 indicate an in vivo virological effect of VRC01 on HIV viral load. VRC 601, subjects were administered only one dose of VRC01 at 40 mg/kg, thus, would not expect a sustained effect on viral load,importantly, there was no evidence of an adverse response or an exaggerated viral rebound in subjects escaping VRC01-mediated VL supression.

3.0 STUDY OBJECTIVES

3.1 PRIMARY OBJECTIVES

1. Safety of VRC01 in acutely HIV-infected viremic individuals
2. Impact of VRC01 on plasma viremia in each mAb arm compared to the ART plus placebo control at day 7 (+/- 1 day)

3.2 SECONDARY OBJECTIVES

1. Impact of VRC01 on viral dynamics in plasma over the course of 24 weeks.
2. Impact of VRC01 on HIV reservoir measures in peripheral blood mononuclear cells and plasma
3. Impact of VRC01 on clinical outcomes/characteristics of AHI
4. Impact of VRC01 on CD4 + T cell count
5. Pharmacokinetics of VRC01 in AHI in peripheral blood

3.3 EXPLORATORY OBJECTIVES

1. Impact of VRC01 on reservoir measures in CSF, mucosal, and tissue compartments
2. Comparison of peripheral blood reservoir measures to those in the CSF, mucosal and tissue compartments
3. Impact of VRC01 on reservoir measures in T cell subsets
4. Pharmacokinetics of VRC01 in CSF and mucosal compartments
5. Anti-VRC01 antibody detection
6. Impact of VRC01 on immune responses and activation
7. Effect of VRC01 on viral sequence, phenotype, and evolution
8. Predictors of favorable viral dynamics, immune responses, and reservoir seeding
9. Impact of VRC01 on neuropsychological testing outcomes

NOTE: Exploratory objectives involving additional specimen collections will only be performed on specimens of those participants who consent to these optional procedures.

4.0 ENDPOINTS

4.1 PRIMARY

1. Safety: ≥ grade 3 mAb-related reactogenicity and mAb-related AEs
2. Plasma viral load change from day 0 to day 7 (+/- 1 day)

4.2 SECONDARY

1. Time to virologic suppression (<50 copies/ml) in plasma
2. Total viremic copy days (area under viral load curve) from day 0 to week 24
3. Plasma viremia including single copy HIV RNA quantification in samples with HIV RNA < 50 copies/ml at day 7, day 14 and week 24
4. Cell-associated HIV RNA and DNA in the peripheral compartment
5. Frequency, severity and duration of acute retroviral syndrome
6. Frequency of hospitalization and incidence of opportunistic infections and non-AIDS related conditions
7. CD4 + T cell decrease from baseline to nadir, increase from nadir to week 24, and overall change from baseline to week 24
8. VRC01 levels in peripheral blood

4.3 EXPLORATORY

1. HIV RNA quantification in the CSF and mucosal compartment (to include single copy measurements from samples with HIV RNA < 50 copies/ml)
2. Cell-associated HIV RNA and DNA in the CSF, mucosal and tissue compartments
3. Correlation of HIV RNA and DNA measurements across biologic compartments that include the peripheral blood, CSF, mucosal, and tissue compartments
4. HIV RNA and DNA in sorted memory CD4+ T cell subsets (for subjects with sufficient PBMC collections)
5. Viral outgrowth in total CD4+ T cells and sorted memory CD4+ T cell subsets (for subjects with sufficient PBMC collections)
6. VRC01 levels across biologic compartments that include the CSF and mucosal secretions
7. Presence of anti-mAb antibody
8. Biomarkers of immune activation (soluble and cellular markers, gene expression)

9. Neutralization sensitivity of virus isolates (including to VRC01)
10. Binding and neutralizing activities of elicited antibodies
11. Non-neutralizing antibody functional assays including antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cell-mediated phagocytosis (ADCP), antibody-dependent cell-mediated viral inhibition (ADCVI), and viral capture
12. Cellular immune responses: percentage of peptide specific T cell responses as measured by enzyme-linked immunospot (ELISPOT); breadth and magnitude of epitope-specific T cell responses; polyfunctionality of cellular responses
13. Innate immune response characterization including assessment of natural killer cell activity
14. Viral sequence evolution and the development of VRC01 escape mutations
15. Host genetic determinants of virologic suppression
16. Neuropsychological battery performance at week 24 compared to cohort data from pre-infection and baseline AHI timepoints, where available.

5.0 STUDY DESIGN AND POPULATION

This is a placebo-controlled study of the safety and impact of broadly neutralizing monoclonal antibody therapy with VRC01 on viremia in acute HIV infection, alone or in combination with antiretroviral therapy (ART). Twenty-four subjects will be enrolled during early acute HIV infection, as defined by either 1) two positive nucleic acid amplification tests (NAATs) for HIV-1 RNA within 21 days of a prior negative NAAT, or 2) a positive NAAT or a positive 4th generation EIA in the context of a negative 2nd or negative 3rd generation HIV EIA. They will be randomized to three groups:

Group	Number of Subjects	Day 0	Day 7
1	**8**	**ART initiation and single placebo infusion**	**(continued ART)**
2	**8**	**ART initiation and single infusion 40mg/kg VRC01**	**(continued ART)**
3	**8**	**Single infusion 40mg/kg VRC01**	**ART initiation**

For groups 1 and 2, participants and the study team will be blinded to active mAb vs. placebo infusion. Product safety and day 7 viral load in each mAb-containing arm (Groups 2 and 3) will be compared to the control arm (Group 1). Secondarily, viral dynamics across the duration of the study, sequential evaluations of the reservoir, evolution of CD4+ T cell count, and clinical characteristics will be compared along with exploratory evaluations of the impact of the mAb on the immune response to HIV infection, viral sequence, and on the reservoir in T cell subsets and optional specimen collections.

Study participants from acute infection cohorts will be co-enrolled in the cohort studies and RV 398, although study visits for the acute infection cohorts will be suspended during the RV 398 study participation. Once the study participant has completed their participation in RV 398, they will resume cohort study activities.

5.1 SOURCE COHORTS

5.1.1 RV 217 ECHO

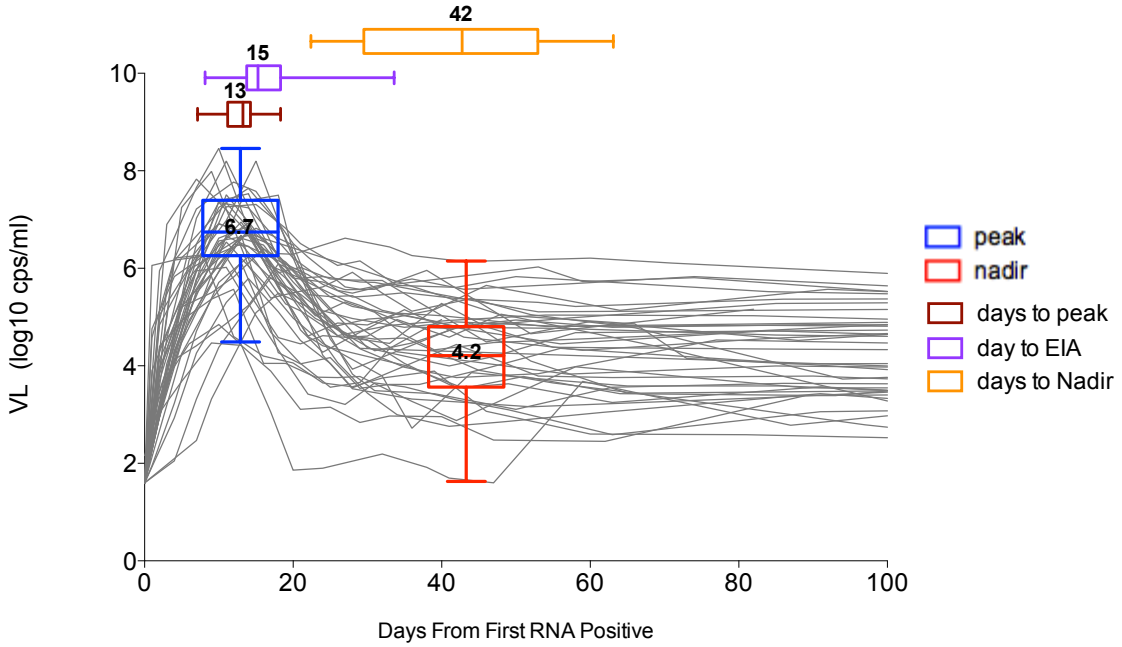
RV 217, entitled HIV Prevalence, Incidence, Cohort Retention, Host Genetics and Viral Diversity in Cohorts in East Africa and Thailand - Short Title: Early Capture HIV Cohort (ECHO), is an ongoing acute infection cohort study enrolling in Kenya, Uganda, Tanzania, and Thailand. The purpose of the study is to characterize recruitment, retention, human immunodeficiency virus (HIV) prevalence, HIV incidence, and biological characteristics of acute HIV infection in high-risk volunteers in Africa and Southeast Asia. The study incorporates two

phases. The main study activity, or phase I, is the observational cohort or surveillance activity during which the study collects very small blood samples via finger stick. These frequent "small blood volume" (SBV) visits afford the opportunity to diagnose HIV infection by nucleic acid amplification testing (NAAT) prior to the advent of detectable antibody by the most sensitive techniques available. Volunteers are also screened for viral hepatitis and other sexually transmitted infections; hematology and chemistry parameters are also recorded. Participants with incident HIV infections observed during phase I are the source of potential volunteers for this mAb intervention study. They will be briefed about this study as well as other acute HIV intervention studies during this surveillance phase.

Based on accumulating RV 217 data, those testing with 2 positive NAATs within 21 days of last negative NAAT correspond to diagnosis in Fiebig Stages I, II, and III. The RV 217 study has defined early Priority 1 cases as those with a known last negative NAAT and having at least 2 positive NAATs within Fiebig stage I or II. Those Priority 1 volunteers have had a median interval of 4 days between last negative to first positive NAAT, and the median day of EIA positivity from the day of first NAAT is day 15 (Interquartile (IQR) 13.5-18).

Following acute HIV infection, RV 398 participants recruited from RV 217 may elect to continue frequent visits for small blood volume draws at the RV 217 study site for masking purposes to avoid stigma associated with disclosure of new HIV positive status.

Figure 1: RV 217 Priority 1 Viral Loads (n = 42)



5.1.2 RV 254/SEARCH 010

RV 254/SEARCH010, entitled "Establish and characterize an acute HIV infection cohort in a high risk population," is an ongoing acute infection cohort study enrolling adult volunteers at the

Thai Red Cross AIDS Research Centre (TRCARC) in Bangkok, Thailand. The primary objectives of the study are to describe clinical, immunological and virological characteristics of persons with acute HIV infection and to identify and follow volunteers who may be candidates for future HIV intervention or treatment protocols due to diagnosis of HIV during acute infection.

In this cohort, patients presenting for HIV testing at the Thai Red Cross are consented for their phlebotomy specimens to be tested for HIV by nucleic acid testing well as by serologic assays. Those with a positive NAAT or positive 4th generation HIV EIA with a negative 2nd or negative 3rd generation EIA are classified as having acute HIV infection (this corresponds to Fiebig stages I-III of HIV infection) and return for the study's Week 0 visit. They will be briefed about this study (RV 398) as well as other acute HIV intervention studies at this Week 0 visit.

5.2 STUDY DURATION

After the first 7 days of the study, all groups will be on ART and followed for 24 weeks for a total study duration of 25 weeks.

5.3 ELIGIBILITY

5.3.1 INCLUSION CRITERIA

1. Able and willing to complete the informed consent process.
2. Passes Test of Understanding
3. 18 to 50 years of age.
4. Experiencing early acute HIV-1 infection as defined by

blood samples on at least two separate days positive by nucleic acid testing within 21 days of a negative nucleic acid HIV-1 test.

OR

by a positive nucleic acid test or a positive 4th generation EIA in the context of a negative 2nd or negative 3rd generation HIV EIA test

5. No history of antiretroviral therapy for any indication in the last 30 days.
6. In general good health
7. Willing to have blood samples collected and stored.
8. Able to participate for 25 weeks for study visits
9. Willing to have photo or fingerprint taken for identification purposes

Female-specific Criteria:

10. Agrees not to become pregnant from the time of study enrollment until the last study visit. If a woman has no history of hysterectomy, tubal ligation or menopause, she must

agree to use an effective birth control method: abstinence; male or female condoms; diaphragm or cervical cap with spermicide; intrauterine device; contraceptive hormones delivered by pills, patch, injections, or vaginally; and hormonal implants under the skin; or a male partner who has previously undergone a vasectomy.

11. Negative β-HCG (human chorionic gonadotropin) pregnancy test (urine or serum) on day of enrollment for any woman unless she is post-menopause for 24 consecutive months or has undergone a surgical procedure that precludes pregnancy.

5.3.2 EXCLUSION CRITERIA

1. Weight < 46 kg or >115kg
2. Previous receipt of humanized or human monoclonal antibody whether licensed or investigational.
3. Ongoing AIDS-related opportunistic infection (including oral thrush or active tuberculosis).
4. Severe acute retroviral syndrome (as defined in Appendix I) or clinical condition (other than HIV infection) constituting an indication for immediate antiretroviral therapy per local country guidelines.
5. Active injection drug use within previous 12 months.
6. History of a severe allergic reaction with generalized urticaria, angioedema or anaphylaxis in the 2 years prior to enrollment.
7. History of chronic urticaria
8. Physical finding on examination considered indicative of significant disease such as murmur (other than functional), hepatosplenomegaly, focal neurological deficit.
9. Hypertension that is not well controlled by medication.
10. Positive Hepatitis B surface antigen at any time in the past
11. History of hepatitis C infection
12. Untreated syphilis infection
13. Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) > 2x the upper limit of normal (ULN).
14. Absolute neutrophil count (ANC) <740 cells/mm^3
15. Estimated GFR < 50 ml/min within the past 90 days
16. Breast-feeding.
17. Pregnancy
18. Receipt of licensed vaccine or other investigational study agent within 28 days prior to enrollment or past participation in an investigational HIV vaccine study with receipt of active product.

19. Current or planned participation in another interventional clinical trial during the study period.
20. Chronic or recurrent use of medications that modify host immune response, e.g., oral or parenteral steroids, cancer chemotherapy.
21. Any other chronic or clinically significant medical condition that in the opinion of investigator would jeopardize the safety or rights of the volunteer. Including, but not limited to: diabetes mellitus type I, chronic hepatitis, renal failure; OR clinically significant forms of: drug or alcohol abuse, mental illness, severe asthma, autoimmune disease, psychiatric disorders, heart disease, or cancer.
22. Study site employee

There are no inclusion/exclusion criteria based on CD4 count because the delay of ART by 7 days does not incur significant risk to the subject compared to immediate ART. Additionally, we have not observed sustained low CD4 counts in the early Fiebig Stages thus far in RV 217. Among 42 early acute cases in RV 217, low CD4 counts rebounded rapidly and the lowest observed CD4 count at mean day 17 post positive NAAT was 395 cells/ul.

6.0 STUDY PROCEDURES

6.1 ENROLLMENT AND RANDOMIZATION

6.1.1 ENTERING FROM RV 217

RV 217 ECHO volunteers, at the time of their first positive HIV test will be re-briefed about this study and other acute HIV infection intervention studies, including an early ART-only protocol, such that participation in this study will not be the only available avenue for accessing antiretroviral therapy in acute HIV. After determination of acute HIV infection according to RV 398 inclusion criteria, interested volunteers will be referred to Visit 1 of this study.

6.1.2 ENTERING FROM RV 254

RV 254 volunteers determined to have acute HIV infection return for the RV 254 Week 0 visit, during which they undergo medical history and physical; hematology, chemistry, HIV RNA and lymphocyte profiles are also obtained. Volunteers also undergo neuropsychological testing and questionnaire administration, are screened for viral hepatitis and other sexually transmitted infections, and have the option of consenting for procedures to further characterize their acute HIV infection (genital secretion collection, CSF collection, brain MRI, colon biopsy, and lymph node biopsy). They will be informed about RV 398 as well as other acute HIV intervention studies at this Week 0 visit. Those volunteers interested in this study will then be referred to RV 398 visit 1 procedures within 5 days. To avoid duplication of study procedures between the Week 0 visit for RV 254 and Visit 1 of RV 398, volunteers enrolling from RV 254 will have a reduced schedule of events for visit 1 of RV 398 (Appendix II).

6.1.3 RV 398 VISIT 1

At RV 398 Visit 1, volunteers will undergo the informed consent process with a test of understanding (TOU) and verification of eligibility, with same visit enrollment and randomization of eligible volunteers on day 0 of the study into one of three groups:

- Group 1: Immediate ART Only (n=8): start ART with single infusion of placebo in AHI on day 0
- Group 2: Immediate ART and mAb Therapy (n=8): start ART with single infusion of 40 mg/kg VRC01 in AHI on day 0
- Group 3: Immediate mAb Therapy and Subsequent ART (n=8): single infusion of 40 mg/kg VRC01 in AHI on day 0 followed by ART initiation on day 7

Participants are allowed to take the TOU three times but must have a passing score (90% or greater) by the third attempt to participate in the study.

The randomization list will be prepared by the study statistician according to a separate randomization plan generated by the statistician. The site pharmacist will be responsible for maintaining access to the list in a secure manner. The total number of treatment assignments may not be the same for each site, in order to accommodate differences in each site's eligible population, pace of enrollment and operational issues. Furthermore, randomizations by arm at each site will likely not be equal and the Protocol Chair may reallocate enrollment slots during the course of the study.

Volunteers will undergo IV infusion of either mAb or placebo during the enrollment visit at the clinic site. The infusion will typically last an hour, however the infusion rate may be reduced to mitigate infusion-related side effects. Following infusion, the volunteers will be observed for a minimum of one hour at the clinic site, longer if indicated by demonstrated signs and symptoms. The infusion will occur prior to the first dose of oral ART. Enrollment (Visit 1) procedures may encompass two days. Day 0 is the day of first product administration.

6.2 ANTIRETROVIRAL THERAPY

Antiretroviral therapy is described in each Site Specific Addendum and consists of country guideline-recommended first line combination therapy: either efavirenz 600 mg/emtricitabine 200 mg/tenofovir disoproxil fumarate 300 mg or efavirenz 600 mg/lamivudine 300 mg/tenofovir disoproxil fumarate 300 mg. The only regimen difference between the sites would be the use of lamivudine or emtricitabine, nucleoside reverse transcriptase inhibitors that are generally considered to have equivalent clinical and virological efficacy and safety profiles [23-24]. Volunteers with ART regimen intolerance or inadequate virologic response may require prescription of an alternate regimen recommended by local country guidelines, typically based on the boosted protease inhibitor lopinavir/ritonavir. Genotype resistance testing will be performed as clinically indicated, including failure to achieve virologic suppression to <1,000 copies/mL after 12 weeks or confirmed viral load over 1,000 copies/mL after initial suppression. Results will be used to guide subsequent regimen choice.

Following the study, antiretroviral therapy will be continued either at the study site or through local referral, with procedures detailed in the site-specific addenda. Volunteers may also be offered enrollment into an early ART study or other intervention studies upon completion of this protocol. For those volunteers electing local referral, site investigators have confirmed with local HIV care program authorities that continued treatment of volunteers beginning ART during this study will be supported. Upon local referral, volunteers will continue treatment with locally available ART regimens.

6.3 NEUROPSYCHOLOGICAL TESTING

The week 24 visit will include a set of thinking tests (neuropsychological (NP) testing), questions about mood, and questions about ability to function in day-to-day activities. During this testing patients will be asked to do easy tasks as fast as possible (such as put pegs in holes and draw lines between sequential circled numbers). Mood and anxiety measures are necessary for the accurate interpretation of the neuropsychological measures and will be captured as covariates, as will information about recent sleep patterns and use of intoxicants. We will also

capture a brief assessment of quality of life and stress as it may be impacted by treatment.

A trained psychometrist completes this cognitive testing using a modification of published international neuropsychological battery [25]. For this study we will use a brief NP battery consisting of Non-Dominant Hand Grooved Pegboard Test, Trails A Test, and Color Trails 1 and 2. To assess stress and depression the following tools will be used: the Hospital Anxiety and Depression Scale (HADS), the Patient Health Questionnaire-9 (PHQ-9), the World Health Organization-Quality of Life (WHO-QOL), and the Distress Thermometer.

Neuropsychological test results, mental health and quality of life measures for these participants will be compared to similar data collected in RV 217 and RV 254.

6.4 OPTIONAL PROCEDURES

Volunteers will have the option of participating in separately consented collections and procedures to characterize the impact of VRC01 and ART on the HIV-1 reservoir and pathogenesis. The availability of these procedures varies across sites, and is detailed in each site-specific addendum. If any of the procedures provides evidence of an abnormal finding, the participant will be referred for diagnosis and care. Specifics on referral are provided in the site-specific addendum.

6.4.1 MUCOSAL SECRETIONS

Collection of these samples will be offered at all sites at baseline and subsequent intervals per the SOE.

- **Female participants**

  Consenting women will be instructed on how to use a Softcup device to collect cervical and vaginal secretions. The cup will remain in place for 4 -12 hours. Pregnant women and those with a history of toxic shock syndrome will be excluded. Date of last menstrual period will be measured/recorded at each collection visit. Collection will not take place if the woman is menstruating or has symptoms of active inflammation or infection of the vagina or cervix. Blood samples will be drawn for hormone levels at each collection visit. Rectal sponge secretions will also be collected, however will be deferred if there are signs or symptoms of perianal inflammation. Volunteers may participate in cervicovaginal secretion collection if they choose to defer rectal sponge secretion collections.

- **Male participants**

  Consenting men will be asked to ejaculate into a sterile container for semen collection. Rectal sponge secretions will also be collected. Semen and rectal sponge collections will be deferred from men if there are signs or symptoms of urethral or perianal inflammation, respectively. Volunteers may participate in semen collection if they choose to defer rectal sponge secretion collections.

6.4.2 RECTOSIGMOID BIOPSY

Gut mucosal biopsy will be performed on consenting individuals at baseline, day 14, and week 24 visits. This will most commonly be a clinic-based rectal biopsy procedure. In Thailand, at the Pattaya site, volunteers may be referred to Bangkok for flexible sigmoidoscopy with biopsy. This will be conductd by a licensed physician trained in the procedure. The biopsy is anticipated to take 5 hours.

6.4.3 LYMPH NODE BIOPSY

Inguinal lymph node biopsy will be offered at the 24 week visit and performed by a qualified surgeon. The procedure will be performed under local anesthesia and last approximately 30-40 minutes. Subjects will then be asked to rest under observation for another 4 hours.

Subjects agreeing to inguinal lymph node biopsy may be asked to undergo photography of the biopsy wound in order to provide an objective reference for assessment of wound healing and for possible use as volunteers' educational material. Subjects are free to refuse photography of their wound at any point should they not be amenable for any reason.

6.4.4 LEUKAPHERESIS

At baseline, day 7, and the week 24 visit, volunteers at capable sites will have the option of undergoing apheresis to collect PBMC and plasma. Leukocytes and plasma will be obtained using automated apheresis techniques conducted by a qualified apheresis nurse/technician. During apheresis, whole blood is withdrawn through a catheter placed in an antecubital vein and channeled into a cell separator where cellular and plasma fractions are separated by centrifugation. The component to be harvested (*i.e.* leukocytes) will be directed into a collection bag while the erythrocytes, platelets, and other plasma components are returned to the donor. The return is accomplished through a second needle placed at another site, usually in the other antecubital vein. Approximately 50 mL of red blood cell volume will be lost as residual loss in the machine during the procedure. Those volunteers who undergo leukapheresis will have a reduced volume of blood (only one 8.5 mL tube) collected via peripheral blood phlebotomy for the PBMC and plasma storage and additional testing blood draw scheduled at the same visit. Leukapheresis will not be performed on participants with platelet counts <150,000 or with hemoglobin < 12.0 mg/dL.

6.4.5 LUMBAR PUNCTURE

Volunteers will undergo lumbar puncture at baseline, day 7 and at the week 24 visit via standard clinical procedure using a standard needle or a specialized atraumatic needle, at sites with capability, to capture up to 20cc of CSF. All individuals will undergo neurological examination by a physician prior to the procedure to ensure no focal neurologic findings that would preclude the procedure. Neurologic examination by the physician will also take place after the lumbar puncture. The full neurologic exam and lumbar puncture is anticipated to take 1.5 hours. The participant will be advised to lie prone in the clinic for one (1) hour after the procedure has finished.

6.5 CLINICAL AND LABORATORY EVALUATIONS

This study is exploratory and will evaluate safety and virologic impact (including on the reservoir) of a mAb therapeutic intervention during acute HIV infection. The primary safety and virologic endpoints will be assessed by medical history, physical exam and collection of peripheral blood at intervals throughout the study. Additional study visits may be required if, in the investigator's opinion, repeat laboratory or additional clinical assessment is indicated. After Day 0, deviations from the visit windows are discouraged and will be recorded as protocol deviations, but will be permitted at the discretion of the site Principal Investigator (or designee) in the interest of obtaining subject safety evaluations following exposure to the investigational product.

The study will also include optional collections of peripheral blood mononuclear cells (PBMC) and plasma via apheresis, of mucosal secretions, of cerebrospinal fluid (CSF) via lumbar puncture, and of tissue samples from other compartments (inguinal lymph node, rectosigmoid tissue), which will contribute to evaluation of additional objectives. Please refer to the Schedule of Evaluations (SOE) in Appendix II for more details.

6.5.1 ASSESSING PRIMARY ENDPOINTS: SAFETY AND VIROLOGIC IMPACT

- Safety and tolerabililty of the study product will be assessed along the following parameters:
  - History and physical exam
  - Adverse events during the course of the study
  - mAb reactogenicity
  - Serum chemistry: Creatinine, ALT, AST, ALP
  - Hematology safety parameters: hemoglobin, hematocrit, white blood cell (WBC) and differential count, red blood cell count, platelet count
  - Plasma HIV RNA and lymphocyte subsets (CD4+ T cell count)

- CD4+ T cell count, chemistry and hematology safety parameters will be performed at baseline (day 0); on days 3, 7, 14, and 28; and then every 4 weeks until study termination. HIV RNA will be measured on days 0, 1, 3, 7, 10, 14, 18, 21, 25 and 28, 42, 56 and then every 4 weeks until study termination. These viral load measurements also evaluate the second primary objective: impact of VRC01 on plasma viremia.

6.5.2 ASSESSING SECONDARY ENDPOINTS

- Secondary objectives, such as the impact of VRC01 on clinical outcomes/characteristics of AHI, viral dynamics, and CD4 + T cell preservation, will be assessed by periodic monitoring of HIV-1 plasma RNA and CD4 + T cell count as described above. Assessment of clinical outcomes such as the incidence of acute retroviral syndrome, hospitalization, opportunistic infections, and non-AIDS related conditions will be assessed according to pre-defined criteria.

- Viral dynamics will also be assesed via single copy HIV RNA assay in plasma samples with HIV RNA<50 copies/mL.

- The impact of the mAb on reservoir seeding in the peripheral compartment will be evaluated by assessments of the HIV reservoir at baseline, on day 7 and week 24. Additional timepoints may also be explored. These evaluations will be done on total PBMC and total CD4+ T cells. Reservoir measurements will include:
  - Cell associated HIV RNA
  - Total and integrated HIV DNA and 2 long terminal repeat (LTR) circles
  - Viral out-growth assay (a 100 mL collection is included at the week 24 visit for this assay)
  - TILDA (Tat/Rev Induced Limiting Dilution Assay) to measure frequency of CD4+ cells producing multi-spliced HIV RNA upon maximal stimulation
  - Digital droplet PCR targeting highly conserved regions of the integrated HIV-1 genome

- Blood samples for PK analysis will be collected pre-dose and at the end of infusion (EOI) of the mAb administration; and then days 3, 7, 14, 21, 28, and 56 after mAb administration.

- Plasma and PBMC will be collected for storage on enrollment (day 0), and days 3, 7, 14, 21, and 28, 56 and then every 4 weeks for the remainder of the study. These stored specimens will be used to evaluate the remainder of the study objectives.

6.5.3 ASSESSING EXPLORATORY ENDPOINTS

- The impact of the mAb on reservoir seeding will also be assessed in tissue and CSF, when available, among participants who separately consent to collection of those specimens. In participants with sufficient PBMC collection (as with leukapheresis), measurement of cell-associated HIV RNA and HIV DNA, as well as viral outgrowth, may be performed in memory CD4+ T cell subsets, as well as other cell subsets that could contribute to latency. Larger volume PBMC/plasma and 20mL CSF collections are scheduled for day 0, 7, and week 24 to support these analyses, although other timepoints may also be explored.

- The impact of VRC01 on immune responses and activation will be evaluated by humoral and cellular immunoassays as well as by measurement of biomarkers of immune activation, conducted at baseline and following mAb administration. These will be evaluated across biological compartments as specimen availability permits.

  - Biomarkers
    - Activation and functional markers (as measured by intracellular cytokine staining (ICS))
    - Frequency and phenotype of virus-harboring cells among collected PBMCs
    - Flow cytometric sorting of $CD4^+$ T-cells, combined with quantitative PCR, to assess viral gene expression, latent viral DNA content, and infected cell phenotype

- Soluble markers of immune activation
- Cellular gene expression profiles of total PBMC and cell-sorted compartments

▪ Humoral immunity

- Neutralization sensitivity of founder vs post-VRC01 virus
- Binding (host-generated) antibodies to relevant antigens, including HIV envelope proteins
- Neutralizing antibody activity using a representative panel of tier 1 and tier 2 HIV strains with emphasis on the subtype infecting the participants and, where possible, the infectious molecular clone and pseudovirus expressing the participant's founder virus and, if relevant, breakthrough virus.
- Non-neutralizing antibody functional assays including antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cell-mediated phagocytosis (ADCP), antibody-dependent cell-mediated viral inhibition (ADCVI), and viral capture

- Cellular immune responses
  - ELISPOT and polyfunctional ICS of collected PBMC
  - Epitope mapping of CD4+ and CD8+ T lymphocyte responses
  - Immunophenotyping and functional characterization of cellular subsets of interest, including natural killer (NK) cells
- Viral evolution
  - The effect of VRC01 on virus evolution will be assessed via viral sequencing, to include envelope, whole genome, and targeted deep sequencing, on baseline and post-mAb specimens, with a particular focus on poor responders as compared to those who have favorable viral load responses to VRC01.

- Exploratory, hypothesis-generating data analysis will identify potential predictors of favorable viral dynamics and reservoir seeding.
  - Host genetics: Host immune-genotyping will be performed as resources are made available. Existing host genetic data from the parent RV 217 or RV 254 cohort will also be employed.
- Optional Specimen Collection Assessments:

Mucosal secretion samples will be assayed for HIV RNA by nucleic acid amplification. Residual samples will be stored at –80°C and will be used to detect VRC01 levels, to isolate and sequence HIV, to assay cytokines and inflammatory markers, and to detect sexually transmitted infections. Other HIV-specific immune responses may also be evaluated. Gut biopsy specimens will be cryo-preserved on the day of collection for mucosal mononuclear cell isolation and phenotyping by flow cytometry and immunohistochemistry. Specimens will also be analyzed for HIV RNA/DNA, cytokine expression by ICS, ELISPOT, and transcriptomics. Inguinal lymph nodes will be divided and sent for immuno-fluorescence confocal microscopy, flow cytometry, HIV RNA/DNA measurement, gene expression/whole transcriptome analysis, cytokine production, and frequency of and phenotype of virus-harboring cells. Other HIV-specific immune responses

may also be evaluated. CSF will be pelleted to obtain samples of cellular product, which, along with supernatant, will be stored for markers of neuronal damage, other immunologic and virologic studies as well as for measurement of VRC01 levels.

Anti-VRC01 antibody will be quantified by bridging electrochemiluminescence technique or alternative sensitive assay. The anti-VRC01 B cell response will also be characterized.

NP data will be summarized using z-scores based on age-matched normative data when such data is available or as raw scores when not available. With either approach, individual performance can be compared longitudinally to determine the impact of the intervention on cognitive performance, the primary interest of the neuropsychological testing portion of this study. Results from the week 24 of this study will be compared to NP battery results from pre-infection and AHI baseline timpoints in the RV 217 and RV 254 studies.

6.6 CONCOMITANT MEDICATIONS

Participants will be asked about concomitant medication use during the 45 days prior to study enrollment and at every study visit. Study participants can receive medications such as acetaminophen, non-steroidal anti-inflammatory drugs (NSAIDs), or antihistamines as required, although they must be documented.

6.7 SOLICITED ADVERSE EVENTS

All participants receiving infusions (VRC01/placebo), temperature and solicited systemic symptoms will be recorded in the clinic prior to infusion and at a minimum of 30 minutes post procedure and then daily by the participant for 3 days.

All participants will be given a diary card on which to record temperature and systemic symptoms daily for 3 days. The diary card will act as a memory tool for the subject but is not considered a source document.

For this study, solicited adverse events occurring during the 3 days after receipt of study agent will include: unusually tired/feeling unwell, muscles aches, headache, chills, nausea and joint pain. Subjects will also record highest measured temperature daily. The diary cards are reviewed for accuracy and completeness at follow-up visits and are recorded without an attribution assessment (unless they are Grade 3 or higher in severity in order to assess whether the event is relevant to the study pause criteria). Clinicians will collect resolution information for any systemic symptoms that are not resolved after 3 days.

Clinician assessment of the local IV administration site will be conducted on day of study agent administration and during the scheduled follow-up timepoints on days 1, 3 and 7 after administration.

At every visit all participants will be asked about other adverse experiences, which will be recorded and entered the same day into the study database. The local PI will assess the relationship of the study products to the events.

6.8 STUDY DISCONTINUATION

6.8.1 EARLY DISCONTINUATION OR WITHDRAWAL OF STUDY PARTICIPANTS

A participant will be taken out of the study entirely under the following circumstances:

- Repeated failure to comply with protocol requirements
- Decision by the study sponsor (DAIDS) or the Protocol Chair to stop or cancel the study
- Decision by Walter Reed Army Institute of Research Institutional Review Board (IRB) and the United States Army Medical Research and Materiel Command's (USAMRMC) Office of Research Protections (ORP), Human Research Protection Office (HRPO), DAIDS, the U.S. FDA, the Office for Human Research Protection (OHRP), or local regulatory authorities and IRBs to stop or cancel the study
- Participant requests withdrawal

Each subject has the right to withdraw from the study at any time for any reason without affecting the right to treatment by the investigator. The investigator should make an attempt to contact subjects who did not return for scheduled visits or follow-up. Although the subject is not obliged to give reason(s) for withdrawing prematurely, the investigator should make a reasonable effort to ascertain the reason(s) while fully respecting the subject's rights.

For those subjects who are unable to continue participation in the study, but who do not withdraw consent, an exit visit will be conducted (refer to SOE for list of procedures to be conducted). Any subject who withdraws consent will not have any further data collected after consent has been withdrawn. However, data that was collected prior to a subject's withdrawal of consent will be included in data analysis.

The investigator also has the right to withdraw a subject, e.g., because of worsening health status, intercurrent illness, or AEs.

If a participant withdraws from the study, the next available participant will be placed in the vacated randomization slot. A study participant will be considered non-evaluable for the primary virologic endpoint if they miss the Visit 4 (day 7) visit window and will be replaced. Replacements for participants who withdraw from the study will only be permitted while enrollment is open.

6.8.2 PREMATURE TERMINATION OF THE STUDY

Walter Reed Army Institute of Research IRB and the USAMRMC ORP, HRPO, DAIDS, the U.S. FDA, and OHRP, and other applicable regulatory authorities reserve the right to terminate the study. The investigator will notify reviewing IRBs in writing of the study's completion or early termination.

6.8.3 MANAGEMENT OF VOLUNTEERS WHO BECOME PREGNANT

Pregnant women are excluded from enrollment, however if a volunteer becomes pregnant during the course of the study, any volunteer who has not yet initiated ART per protocol will be offered ART and referred for maternal and child health services. Volunteers will continued to be followed for the remainder of the study period for safety and the study team will follow the course of the pregnancy until the outcome is documented. During subsequent study visits, blood draws will be conducted for safety and HIV disease monitoring purposes only. No optional collections or procedures will be performed. Pregnant volunteers prescribed ART will also be advised to enroll in the antiretroviral pregnancy registry (http://www.apregistry.com/)

The site PI or designated associate investigator will be responsible for reporting any pregnancy to the PSRT. This information will be reviewed by the PSRT weekly in aggregate with other safety data. The PSRT will forward notification as necessary to IRBs, study sponsors, and regulatory agencies.

Pregnancy outcomes will be recorded via a standardized case report form. Information documented on this form will include date of last menstrual period, date pregnancy confirmed, history of complications during prior pregnancies (such as congenital abnormalities or spontaneous abortions), and the outcome of the pregnancy including date of termination or delivery, any complications of pregnancy, and the status of the child. A separate case report form will be completed for the delivered child to document date of delivery, gender, weight, presence of any congenital abnormalities, American Pediatric Gross Assessment Record (APGAR) score, HIV status, and any other complication of delivery.

6.8.4 UNBLINDING PROCEDURE

Once all study visits have completed and the study safety database lock is approved by the sponsor, the site investigators will contact blinded study participants by phone or through home visit to invite them to come back to the clinic to find out whether they received the study product or placebo. When the participant returns to the clinic, they will be given a letter, which indicates their study arm. Site investigators, in consultation with the PSRT, may elect to unblind a study participant if treatment group information would substantively inform the clinical management of an adverse event.

7.0 STUDY TREATMENT

Study treatment is defined as VRC-HIVMAB060-00-AB (VRC01) or placebo for VRC01. Standard first line combination ART will be provided to participants as part of the study but is not an investigational product (Section 6.2).

7.1 STUDY TREATMENT REGIMENS, ADMINISTRATION, AND DURATION

7.1.1 REGIMENS

Twenty-four men and women 18 to 50 years of age, in early acute HIV infection (AHI) will be randomized to one of three groups: Group 1: Participants will start ART and receive placebo for VRC01 (0.9% sodium chloride for injection, USP) administered intravenously at Day 0; Group 2: participants will start ART and receive 40 mg/kg VRC01 administered intravenously in 100 ml of 0.9% sodium chloride for injection, USP, at day 0; Group 3: Participants will receive 40 mg/kg VRC01 administered intravenously in 100 ml of 0.9% sodium chloride for injection, USP, at day 0 followed by ART initiation at day 7.

7.1.2 ADMINISTRATION

VRC01 or placebo for VRC01 will be administered as an intravenous infusion over 30 to 60 minutes using a volumetric pump. The infusion rate may vary based on the total volume needed to administer the full dose The total time needed to administer the dose may be longer based on factors such as participant tolerance.

An in-line filter infusion set must be used for IV administration.

The in-line filter must comply with the following specifications:
- 1.2 micron PES (polyethersulfone) filter membrane
- DEHP-free
- Latex-free

When the in-line filter is added to the tubing, prime the administration set. Flush the administration set with about 30 mL or appropriate volume of normal saline at the end of product administration.

7.1.3 DURATION

Participants will be followed for a total of 25 weeks.

7.2 STUDY PRODUCT FORMULATION AND PREPARATION

7.2.1 FORMULATION

VRC01 (labeled as VRC01 HIV MAb Drug Product VRC-HIVMAB060-00-AB) is supplied at a

concentration of 100 ± 10 mg/mL in an isotonic, sterile solution. Two fill volumes are available: 10 mL glass vials with a 6.25 ± 0.10 mL fill volume and 3 mL glass vials with a 2.25 ± 0.10 mL fill volume.VRC01 is a sterile clear, colorless to yellow liquid, essentially free of visible particles; some opaque or translucent particles may be present. The formulation buffer is composed of 25 mM sodium citrate, 50 mM sodium chloride, and 150 mM L-arginine hydrochloride at pH 5.8. Vials are intended for single use only and thus do not contain a preservative.

VRC01 is a highly concentrated protein solution and may develop white, opaque to translucent particles after thawing. When particles are observed, they may disappear after a few hours at room temperature or storage at 2°C to 8°C.

Note: All dose calculations should be based on a concentration of VRC01 of 100 mg/mL and a volume of 2 mL per vial (200 mg per vial) or 6ml per vial (600 mg per vial) that may be withdrawn.

Placebo for VRC01 will be sodium chloride for injection 0.9%, USP.

7.2.2 PREPARATION OF STUDY PRODUCTS

VRC01 Thawing Instructions

Please refer to the RV398 pharmacy SSP for detailed instructions on VRC01 thawing instructions.

IV infusion preparation instructions

The pharmacist must receive a prescription that states the participant's weight obtained at the most recent visit where weight was measured. Participants' screening weight, entry weight, or last obtained weight may be used for estimating the dose to thaw vials. Calculate the total milligrams of VRC01 required based on the participants weight and the dose of 40 mg/kg and the total number of vials required based on a 6 mL withdrawal volume containing 600 mg of VRC01 or 2 ml withdrawal volume containing 200 mg of VRC01

1. Prior to preparation for administration, vials should be swirled for 30 seconds with sufficient force to resuspend any visible particles, yet avoiding foaming. DO NOT SHAKE THE VIALS. Keep the vials upright at all times until ready to withdraw the contents. Do not invert the vial during inspection.

2. Add the required volume for the calculated total milligrams needed to 0.9% Sodium Chloride Injection, USP 100 mL using aseptic technique.

3. The prepared IV bag should then be covered with an amber bag and labeled to maintain blinding. The IV bag will be labeled as "VRC01 or Placebo in Normal Saline, total volume in ml (eg: 128 ml)", participant weight used for calculating the dose, and a DO NOT INFUSE AFTER date and time. A bag cover will be placed over the bag and should not be removed by the clinic staff.

Placebo

Placebo for VRC01 will be 0.9% sodium chloride for injection, USP and should be added to a 100 mL bag of 0.9% sodium chloride for injection, USP, using aseptic technique.. The volume of placebo to be infused will be approximately 100 mL plus 0.4 mL/kg to be consistent with the volume infused in the active VRC01 arms. The IV bag should then be covered with an amber bag. The IV bag will be labeled as "VRC01 or Placebo in Normal Saline, total volume in ml (eg: 128 ml)", participant weight used for calculating the dose and a DO NOT INFUSE AFTER date and time. A bag cover will be placed over the bag and should not be removed by the clinic staff.

ASSIGNING THE DO NOT INFUSE AFTER DATE AND TIME

After product preparation in IV bags:

VRC01 IV bags or Placebo for VRC01 may be stored at 2°C to 8°C for up to 48 hours (equilibrate to room temperature for a minimum of 30 minutes prior to product administration).

VRC01 IV bags or Placebo for VRC01 may be stored at room temperature (maximum 27°C) for no longer than 4 hours, including infusion time.

Product may not be stored in direct sunlight.

Storage of Vials of VRC01

VRC01 vials should be stored in a qualified, continuously monitored, temperature-controlled freezer at -35°C to -15°C. Freezer temperature from -45°C and -10°C is acceptable.

Placebo for VRC01 (0.9% sodium chloride for injection, USP) should be stored as per manufacturer instructions .

7.3 PHARMACY: PRODUCT SUPPLY, DISTRIBUTION, AND ACCOUNTABILITY

7.3.1 STUDY PRODUCT ACQUISITION/DISTRIBUTION

VRC01 is manufactured by the VRC and will be available through the National Institute of Allergy and Infectious Diseases (NIAID) Clinical Research Products Management Center (CRPMC). The site pharmacist should obtain the VRC01 product(s) for this protocol by following the instructions in the manual Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks.

The placebo for VRC01 (0.9% sodium chloride for injection, USP) can be obtained locally.

Sodium chloride for injection 0.9%, USP, 100 ml bags required to prepare the dose for VRC01 and placebo for VRC01, can be obtained locally.

For the sites that are unable to obtain the Sodium chloride for injection 0.9%, USP,MHRP will supply the Sodium chloride for injection 0.9%, USP and will be distributed to the sites by CRPMC.

7.3.2 STUDY PRODUCT ACCOUNTABILITY

The site pharmacist is required to maintain complete records of all study products received from the NIAID CRPMC and subsequently dispensed. All unused study products at the study sites must be returned to the NIAID CRPMC or destroyed (as directed by the sponsor) after the study is completed or terminated. The procedures to be followed are in the Pharmacy Guidelines and Instructions Manual.

7.3.3 DISPOSITION

The empty vials and the unused portion of a vial will be discarded in a biohazard containment bag and incinerated or autoclaved. Any unopened vials that remain will be returned to the production facility or discarded at the discretion of DAIDS in accordance with policies that apply to investigational agents. Partially used vials will not be administered to other subjects or used for *in vitro* experimental studies. They will be disposed of in accordance with institutional or pharmacy policy.

Vials or IV infusion bags of VRC01 with particles should be quarantined and stored at 2-8ºC. Contact the protocol pharmacist for further instructions regarding management of VRC01 vials or infusion bags VRC01 with particles..

7.4 ANTIRETROVIRAL THERAPY

Antiretroviral therapy is described in each Site Specific Addendum and consists of country guideline-recommended, available first line once-daily oral combination therapy: currently either efavirenz 600 mg/emtricitabine 200 mg/tenofovir disoproxil fumarate 300 mg or efavirenz 600 mg/lamivudine 300 mg/tenofovir disoproxil fumarate 300 mg. This initial ART regimen may be adjusted or switched to an alternate regimen as clinically indicated for regimen intolerance or failure. ART will be prescribed by study clinicans, pharmacy-dispensed and labelled for each individual participant. It will be provided at the study site and stored at 15-30°C with continuous temperature monitoring. The site pharmacist is required to maintain complete records of all ART that is dispensed to study participants.

8.0 PHARMACOVIGILENCE, SAFETY, AND ADVERSE EXPERIENCE REPORTING

8.1 DEFINITIONS

An adverse event (AE) is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and does not necessarily have a causal relationship with this product. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product. (International Conference on Harmonization (ICH) E6) (Synonym: Adverse Experience).

A serious adverse event (SAE): An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: Death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse (21 CFR 312.32).

“Life-threatening” refers to an adverse event that at occurrence represents an immediate risk of death to the subject. An event that may cause death if it occurs in a more severe form is not considered life-threatening. Similarly, a hospital admission for an elective procedure is not considered an SAE.

8.2 ADVERSE EVENT GRADING AND RECORDING

Recording of all AEs will occur during the period from study agent administration through 175 days after study agent administration.

All recorded AE and lab data will be coded for severity using the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table), Version 2.0 dated November 2014 included as Attachment IV and found on the website https://rsc.niaid.nih.gov/clinical-research-sites/daids-adverse-event-grading-tables, and modification to the DAIDS AE Grading table as per Section 8.9.

For adverse events not identified in the grading table, the following guidelines will be applied:

Table 2. Additional category for grading the AE

Mild	Grade 1	Symptoms causing no or minimal interference with usual social & functional activities
Moderate	Grade 2	Symptoms causing greater than minimal interference with usual social & functional activities
Severe	Grade 3	Symptoms causing inability to perform usual social & functional activities
Potentially Life-Threatening	Grade 4	Symptoms causing inability to perform basic self-care functions OR medical or operative intervention indicated to prevent permanent impairment, persistent disability or death
Death	Grade 5	Symptoms resulting in fatal outcome

The site clinical research team will ascertain accurate recording of AEs during the study. AE case report forms (CRFs) will be completed by the research staff on a daily basis as the data become available from the clinic or laboratory.

The clinical investigators will monitor and analyze study data including all AE and lab data as they become available and will make determinations regarding the severity of the adverse experiences and their relation to study product. To insure that all AEs are captured in a timely manner, CRFs will be entered in real-time and also subjected to analysis to identify AEs that may invoke study pause rules.

Although post injection reactogenicity (PIR)/Solicited AEs are documented separately from unsolicited AEs, they are reported to DAIDS if they meet SAE or study pause rule definitions as noted below. Therefore the PI or designee must review both PIR and AE CRFs to insure prompt and complete identification of all events that require expedited reporting as SAEs, study pause rules or other serious and unexpected events.

AEs will be followed by the clinical research team until resolved or stable.

8.3 PROTOCOL SAFETY REVIEW TEAM (PSRT) AND PSRT REVIEWS

The PSRT will review all AEs (including reportable AEs) on a regular and expedited basis as needed. In addition, the PSRT will review aggregate safety data reports from all sites on a weekly basis. This team includes the following: Study Chair, Site PIs, a research monitor, and the DAIDS medical officer or their designees. Additional participants could include associate investigators, and senior clinical research nursing staff. A quorum is established with the Protocol Chair, 2 of 4 site PIs and the DAIDS medical officer (MO) or their designee.

8.4 SAFETY MONITORING COMMITTEE (SMC) REVIEWS

The SMC for this study will be comprised of an independent group of experts established with consultation of DAIDS to review safety data during the clinical trial. The Protocol Chair (or designee) will submit written recommendations and cumulative safety data to the SMC Executive Secretary after the first enrollee has completed 90 days of the study. Subsequently, the SMC will convene every six months to review the completeness of the study data collected, the adherence to the protocol, and the Protocol Chair's review summaries. The SMC will request review of unblinded data if needed to evaluate a safety signal or serious adverse event. The SMC will also meet as needed to deliberate upon the initial interim safety data, the disposition of study pauses, and/or to provide other recommendations regarding the safe conduct of the study as requested by the Protocol Chair and/or DAIDS. The SMC Executive Secretary will provide the Protocol Chair and DAIDS Medical Officer with SMC recommendations, and the Protocol Chair and site PIs will inform IRBs and regulatory authorities as appropriate.

8.5 CRITERIA FOR STUDY PAUSE OR TERMINATION

If the trial is placed on safety pause, all enrollment and infusions will be suspended until further notice. Study pauses should not delay the initiation of ART according to study schedule.

Table 3 summarizes the AEs, which when experienced by at least one participant will lead to a safety pause or prompt PSRT AE review. For any AE where the outcome is death, the severity of the AE is classified as Grade 5. Related AEs refer to AEs deemed to be definitely, probably, or possibly related to the study; Not related AEs refer to AEs deemed to be unlikely related or not related to the study.

Grade 5 or 4 events that are judged related to study product administration should be reported immediately (within hours of the site learning of the event). These events require immediate pause.

Grade 3 events that are judged related to study product administration should be reported promptly i.e., within 24 hours. These events require prompt review by the PSRT.

All events that are related to study product administration and are similar grade 2 AEs in two or more participants will be routinely reviewed by the PSRT but will not necessarily prompt a pause. The PSRT may take that action in consultation with DAIDS after review.

When the administration of study products has been paused, mAb administration and enrollments would resume only if review of the triggering AEs by the PSRT, DAIDS, (and where applicable, the SMC), result in a recommendation to permit further study product administration and study enrollments.

Table 3. ADVERSE EVENT NOTIFICATION AND INFUSION PAUSING GUIDELINES

Toxicity	Post-Injection Reactogenicity (PIR) and Study Treatment Related Adverse Events (AEs)	Action [1]
Grade 5/Grade 4 Related[2]	"Verified" [3] abnormal laboratory values, systemic reactogenicity, or other study mAb infusion related AEs (if fever, must persist for ≥ 48 hours)	Automatic pause Immediate reporting[2] (Concurrent with observation or report)
Grade 3 Related	"Verified"[3] abnormal laboratory values, fever for ≥ 48 hours, vomiting or other clinical study mAb infusion related AEs (Except subjective symptoms)	PSRT considers pause Prompt reporting (within 24 hours of observation or report)

[1]Follow-Up and Resolution: All promptly or immediately reportable AEs are followed until resolution or condition is medically stable.

[2]Related AEs refer to AEs deemed to be definitely, probably, or possibly related to the study; Not related AEs refer to AEs deemed to be unlikely related or not related to the study

[3]If no evidence of disease is present other than the abnormal laboratory value, the test must be repeated at least one time in order to be considered "verified". The verification period will be a maximum of 48 hours after initial awareness of the abnormal laboratory value. When signs and symptoms are present, repeat test WILL NOT be needed.

For events in the table above, the Site PI notifies the PSRT, which includes the Study Chair and DAIDS MO, within a few hours after the site is aware of the AE. The PSRT will convene within one business day to review these adverse events and determine disposition (including whether the SMC needs to review the event).

If a decision to resume study enrollment and study treatment administration is made, the PSRT and/or SMC will record its judgment in a memorandum to the study file and notify DAIDS. MHRP COO will then forward the memorandum to the principal investigators. The clinical sites will be allowed to resume activities upon receipt of written notification from DAIDS. As needed, the appropriate regulatory authorities will be informed in writing of the decision to resume or discontinue study activities. Sites are responsible for notifying their respective IRBs according to local standards and regulations. The sponsor (DAIDS) is responsible for notifying the FDA.

8.6 EXPEDITED REPORTING TO IND SPONSOR (DAIDS)

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

The SAE Reporting Category, as defined in Version 2.0 of the DAIDS EAE Manual, will be used for this study. All SAEs will be reported by the site PI or designee to the DAIDS RSC Safety Office and MHRP COO (email: reportable_events@hivresearch.org).

The DAIDS Adverse Experience Reporting System (DAERS), an internet-based reporting system, must be used for expedited AE reporting to DAIDS. In the event of system outages or technical difficulties, expedited AEs may be submitted via the DAIDS EAE Form. This form is available on the RSC website: https://rsc.niaid.nih.gov/clinical-research-sites/paper-eae-reporting.

For questions about DAERS, please contact DAIDS-ES at CRMSSupport@niaid.nih.gov. Site queries may also be sent from within the DAERS application itself. For questions about EAE reporting, please contact the RSC (DAIDSRSCSafetyOffice@tech-res.com).

At the time of SAE submission, any supplemental forms as required by local and U.S. Department of Defense (DoD) IRBs must also be submitted to DAIDS RSC and MHRP COO. Outcomes of the SAE will need to be provided as updates through DAERS or on the EAE form until the adverse event is resolved, is stable, or chronicity has been established and documented.

For questions or other communication with RSC on SAEs, please note the following:

Website:	https://rsc.niaid.nih.gov/
Office Phone*:	1-800-537-9979 (U.S. only) or +1-301-897-1709
Office Fax*:	1-800-275-7619 (U.S. only) or +1-301-897-1710
Office Email:	DAIDSRSCSafetyOffice@tech-res.com
Office Hours:	Monday through Friday, 8:30 AM to 5:00 PM (U.S. Eastern Time)
Mailing Address:	DAIDS Safety Office 6500 Rock Spring Drive Suite 650 Bethesda, Maryland 20817
*Office phone and fax are accessible 24 hours per day	

8.6.1 STUDY AGENTS FOR EXPEDITED REPORTING TO DAIDS

The study agents that must be considered in determining relationships of AEs requiring expedited reporting to DAIDS is the VRC01 MAb (VRC-HIVMAB060-00-AB) and placebo for VRC01 (0.9% sodium chloride for injection, USP).

8.6.2 STUDY RECORDING PERIOD FOR SAES

The protocol-defined expedited event reporting period for this protocol is 175 days after study agent administration until study completion or discontinuation of the subject from study participation for any reason. After the end of the protocol-defined EAE Reporting Period stated above, sites must report serious, unexpected, suspected adverse drug reactions (SUSARs) if the study site staff becomes aware of the event on a passive basis (i.e., from publicly available information).

8.6.3 TIMEFRAME FOR EXPEDITED REPORTING OF INDIVIDUAL ADVERSE EVENTS

The timeframe for expedited reporting of an individual AE begins when the site recognizes that an event fulfills reportable AE criteria. For SAEs sites must submit adverse events requiring expedited reporting to the DAIDS RSC Safety Office as soon as possible, but no later than 3 reporting days (as defined in the DAIDS EAE Manual v2.0), after the site's recognition that the event fulfills the criteria for SAE reporting. The RSC will submit written IND Safety Reports to the FDA in accordance with 21 CFR 312.32 as soon as possible and, in no event, later than 7 or 15 calendar days following receipt of reportable adverse event information.

For IND Safety Reports submitted to the FDA and received from DAIDS, the MHRP COO will complete the necessary reporting to the Walter Reed Army Institute of Research (WRAIR), including telephone contact and a written report generated within 24 hours of the initial safety report. WRAIR HSPB will forward to USAMRMC ORP.

The COO will also submit the necessary AE reporting documents and any study pauses to the WRAIR IRB and the USAMRMC ORP.

8.7 REPORTING SERIOUS AND UNEXPECTED ADVERSE EVENTS TO THE DOD IN ADDITION TO DAIDS

The WRAIR IRB provides DoD-required human subjects protection review of clinical trials involving WRAIR investigators and employees. It is the responsibility of the MHRP COO to fulfill the reporting requirements. .The local DoD research monitor should also review the events and provide an independent assessment to the WRAIR HSPB (as a DoD research monitor's report).

Per DAIDS new EAE Manual and 21 CFR 312.32, serious and unexpected adverse events may be categorized as related to the study agent or not related. All AEs that are serious and unexpected and related to the product are reported as described above and become IND Safety reports. Any serious and unexpected adverse events assessed as related to the study agent will be reported to the WRAIR HSPB immediately to the Director of HSPB (UWZ-C) (301-319-9940). WRAIR HSPB will report SAEs to USAMRMC ORP HRPO as per SOP UWZ-C-636.

8.7.1 SOCIAL HARMS REPORTING

Unanticipated events and social harms may occur during the course of the study. When such events are related to study participation, the study staff, informed of these events, will inform the PI or his/her designee. The PI or designee will then prepare a narrative summary of the event and report to the local IRBs, MHRP COO, and PSRT including DAIDS Medical Officer. WRAIR HSPB will then be informed. The DoD research monitor should also review the social harms and provide an independent assessment of these to the WRAIR HSPB. WRAIR HSPB will report these summaries to USAMRMC ORP HRPO.

8.7.2 UNANTICIPATED PROBLEMS REPORTING TO WRAIR

All unanticipated problems related to the study and involving risk to subjects or others (UPIRTSOs) and all subject deaths should be promptly reported to the WRAIR IRB (phone +1 301-319-9940, facsimile +1 301-319-9961) by the MHRP COO. A complete written report should follow the initial notification. The complete report will be sent to the Director, Human Subjects Protection HSPB, Walter Reed Army Institute of Research, 503 Robert Grant Ave., Silver Spring, Maryland 20910-7500. The DoD research monitor should also review the unanticipated events and provide an independent assessment to the WRAIR HSPB (as a DoD research monitor's report). WRAIR HSPB will report these summaries to USAMRMC ORP HRPO. MHRP COO will forward the report to the Division of AIDS.

8.8 REPORTING REQUIREMENTS TO THE LOCAL IRB

The site PI will be responsible for providing all Safety Reports and reporting all SAEs, study pauses, social harms, UPIRTSOs, and major deviations to the local regulatory authority, such as a local IRB, and any country-specific regulatory agencies, in a timely manner according to the institution's guidelines. Reporting requirements to the local IRB can be found in the site-specific addenda.

8.9 MODIFICATION TO THE DAIDS AE GRADING TABLE

The only modification to the DAIDS AE Grading Table is a change in the grading of absolute neutrophil count. Individuals of African descent have lower neutrophil counts than individuals of other ethnicities. A study evaluating the reasons for ineligibility in phase I and II HIV vaccine trials in East Africa found that approximately one third of subjects were excluded because of neutropenia. The phase I and II studies used normal ranges from U.S. populations, not from African ones. If neutrophil ranges from East/South Africa populations were used instead, over one half of the subjects excluded because of hematologic abnormalities could have been included [26]. Since this study will be conducted in part in East Africa, we will use the normal neutrophil range from East/South Africa ($1.0 – 5.3 \times 10^3$ cells/ul, [27]) for subjects in East Africa (Kenya, Uganda, Tanzania) or other subjects of African descent. Grades for absolute neutrophil count in these subjects will be as follows:

	Grade 1	Grade 2	Grade 3	Grade 4
Absolute Neutrophil Count (cells/ul)	750 – 999	500 – 749	250 – 499	<250

For other subjects, the usual DAIDS AE grading scale will be used as follows:

	Grade 1	Grade 2	Grade 3	Grade 4
Absolute Neutrophil Count (cells/ul)	800 - 1000	600 – 799	400 - 599	<400

9.0 STATISTICAL CONSIDERATIONS

9.1 POWER AND SAMPLE SIZE

Data from twenty-two RV 254 and RV 217 participants starting ART in AHI and with a viral load measurement between days 6 and 8 post-ART initiation show a typical decline of 1.5 logs with a standard deviation of 1.

Table 4. Log Viral Load Prior to ART Initiation and Approximately 7 Days Post-ART Initiation in Subjects Receiving Between 6 – 8 Days of ART.

Measurement	N	Mean	Std Dev	Median	Minimum	Maximum
Pre-ART Log Viral Load	22	5.96	1.08	5.88	3.31	7.49
Day 7 Log Viral Load	22	4.46	0.78	4.25	3.23	5.75
Change From Pre-ART to Day 7	22	-1.49	1.01	-1.58	-3.33	0.70

The study design features 3 arms with varying treatment in the first 7 days: ART only, mAb only, and mAb + ART. The ART only arm functions as a control. Pairwise comparisons of the mAb only arm to the ART only arm and of the mAb + ART arm to the ART only arm are planned. Table 5 shows the approximate detectable effect sizes for various sample sizes using a two-sided non-parametric test with 85% power and a 5% Type I Error. The assumed standard deviation is 1 in both groups based on the above data. This demonstrates that a sample size of 24 (8 per arm) would have 85% power to detect a difference in mean viral load decline of 1.7 logs between the control arm and a mAb treatment arm. If the assumptions of the t-test are met, this represents a conservative estimate of the detectable difference as the asymptotic relative efficiency of the non-parametric test to the t-test is approximately 95%. Since this is an early phase, exploratory study no adjustment for multiple pairwise comparisons will be made. Given the short time between treatment and the primary endpoint, no loss to follow-up for this endpoint is anticipated. Note that this study is not powered to detect differences between the two monoclonal arms.

Table 5: Effect Size for Two-Arm Comparison Detectable with 85% Power and 5% Type I Error

N per Arm	Effect Size (Mean Difference/SD)*
6	2.1
7	1.9
8	1.7
9	1.6
10	1.5

To assess safety, exact two-sided 95% confidence intervals will be constructed for the proportion of subjects experiencing a mAb related - grade 3 or greater reactogenicity. Table 6 below shows the expected confidence intervals based on number of subjects experiencing an event within arm (n=8) and collapsed across arms (n=16).

Table 6. Exact Two-Sided 95% Confidence Intervals for Number of Subjects Experiencing a mAb Related - Grade 3 or Greater Reactogenicity Within Arm and Across mAB Arms

Number of Subjects with Event	Single Arm (n=8)	Combined mAB Arms (n=16)
0	(0%, 36.9%)	(0%, 20.6%)
1	(0.3%, 52.7%)	(0.1%, 30.2%)
2	(3.2%, 65.1%)	(1.6%, 38.5%)
3	(8.5%, 75.5%)	(4.0%, 45.7%)
4	(15.7%, 84.3%)	(7.3%, 52.4%)
6	(34.9%, 96.8%)	(15.2%, 64.6%)

9.2 ANALYSIS

All data from randomized participants will be included in the intention-to-treat analysis. Data from all randomized participants who receive the assigned study treatments and complete the study through the 24 week visit will be included in the per-protocol analysis. Endpoints are listed in section 4.0.

Baseline characteristics including demographics and laboratory measurements will be summarized using descriptive statistics. Summaries of the number and percentage of subjects experiencing any AE or reactogenicity will be tallied by group and presented along with exact 95% confidence intervals for the proportion. For the following continuous endpoints appropriate transformations (e.g. log, square root, or arcsin) will be applied as necessary.

9.2.1 PRIMARY ENDPOINTS:

1. Safety: mAb related - grade 3 or greater reactogenicity and product related AEs

For solicited AEs/reactogenicity, number and percentage of subjects experiencing each type of solicited sign or symptom will be tabulated by severity. For a given sign or symptom, each subject's solicited AEs will be counted once under the maximum severity for all assessments.

For unsolicited AEs, number and percentages of participants experiencing each specific adverse event will be tabulated by severity and relationship to treatment. For the calculations in these tables, each participant's adverse experience will be counted once under the maximum severity or strongest recorded causal relationship to treatment.

A complete listing of adverse experiences for each participant will provide details including severity, solicited or unsolicited, onset, duration, outcome.

Proportions of subjects experiencing a reaction will be summarized and compared across all 3 treatment groups using a chi-square test or Fisher's exact test.

2. Log change in plasma viral load from day 0 to day 7

The log change in plasma viral load over the first seven days will be compared among treatment groups using an Analysis of Variance (ANOVA) model or the non-parametric equivalent if

necessary. Regardless of the significance of the global test, pairwise comparisons between the ART arm and the monoclonal arms will be made at the 5% significance level using a two-sided t-test or non-parametric methods if assumptions are not met.

9.2.2 SECONDARY ENDPOINTS:

1. *Time to virologic suppression (<50 cp/mL)*

Time to virologic suppression as defined above will be compared between groups using time to event methods (Kaplan-Meier) to account for the possibility of subjects not achieving viremic control prior to 24 weeks and will be tested among groups using a log-rank test.

2. *Total area under viral load curve [28] (viral load over time)*

Area under the viral load curve will be calculated as described in the referenced paper and compared between groups using an ANOVA model followed by pairwise contrasts (without adjustment for multiple comparisons) where appropriate or using non-parametric methods if necessary.

3. *HIV RNA quantification, including single copy HIV RNA in samples with HIV RNA < 50 copies/ml, at week 24*

HIV RNA quantification will be compared among treatment groups using an ANOVA model or the non-parametric equivalent if necessary. Pairwise comparisons between the ART arm and the monoclonal arms will be made at the 5% significance level using a two-sided t-test or non-parametric methods if assumptions are not met.

4. *Cell-associated HIV RNA and DNA in the peripheral compartment*

Cell-associated HIV RNA and DNA in the peripheral compartment will be compared among treatment groups using an ANOVA model or the non-parametric equivalent if necessary. Pairwise comparisons between the ART arm and the monoclonal arms will be made at the 5% significance level using a two-sided t-test or non-parametric methods if assumptions are not met.

5. *Frequency, severity, and duration of acute retroviral syndrome*

Frequency and percentage of subjects experiencing acute retroviral syndrome will be tabulated by treatment group and severity. For the per subject analysis, maximum severity will be considered. Percentage of subjects experiencing ARS will be compared across treatment groups using a chi-square test of Fisher's Exact Test.

6. *Frequency of hospitalization and incidence of opportunistic infections and non-AIDS related conditions.*

Frequency and percentage of subjects experiencing a hospitalization will be tabulated by treatment group. Similar methods will be used for infections and conditions. Percentage of subjects experiencing a hospitalization, infection, or non-AIDS related condition (each endpoint will be considered separately) will be compared across treatment groups using a chi-square test of Fisher's Exact Test.

7. CD4 + T cell decrease from baseline to nadir, increase from nadir to week 24, and overall change from baseline to week 24

Change from baseline to nadir, change from nadir to week 24, and change from baseline to week 24 in CD4+ T-Cells will be compared across treatment groups using an ANOVA model or the non-parametric equivalent if necessary. Pairwise comparisons between the ART arm and the monoclonal arms will be made at the 5% significance level using a two-sided t-test or non-parametric methods if assumptions are not met.

8. VRC01 Levels in Peripheral Blood - Pharmacokinetics

Cmax, Cmin, Tmax, and Tmin will be described by group and overall. Slope of elimination will be estimated using WinNonlin PK[Pharsight Corporation, CA, USA] software or comparable software and/or methods.

9.2.3 EXPLORATORY ENDPOINTS:

Exploratory endpoints will be described over time by treatment arm in tabular and graphical format. Continuous variables will be described at each timepoint using means, standard deviations, medians and interquartile ranges. Categorical variables will be presented as proportions and confidence intervals at each timepoint. If hypothesis testing is possible, similar methods to those for the primary and secondary endpoints can be employed. Further for repeated measures data (e.g. HIV RNA and DNA collected at 10 timepoints) models will be fit to compare treatment groups accounting for within subject correlation. If the number of subjects consenting to optional procedures is low, then analysis of exploratory objectives will be primarily descriptive.

More specifically, the key exploratory endpoints will be analyzed according to the following plan:

1. *HIV RNA quantification in the CSF and mucosal compartment (to include single copy measurements from samples with HIV RNA < 50 copies/ml) at days 0, 7, and 168. Cell-associated HIV RNA and DNA in the CSF, mucosal and tissue compartments at days 0, 7, and 168.*

HIV RNA quantification in the CSF and mucosal compartments will be compared among treatment groups using an ANOVA model or the non-parametric equivalent if necessary at days 7 and 168. Change from baseline will also be considered as an outcome. Pairwise comparisons between the ART arm and the monoclonal arms will be made at the 5% significance level using a two-sided t-test or nonparametric methods if assumptions are not met.

2. *Correlation of HIV RNA and DNA measurements across biologic compartments (at baseline, day 7, day 168) that include the peripheral blood, CSF, mucosal, and tissue compartments.*

Correlation of viral loads across different biologic compartments will be measured using Spearman's correlation coefficient at each time point.

3. *HIV RNA and DNA in sorted memory CD4+ T cell subsets (for subjects with sufficient PBMC collections) at each days 0, 3, 7, 14, 21, 28, 56, 84, 112, 168. Viral outgrowth in total*

CD4+ T cells and sorted memory CD4+ T cell subsets (for subjects with sufficient PBMC collections) at day 168.

HIV RNA and DNA will be compared between treatment groups over time using repeated measures models accounting for within subject correlation. Viral outgrowth will be compared between treatment groups at day 168. Viral outgrowth will be described and compared between groups at day 168.

4. *VRC01 levels across biologic compartments that include the CSF and mucosal secretions at days 0, 7 and 168. Presence of anti-mAb antibody at days 0, 7, 14, 28, 56, 84, 112, 168. Biomarkers of immune activation (soluble and cellular markers, gene expression).*

As with comparisons of virologic measures, VRC01 levels, anti-mAb levels, and biomarkers of immune activation will all be compared across groups using an ANOVA model or the non-parametric equivalent at days 7 and 168. Pairwise comparisons will be made at the 5% significance level using a two-sided t-test or nonparametric methods. Additionally presence and magnitude of anti-mAB antibody will be assessed longitudinally adjusting for within subject correlation due to repeated measures.

5. *Remaining exploratory outcomes*

The remaining exploratory outcomes that address the predictors of favorable viral dynamics, immune responses, and reservoir seeding will be analyzed with the appropriate statistical measures as the data become available.

10.0 DATA COLLECTION

Data collection will be conducted on tablets or computers with daily upload to a central database. Access to the data collection software is limited by individual username and password. Data is stored in encrypted fashion on the local computer. After the data is uploaded to the central database it is deleted from the local tablet or computer.

Data obtained in the conduct of this study are housed in a secure database maintained by the DCAC, MHRP, WRAIR. All research data are entered in a secure database with standardized quality assurance review procedures in accordance with Good Clinical Practices (GCP).

The DCAC serves as the central data management facility for MHRP research protocols. Data managed by DCAC are entered into and maintained in a password-protected database. Data are accessible only to participating sites, DCAC, and Information Technology staff authorized to work on the protocol. The database is located at the Bethesda campus of MHRP and is protected by a firewall.

This data does not contain participant names or Social Security or other national identification number, but is referenced only by the study specific identification code. All subjects consented will be assigned a 9-digit Subject Identification (ID). The first three digits are the protocol number (398), followed by a 2-digit site code, and then a 4-digit number which, will be assigned sequentially**.**

Every attempt must be made to follow the protocol and to obtain and record all data requested for each subject at the specified times. However, ethical considerations or other events may result in the failure to obtain and record certain data, or to record data at the times specified. If this occurs, the events and, the reasons for the event must be clearly documented on the case report form for deviations and reported as described above.

Analysis files are created on a periodic basis and made available to the Protocol Chair, site Principal Investigators (PIs), and Associate Investigators (AIs) at the direction of the Protocol Chair. Other collaborators may be given access to these analysis files, or data gathered from them, at the direction of the Protocol Chair. Data may be made available as a listing, external file, or through a query program.

11.0 ETHICAL CONSIDERATIONS

11.1 PARTICIPATION OF CHILDREN

Children are not eligible to participate in this clinical trial because it does not meet the guidelines for inclusion of children in research. These guidelines (45 CFR 46, Subpart D, 401-409) state the Department of Health and Human Services protections for children who participate in research. Generally, healthy children can be studied when the research is considered as "not greater than minimal risk." Children can be involved in research with greater than minimal risk only when it presents the prospect of direct benefit to the individual child or is likely to yield generalizable knowledge about the child's disorder or condition.

11.2 PARTICIPATION OF PRISONERS

Participation of prisoners is not planned and any volunteer will be suspended from study visits while incarcerated. The IRB will be notified of the period of incarceration. If possible, site teams will coordinate with correctional authorities to ensure that antiretroviral therapy is not interrupted.

11.3 RISKS

Risks from IV infusions:
General risks of IV infusion include stinging, discomfort, pain, soreness, redness, bruising, swelling or small laceration at the needle insertion site.

Risks of the VRC01 study agent:
Safety experience:

As described in section 2.6 and in the Investigator's Brochure, as of January 10, 2017, VRC01 administrations in the dose range from 1 to 40 mg/kg IV and 5 to 40 mg/kg SC have been assessed as well-tolerated in adults and infants and safe for further evaluation.

In phase 2 trials, most subjects had no local or systemic reactogenicity symptoms. When present, local and systemic reactogenicity symptoms were of mild or moderate severity. Infusion site pain and/or tenderness developed in 26% of study subjects. Infusion site erythema and/or induration developed in 9% of study subjects. The 3 most common systemic reactogenicity events were malaise (21%), headache (19%), headache followed by nausea (8%) and myalgia (7%).

In phase 2 trials, there have been 22 related AEs of mild or moderate severity in 15 subjects (2 AEs of change in sleep pattern, 2 AEs hypogeusia, 5 AEs of urticaria and 1 event each of wheals and erythema-left upper arm/right calf/under right breast, urticaria of upper limbs, urticaria of the face, infusion site pruritus, generalised body itchiness, low neutrophil count, dizziness during infusion, lightheadedness, loose stools, dermatitis, headache, nausea and malaise). There has been one related severe (grade 3) AE of urticaria in 1 subject.

Potential safety concerns:

Administration of mAb may have a risk of immune reactions such as acute anaphylaxis, serum sickness and the generation of antibodies; however, these reactions are rare and more often associated with mAb targeted to human proteins or with the use of murine monoclonal antibodies, which would have a risk of human anti-mouse antibodies. In this regard, as VRC01 is targeted to a viral antigen and is a human monoclonal antibody, it is expected to have a low risk of such side effects.

Typically, the side effects of mAbs are mild but may include fever, chills, rigors, nausea, vomiting, pain, headache, dizziness, shortness of breath, bronchospasm, hypotension, hypertension, pruritus, rash, urticaria, angioedema, diarrhea, tachycardia or chest pain. Clinical use of monoclonal antibodies that are targeted to cytokines or antigens associated with human cells may be associated with an increased risk of infections [20]; however, this is not expected to be a risk for a mAb targeted to a viral antigen.

It is known from published experience with human mAb directed against the cell surface targets on lymphocytes, that infusion of a mAb may be associated with cytokine release, causing a reaction known as "cytokine release syndrome" (CRS) [29]. Most infusion-related events occur within the first 24 hours after beginning administration. Severe reactions, such as anaphylaxis, angioedema, bronchospasm, hypotension and hypoxia, are infrequent and more often associated with mAbs targeted to human proteins or when a nonhuman mAb, such as a murine mAb, is used [20]. The VRC01 mAb is a human mAb that is directed to a viral target rather than a host cell target. Severe CRS has not been reported in such pathogen-directed human mAbs, and CRS has not been observed in the human experience with VRC01. Specifically, with regard to CRS reactions, these most commonly occur within the first few hours of beginning the infusion and are more common with the first mAb infusion received. This is because the cytokine release is associated with lysis of the cells targeted by the mAb and the burden of target cells is greatest at the time of the first mAb treatment. With licensed therapeutic mAbs, CRS is managed by temporarily stopping the infusion, administration of histamine blockers and restarting the infusion at a slower rate [30].

Delayed allergic reactions to a mAb may include a serum sickness type of reaction, which is characterized by urticaria, fever, lymph node enlargement, and joint pains. These symptoms may not appear until several days after the exposure to the mAb and is noted to be more common with chimeric types of mAbs [31].

There are several FDA-licensed mAbs for which reactions related to the rate of infusion have been described. Some symptoms may be treated by slowing or stopping the infusion. Supportive treatment may also be indicated for some signs and symptoms.

A theoretical risk is that receiving VRC01 might affect the subject's drug sensitivity profile for ARV medications that work through entry or fusion inhibition, or may limit eligibility for some future monoclonal antibody treatments.

Risks of Phlebotomy:
Blood drawing may cause pain, bruising and may, infrequently, cause presyncope. Local

infection at the phlebotomy site is also a risk of phlebotomy. Rarely, it may cause infection at the site where the blood is taken.

Risks of Antiretroviral Therapy:
The side effect profiles of the ART regimens in this study are well characterized. Dizziness, headache, trouble sleeping, drowsiness, trouble concentrating, and unusual dreams are common side effects. These may be reduced by taking the medication at bedtime on an empty stomach and tend to resolve after taking the medication for a few weeks. Rash is also a common side effect that usually resolves without a change in treatment but can be serious in a small number of patients. Other common side effects include nausea, vomiting, and diarrhea. Serious side effects are rare and include kidney injury, liver injury, lactic acidosis, allergic reactions, major depression and decreased bone mineral density [32-33]. Risks of antiretroviral therapy are mitigated by frequent clinical evaluations and monitoring of blood chemistry and liver tests.

Risk of Delaying Antiretroviral Therapy:
Although the risk of delaying antiretroviral treatment for seven days among participants diagnosed with early, acute HIV infection is real, we believe it to be minimal. Data from acute HIV infection studies have shown that antiretroviral therapy initiated later in acute infection still reduces the viral reservoir to the same levels as those treated in Fiebig stage I or II [9]. Additionally, data from RV 254 suggest HIV reservoirs do not change appreciably over one year after infection if ART is initiated in the early Fiebig stages (I-III); a timeframe that covers all participants, including those who will have a seven day treatment delay.

Furthermore, the study is designed to mitigate these risks. Participants will only be enrolled if they are in relatively good health. In addition, participants who exhibit signs and symptoms of severe acute retroviral syndrome (as defined in Appendix I) during the seven-day delay will be started on ART immediately. Participants will be seen three times during the first week and will be instructed to return to the clinic for prompt evaluation and potential treatment initiation if they exhibit signs and symptoms of acute retroviral syndrome at home.

Risks Related to Pregnancy:
The possible effects of VRC01 on a fetus or nursing infant are unknown. Since VRC01 is a human monoclonal antibody and not a chemotherapeutic agent, traditional toxicology and animal reproduction studies are not possible. However, the antibody is directed against a viral protein and showed no cross-reactivity with fetal or adult human tissue in preliminary safety studies. Furthermore, anti-HIV antibodies are routinely transferred from the mother to the fetus during pregnancy without any adverse affect on the mother or infant [34]. As with the majority of IgG antibodies, transfer of anti-HIV antibodies occurs from the mother to the fetus predominantly during the third trimester; since VRC01 infusions will be stopped if pregnancy is diagnosed, they are unlikely to be present at the time of IgG transfer to the fetus [34].

This evidence suggests that adverse affects to the infant of a mother exposed to VRC01 are unlikely, but pregnant women are still conservatively excluded from participation in this phase II study of an investigational new agent. Women who are able to have children must agree to not get pregnant during study participation and must use at least one method of effective birth control during study participation. Effective birth control includes not having sex; male or female condoms; diaphragm or cervical cap with spermicide; intrauterine device; contraceptive

hormones delivered by pills, patch, injections or vaginally; and hormonal implants under the skin; or a male partner who has previously undergone a vasectomy.

Women must have a negative pregnancy test before dosing with VRC01 and before collection of cervicovaginal secretion samples. Women becoming pregnant during the study will be asked to continue with study follow-up visits for clinical evaluation. The outcome of the pregnancy will be recorded in the antiretroviral pregnancy registry (http://www.apregistry.com/who.htm).

Risks related to mucosal secretion collection:
Mucosal secretion samples collected in the clinic (rectal sponges, semen, and cervicovaginal cups) will be obtained noninvasively. Semen will be self-collected and for the collection of cervical-vaginal secretions, women may elect to insert and remove cervical cups themselves, as well. Inserting an instrument or collection device into the anus or the vagina may cause discomfort and slight irritation. There is no evidence of rectal sponge or cervical cup sampling contributing to risk of HIV or other sexually transmitted infection. For these non-invasive mucosal collections, men and women will be asked to refrain from receptive anal or vaginal, intercourse, douching, or inserting any product into the rectum or vagina for 3 days prior to the mucosal collection. Men will be asked not to masturbate nor ejaculate 3 days prior to semen collection.

Risks related to colon biopsy (via sigmoidoscopy):
Rectosigmoid biopsies will be performed through a flexible sigmoidoscopy. Brief cramping and gas pains may be felt as air is inserted or as the scope advances. The passing of gas is necessary and should be expected after the procedures are terminated. Volunteers may choose to receive sedation during the procedure in order to ameliorate the discomfort and anxiety they may feel. There may be slight bleeding from the biopsy site, which generally stops spontaneously. There is a remote possibility that a biopsy may result in significant bleeding or even perforation requiring emergency medical care. A gastroenterologist will perform this procedure in order to minimize these risks. All volunteers will be counseled to avoid sexual rectal intercourse for 3 days prior to and 7 days following biopsy. Volunteers participating in sigmoidoscopy are not eligible to participate in rectal biopsy via anoscopy at the same time point.

Risks related to rectal biopsy (via anoscopy):
Rectal biopsies will be performed with the use of an anoscope, the insertion of which may cause discomfort and slight irritation. There may be slight bleeding from the biopsy site which generally stops spontaneously. There is a remote possibility that a biopsy may result in significant bleeding or even perforation requiring emergency medical care. A trained physician will perform this procedure in order to minimize these risks. Volunteers will be counseled to avoid sexual rectal intercourse for 3 days prior to and 7 days following rectal biopsies. Volunteers participating in this rectal biopsy procedure are not eligible to participate in sigmoidoscopy at the same time point.

Risks related to lymph node biopsy:
Excisional inguinal lymph node biopsy will be performed under local anesthesia by a qualified surgeon. As with all surgical procedures, there is a risk of scarring, bruising or bleeding at the surgical site; these risks are minimized by the small nature of the incision (approximately 1-2 centimeters) and by prior clinical and laboratory evaluation to determine any bleeding risks.

Subjects may experience some discomfort following the procedure despite use of local anesthesia; for subjects who complain of any subsequent discomfort, additional analgesia will be made available. There is a possibility of seroma formation at the biopsy site, which may require subsequent drainage or further management, or sensory nerve injury during the procedure which could result in temporary or permanent local reduction in feeling. Surgical site infection is a possible complication of any procedure involving an incision, however the risk is low [35]. Finally, there is the unlikely possibility that the procedure will be unsuccessful and no lymph node will be recovered.

Risks related to leukapheresis:
Adverse reactions to leukapheresis procedure are rare and include vaso-vagal episodes related to needle insertions and transient volume shifts, peri-oral paresthesias, chills, nausea, and heartburn caused by the citrate anticoagulant used during the procedure. Vaso-vagal reactions are handled by postural manipulation and fluid administration. Volunteers will be observed closely by an experienced technician during the procedure. Citrate reactions are usually relieved by slowing the rate of the anticoagulant infusion and by administering oral calcium carbonate tablets. Seizures and allergic reactions are very rare possible reactions.

Risks related to lumbar puncture:
The risks of lumbar puncture include local soreness at the site of needle entry. There is a risk of headache or decreased blood pressure from removing the small amount of fluid or leaking of cerebral spinal fluid after the procedure. About 1 in 4 or 5 people will experience a headache following lumbar puncture, and this can sometimes require hospitalization for pain management. There is a small risk of infection and a very small risk of damage to nerves in the lumbar spinal roots after the procedure, which could cause pain, numbness, or loss of sensation to the legs. In order to reduce side effects of the lumbar puncture, the volunteers will be asked to remain lying flat for about an hour after the procedure and be given fluid to drink after the procedure. A band-aid will be placed on the skin where the needle went in and the volunteers will be asked to remove it the next day and tell the study doctor right away if any redness or tenderness is present. The investigators will ask the volunteers about history of any allergies to anesthetics and will not perform lumbar puncture in any volunteer with such history.

11.4 BENEFITS

Although study volunteers may benefit from clinical testing and physical examination, they may receive no direct benefit from participation.

11.5 INFORMED CONSENT

The study informed consent describes the investigational product to be used and all aspects involved in protocol participation. A properly executed written site specific informed consent based on the template provided here, in compliance with the Declaration of Helsinki, guidelines of the Council of International Organization of Medical Sciences (CIOMS), the Belmont Report, the U.S. Code of Federal Regulations 21 CFR 50, must be obtained from each subject prior to entering the subject into trial or prior to performing any unusual or non routine procedure that involves risk to the subject. The investigator must provide a copy of the approved informed

consent to the subject and a signed copy must be maintained in the subject's record file. Before a subject's participation in the study, it is the investigator's responsibility to obtain this written informed consent from the subject, after adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the study and before any protocol-specific procedures or study medications are administered.

11.6 LANGUAGE

The ICF and other study documents given to participants will be translated into local language as detailed in the site specific addenda.

11.7 COMPENSATION

Participants will be compensated for time and inconvenience in accordance with the standards and legal obligations for compensation required by each study site. Any applicable guidelines by IRBs/ECs for compensation of research subjects will be sought and followed

There may be compensation for lost time, travel expenses and inconvenience, for each scheduled visit. Compensation for unscheduled visits may be provided at the discretion of the site investigator. Any applicable guidelines by IRBs/ECs for compensation will be sought and followed. Compensation is further detailed in the site specific addenda.

11.8 DOD RESEARCH MONITOR

The role and qualifications of the medical monitor are identified as follows:

The medical monitor will review all unanticipated problems involving risk to volunteers or others, social harms, and all volunteer deaths due to social harms associated with the protocol. The DoD research monitor will provide an unbiased written report of the event. At a minimum, the medical monitor should comment on the outcomes of the problem, and in the case of a death, comment on the relationship to participation in the study. The medical monitor should also indicate whether he/she concurs with the details of the report provided by the study investigator.

11.9 SUBJECT INSURANCE

The US DoD and DAIDS/NIAID/NIH are funding this protocol. As stated in the consent form, participants who experience illness or injury arising from participation in the study will receive medical care for such illness or injury with costs for such care provided by a limited set-aside fund and a clinical trials medical insurance policy that will be obtained by the study funder. While we anticipate the combination of the set-aside fund and the insurance policy is more than enough to pay for the research related injury medical care cost associated with this study, there is a limit to the amount of coverage available. If the limit is exceeded, the study subject may have to pay non-covered costs. Other than medical care, and other payments as stated in the consent form, there is no other compensation available from this research study.

11.10 PARTICIPANT CONFIDENTIALITY

The PI will maintain research records of participant's participation at the site for this study. All participants will receive study numbers that are known only to the investigators and clinic staff. Clinical tests will be identified by study number and the specimen bag will be identified by study number and/or bar code only. Genetic testing results will not contain enough information to independently identify individuals in the study. Clinical and research records may be reviewed by the representatives of DAIDS, USAMRMC, WRAIR, representatives of the USAMRMC ORP, representatives of the FDA, OHRP, and other local, US, and international regulatory entities as part of their responsibilities for insuring the protection of research participants.

Every effort will be made to keep the records as confidential as possible within the limits of the law. All data and medical information obtained about participants as individuals will be considered privileged and held in confidence. Research and clinical information relating to participants will be shared with other investigators and the scientific community through presentation or publication; however, participants will NOT be identified by name or social security number. Electronic data will be stored at least as long as the IND remains open.

11.11 INSTITUTIONAL REVIEW BOARD

A copy of the protocol, proposed informed consent form, other written participant information, and any proposed advertising material will be submitted to the appropriate ethical and scientific review committees in each country for review and approval.

In addition, the protocol will undergo review and approval by both the WRAIR IRB and the USAMRMC ORP at Medical Research and Materiel Command, U.S. Army (MRMC).

11.12 FUTURE USE AND STORAGE OF BLOOD SAMPLES

Each study participant will be asked to separately, and voluntarily consent to their blood samples to be stored for other research studies that may be done after this study is completed. Future testing may involve genetic tests. As stated above, the sample will be labeled with the bar code of the subject ID that can be linked to their study information.

All samples for which consent has been obtained and for which additional material is available after study specified testing is complete will be stored for future testing at the site. However, WRAIR IRB and local IRB approval will be sought before any such samples are used for analysis not specified in the protocol or a protocol amendment approved by the IRB. All samples belong to the site from which they were obtained and MHRP.

11.13 STUDY DOCUMENTATION AND STORAGE

Source documents are original documents, data, and records from which the subject's data are obtained. These include but are not limited to hospital records, clinical and office charts, laboratory and pharmacy records, diaries, microfiches, radiographs, and correspondence.

The investigator and staff are responsible for ensuring maintenance of a comprehensive and

centralized filing system of all study-related (essential) documentation, suitable for inspection at any time by representatives from the WRAIR, MRMC, DAIDS, FDA, and other local, US, and international regulatory entities.

12.0 ADMINISTRATIVE AND LEGAL PROCEDURES

12.1 PROTOCOL DEVIATION REPORTING

A protocol deviation is defined as an isolated occurrence involving a procedure that did not follow the study protocol.

The timeline for reporting protocol deviations to the Division of Human Subjects Protection/ WRAIR IRB is determined by the categorization of the deviation: (1) emergent/significant or (2) non-emergent/minor. Unanticipated problems should be reported in the appropriate timeframe according to the seriousness of the event as a significant deviation or a minor deviation.

Emergent/significant deviations are departures from protocol that have a significant impact on the welfare or safety of a volunteer or on the integrity of the study data. Examples: providing the wrong lab result to a volunteer or failure to obtain a scheduled blood draw for multiple participants. Changes in protocol procedures may be initiated without prior IRB/ethical review committee (ERC) approval,only in cases where the change (s) is /are necessary to eliminate an immediate apparent hazard.

Non-emergent/minor deviations are routine departures that typically involve a volunteer's failure to comply with the protocol. Examples include missing scheduled visits and failing to complete a required questionnaire. Minor deviations will be reported to the sponsor and the Division of Human Subjects Protection/IRB in a summary report with the annual continuing review report.

A cumulative deviation report will be submitted to the HSPB/WRAIR IRB with each protocol continuing review report or with the closeout report, whichever comes first.

12.2 PROTOCOL MODIFICATIONS

Amendments to the protocol will be made only after consultation and agreement between DAIDS, the protocol chair, and the principal investigators. All protocol modifications (including but not limited to changes in the principal investigator, inclusion/exclusion criteria, number of participants to be enrolled study sites, or procedures) must be submitted as a written amendment to DAIDS, local IRB, and the DoD IRB approval before implementation of the changes. The WRAIR IRB will submit protocol amendments and modifications to the USAMRMC ORP HRPO.

Modifications or updates to the investigational brochures (IBs) will also be submitted as protocol amendments to the ERCs/IRBS for review and approval. DAIDS does not consider changes to the IB as protocol amendments and therefore does not provide approval of IBs.

The Informed Consent Form must be revised to concur with any significant amendment that directly affects volunteers, and must also be reviewed and approved with the amendment. New volunteers enrolled in the study will be consented with the most recent approved consent form. Volunteers already enrolled in the study will be informed about the revision and, depending on the impact of the amendment, may be asked to re consent. This may be accomplished by

repeating the consent process with the revised consent form with attention given to the changes, or it may be done using an addendum consent that states the revision or new information. The new document must be signed, placed in the study record, and a copy given to the volunteer.

Prior to implementation of this protocol, and any subsequent full version amendments, each site must have the protocol and the protocol consent form(s) approved, as appropriate, by their local institutional review board (IRB)/ethics committee (EC) and any other applicable regulatory entity (RE). Upon receiving final approval, sites will submit all required protocol registration documents to the DAIDS Protocol Registration Office (DAIDS PRO) at the Regulatory Support Center (RSC). The DAIDS PRO will review the submitted protocol registration packet to ensure that all of the required documents have been received.

Site-specific informed consent forms (ICFs) WILL be reviewed and approved by the DAIDS PRO and sites will receive an Initial Registration Notification from the DAIDS PRO that indicates successful completion of the protocol registration process. A copy of the Initial Registration Notification should be retained in the site's regulatory files.

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

For additional information on the protocol registration process and specific documents required for initial and amendment registrations, refer to the current version of the DAIDS Protocol Registration Manual.

A list of proposed modifications or amendments to the protocol and an explanation of the need for these modifications will be submitted, along with a revised protocol incorporating the modifications. The only exception occurs when the investigator considers that a study participants’s safety is compromised without immediate action. In these circumstances, immediate approval of the chairman of the IRB must be sought, and the investigator should inform DAIDS and the IRB within five working days after the emergency occurred. As previously mentioned, all study amendments will be submitted as a written amendment to DAIDS for possible Clinical Sciences Review Committee (CSRC) review and regulatory submission to the local IRB, and WRAIR. In accordance with 21 CFR 312.30 (b) any change in a Phase 1 protocol that significantly affects the safety of subjects will be submitted to the FDA.

12.3 CONTINUING REVIEWS /CLOSEOUT REPORT

A continuing review report (CRR) will be submitted to all ERCs/IRBs prior to the anniversary date determined at initial IRB review. If the continuing review is not approved by the local ERC/IRB and WRAIR IRB by the anniversary date, all protocol activities must stop at that site until such time as the approval is obtained. A copy of the approved CRR and local IRB approval

notifications will be submitted to the WRAIR HSPB as soon as these documents become available. A copy of the approved closeout report and local IRB approval notifications will be submitted to the WRAIR HSPB as soon as these documents become available.

12.4 VOLUNTEER REGISTRY DATABASE

It is the policy of the USAMRMC that Volunteer Registry Data Sheets are completed on all volunteers participating in greater than minimal risk research for entry into the Command's Volunteer Registry Database. Ordinarily this information would include the individual's unique identifier (e.g. social security number (SSN) in the United States of America (U.S.A.)), study name and dates. But because many countries do not have a national identification system that would uniquely identify each person in the country, the Volunteer Registry Data Sheets will collect the following data on the volunteers:

names (first and last name)
date of birth, home district
study name and study dates

The intent of the database is two-fold: first, to readily answer questions concerning an individual's participation in research sponsored by USAMRMC; and second, to ensure that the USAMRMC can exercise its obligation to ensure research volunteers are adequately warned (duty to warn) of risks and to provide new information as it becomes available. The information will be stored at USAMRMC for a minimum of 75 years. In countries other than the U.S., social security numbers do not exist and cannot be collected; all other data can. If available, country-specific identity numbers may be used.

12.5 SITE-SPECIFIC ADDENDUM

A site-specific addendum will be employed. The core protocol will be followed by all sites except for the differences noted in the site-specific addendum, which must be approved by the sponsor prior to submission to the IRBs and regulatory authorities.

12.6 USE OF INFORMATION AND PUBLICATION

It is understood by the investigator that the information generated in this study may be used in connection with the development of the product and therefore may be disclosed to government agencies in various countries. To allow for the use of information derived from the study, it is understood that the investigator is obliged to provide DAIDS with complete test results, all study data, and access to all study records.

WRAIR recognizes the importance of communicating medical study data and therefore encourages their publication in reputable scientific journals and at seminars or conferences. Any results of medical investigations and or publication/lecture/manuscripts based thereon, shall be exchanged and discussed by the investigator and the USAMRMC prior to submission for publication or presentation.

Results from investigations shall not be made available to any third party by the investigating team outside the publication procedure as outlined previously. WRAIR will not quote from publications by investigators in its scientific information and/or promotional material without full acknowledgment of the source (i.e., author and reference). All publications written by WRAIR investigators must be reviewed and approved through the WRAIR publications approval process.

13.0 CONDUCT OF THE RESEARCH STUDY

This research study will be conducted in accordance with GCP, ICH guidelines, DOD Directive 3216.2, the Declaration of Helsinki, the Belmont Report, the U.S. Code of Federal Regulations 21 CFR 312, 812, 50 and 56, and all applicable local laws and regulations.

13.1 REGULATORY AUDITS

The knowledge of any pending compliance inspection/visit by the US FDA, OHRP, or other local, US or international regulatory entities concerning clinical investigation or research, the issuance of Inspection Reports, FDA Form 483, warning letters or actions taken by any regulatory agencies including legal or medical actions and any instances of serious or continuing noncompliance with the regulations or requirements will be reported immediately to the sponsor (DAIDS) and WRAIR HSPB. The WRAIR HSPB will report knowledge of any pending inspections/audits by regulatory agencies to the USAMRMC ORP HRPO.

13.2 SPONSOR STUDY MONITORING

Site visits by study monitors will be made in accordance with DAIDS' policy to monitor the following: study operations, the quality of data collected in the research records, the accuracy and timeliness of data entered in the database, and to determine that all process and regulatory requirements are met.

Site investigators will allow the study monitors, DAIDS, the IRB/ERCs, MRMC, FDA and other local, US, and international regulatory entities to inspect study documents (e.g., consent forms, drug distribution forms, case report forms), and pertinent hospital or clinic records for confirmation of the study data.

Study data will be closed and final after data cleaning activities are completed and resolutions have been documented.

14.0 PRINCIPAL INVESTIGATOR AGREEMENT

1. I agree to follow this protocol version as approved by the IRBs/ERCs.
2. I will conduct the study in accordance with applicable IRB/ERC requirements, Federal regulations, and state and local laws to maintain the protection of the rights and welfare of study participants.
3. I certify that I, and the study staff, have received the requisite training to conduct this research protocol.
4. I will not modify the protocol without first obtaining an IRB/ERC approved amendment and new protocol version unless it is necessary to protect the health and welfare of study participants.
5. I have read and understand the information in the Investigators' Brochure (or Manufacturer's Brochure) regarding the risks and potential benefits. I agree to conduct the protocol in accordance with Good Clinical Practices (ICH-GCP), the applicable ethical principles, the Statement of Investigator (Form FDA 1572), and with local regulatory requirements. In accordance with the FDA Modernization Act, I will ensure the registration of the trial on the www.clinicaltrials.gov website.
6. In accordance with Command Policy 2008-35, I will ensure that the Commanding General receives a pre-brief (or Executive Summary) and approves the study prior to execution.
7. I will ensure that the data (and/or specimens) are maintained in accordance with the data (and/or specimen) disposition outlined in the protocol. Any modifications to this plan should first be reviewed and approved by the applicable IRBs/ERCs.
8. I will promptly report changes to the research or unanticipated problems to the WRAIR IRB immediately via the WRAIR Human Subjects Protection Branch at (301) 319-9940 (during duty hours) or to the usarmy.detrick.medcom-wrair.mbx.hspb@mail.mil and submit a written report within 10 working days of knowledge of the event.
9. I will prepare continuing review reports at an interval established by the IRB/ERC, and a study closure report when all research activities are completed.
10. I will immediately report to the WRAIR Human Subjects Protection Branch knowledge of any pending compliance inspection by any outside governmental agency.

11. I agree to maintain adequate and accurate records in accordance with IRB policies, Federal, state and local laws and regulations.

RV 398 Protocol Chair Signature	Date (DD/MM/YYYY)
RV 398a PI Signature	Date (DD/MM/YYYY)
RV 398b PI Signature	Date (DD/MM/YYYY)
RV 398c PI Signature	Date (DD/MM/YYYY)
RV 398d PI Signature	Date (DD/MM/YYYY)

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APPENDIX I: CASE DEFINITION FOR ACUTE RETROVIRAL SYNDROME (ARS)

Case definition must include all of the following:

- Occurs within 60 days after diagnosis
- Documented fever (temperature >38.5°C)
- At least two major criteria
- At least two minor criteria
- No other cause for signs and symptoms identified

For severe ARS: at least one major or minor criteria meeting criteria for severe AE per DAIDS grading tables

Major criteria:

- Pharyngitis
- Fatigue
- Morbilliform rash
- Myalgia/arthralgia
- Lymphadenopathy
- Subjective fever as reported by subject (may be intermittent)

Minor criteria:

- Headache
- Nausea/vomiting
- Diarrhea
- Mucocutaneous ulceration (oral, genital, and/or anorectal)
- Meningismus/aseptic meningitis
- Night sweats
- Thrombocytopenia (platelets <150,000)
- Leukopenia (WBC <4,000)
- Malaise
- Abdominal pain
- Weight loss (loss of >5% of body weight)

APPENDIX II: SCHEDULE OF EVALUATIONS

Visit Number		RV217 screening	RV254 screening	1[1]	2	3	4	5	6	7	8	9	10	11	12	13	14	15/exit [7]	Final
Visit Day		−19 to −1	−5 to −1	0	1	3	7	10	14	18	21	25	28	42	56	84	112	168	175
Visit Window (days)				0	1	3 to 4	6 to 8	9 to 11	12 to 16	17 to 19	20 to 23	24 to 26	27 to 34	35 to 49	50 to 63	77 to 91	105 to 119	161 to 174	175 to 196
Visit Type		**RV217 visit 1 [11]**	**RV254 Week 0 [11]**	**ENRL**															
CLINICAL																			
Briefing		x	x																
Test of Understanding				x															
Informed Consent				x															
Physical Exam [2]				x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
Medical history [3]				x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
Contact information and registry form				x															
Enrollment & Randomization				x															
Monoclonal Ab/ Pbo Infusion				**x**															
Positive prevention counseling				x		x	x		x				x					x	x
Pregnancy prevention counseling [4]				x		x	x		x				x					x	x
Solicited Adverse Events (Diary Card)				x	x	x													
ARV medication record				x			x		x		x		x	x	x	x	x	x	
Clinical Labs	Coagulant																		
CBC w/ diff	AC	RV217	RV 254	NB		NB	NB		NB				NB		NB	NB	NB	NB	
Creatinine, ALT/AST/ALP	C	RV217 (Cr/ALT)	RV 254 (Cr/ALT)	NB		NB	NB		NB				NB		NB	NB	NB	NB	
Hepatitis BsAg; Hep C Ab		RV 217	RV 254																
RPR or VDRL	C or AC	RV 217	RV 254																
Pregnancy test; urine or serum [4]		RV217	RV 254	x		x	x		x				x					x	
Fiebig staging (EIA/WB/p24)		RV217		4					4				4						
HIV PCR [10]	AC	RV217	RV 254	6	4	6	6	4	6	4	4	4	6	4	6	6	6	6	
CD4/CD8	AC	RV217	RV 254	NB		NB	NB		NB				NB		NB	NB	NB	NB	
Plasma LH and FSH [5, 9]				NB		NB	NB		NB				NB					NB	
Research																			
PK samples	C			4		2	2		2		2		2		2				
Mucosal secretion collection [4,5]			RV 254	x[12]		x	x		x				x					x	
Rectosigmoid biopsy [5]			RV 254	x[12]					x									x	
Lymph Node biopsy (inguinal) [5]																		x	
Lumbar Puncture(20 ml) [5]			RV 254	x[12]			x											x	
Leukapheresis [5, 14]			RV 254	x[12]			x											x	
Neurocognitive testing		RV217	RV 254															x	
PBMC and plasma for storage and additional testing: reservoir, virologic, and immune assays [8, 15]	AC	RV217	RV 254	68		17	85		25.5				25.5		17	34		153	
Serum for storage and immune assays	C	RV217	RV 254	8		4	4		4		4		4		4	4	4	6	
Daily Volume (mL)		60	70	90	4	29	97	4	41.5	4	10	4	41.5	4	29	44	10	165	0
Cumulative Volume (mL) [6, 13]				150	154	183	280	284	325.5	329.5	339.5	343.5	385	389	418	462	472	637	637

[1] Enrollment visit procedures may encompass two days to allow for optional procedures and/or patient transport. Day 0=day of first product administration. Evaluations prior to VRC01/placebo administration are the baseline for assessing subsequent AEs.
[2] Visit 1 includes physical exam with vital signs, height (ht) and weight (wt). At other visits, if medically indicated, a targeted exam is performed. Otherwise only blood pressure (BP) and temperature are required.
[3] Visit 1 includes past medical history. At other visits only interval medical history will be obtained.
[4] Pregnancy testing for all females at visit 1 and subsequently with invasive procedure or mucosal secretion collection visits for participating females
[5] Optional procedures. Schedule the mucosal sample collection visits for women so that they will occur between menstrual periods; cervical sample collection may be skipped at other mucosal timepoints if blood contamination is likely.
Optional procedures may, at the discretion of the PI, be conducted over 2 days, as long as it fall within the visit window.
Those participants who consent to optional procedures are allowed to select which time points they would like to provide samples as long as the collection is within the visit window for that procedure.
Pre-procedure labs will be drawn according to the requirements of the institutions performing the respective invasive procedures, and the totals listed do not include these volumes.
[6] Residual volume/aliquots will be stored for additional testing and future use
[7] Volunteers will complete Exit visit procedures if they withdraw or are terminated from the study prior to final visit
[8] Includes 100 mL for QVOA at visit 15
[9] Females participating in mucosal secretion collection, from stored samples
[10] Genotype resistance testing will be performed at baseline and as clinically indicated
[11] RV 217 Visit 1 = pertinent study activities for the RV 217 Visit 1 following first positive NAAT. RV 254 Week 0 = shows pertinent study procedures for the RV 254 baseline visit. RV 398 briefs during these visits - remaining activities are conducted under the parent col
]12] Participants from RV217 only
[13] Participants recruited from RV254 in Thailand will have an additional 10 mL tube of blood drawn during RV254 screening, but will have one less 10 mL tube drawn during visit 1 of RV 398 such that the total blood volume drawn throughout the study is the same as all
[14] Approximately 50 mL of red blood cell volume will be lost during apheresis
[15] At visits where leukapheresis is performed, participants who undergo the procedure will only have one 8.5mL tube of blood drawn for "PBMC and plasma for storage and additional testing" instead of the listed blood volume.
If clinical laboratory results from RV 217 visit 1 or RV 254 Week 0 visit are unavailable, they may be drawn at visit 1 of RV 398. If the participant is not enrolled in RV 217 or RV 254, they must sign the ICF prior to collection of screening labs.
NB = No additional blood required - blood from tubes drawn for other tests on the SOE for that visit will be used to perform these assays

APPENDIX III: TEST OF UNDERSTANDING

TEST OF UNDERSTANDING

Please read each question and answer whether the statement is **True** or **False**.

True ☐	False ☐	1. The VRC01 antibody used in this study will protect you from transmitting HIV to others.
True ☐	False ☐	2. You will need to come to the clinic for 15 scheduled visits over the next 25 weeks.
True ☐	False ☐	3. This is the first time that the VRC 01 antibody has been given to participants newly infected with HIV.
True ☐	False ☐	4. One purpose of this study is to determine if VRC01 antibody is safe to administer to humans.
True ☐	False ☐	5. All study participants will receive antiretroviral therapy immediately.
True ☐	False ☐	6. You may take other experimental (test) products while you are taking part in this study.
True ☐	False ☐	7. You may withdraw from the study at any time if you choose or your participation may be stopped if the study team decides it is in your best interest.
True ☐	False ☐	8. Women are asked to not become pregnant while in the study.
True ☐	False ☐	9. Some participants in this study may experience side effects from VRC01 antibody infusion.
True ☐	False ☐	10. Some participants in this study may get placebos or “salt water”.
True ☐	False ☐	11. At the end of this study, I will no longer have access to antiretroviral therapy (ART).
True ☐	False ☐	12. The VRC01 antibody infusion is only administered once during the study.
True ☐	False ☐	13. The VRC01 antibody is a cure for HIV so I will not need ART anymore once I finish the study.

TEST OF UNDERSTANDING

Please read each question and answer whether the statement is **True** or **False**.

True ☐	False ☒	1. The VRC01 antibody used in this study will protect you from transmitting HIV to others.
True ☒	False ☐	2. You will need to come to the clinic for 15 scheduled visits over the next 25 weeks.
True ☒	False ☐	3. This is the first time that the VRC 01 antibody has been given to participants newly infected with HIV.
True ☒	False ☐	4. One purpose of this study is to determine if VRC01 antibody is safe to administer to humans.
True ☐	False ☒	5. All study participants will receive antiretroviral therapy immediately.
True ☐	False ☒	6. You may take other experimental (test) products while you are taking part in this study.
True ☒	False ☐	7. You may withdraw from the study at any time if you choose or your participation may be stopped if the study team decides it is in your best interest.
True ☒	False ☐	8. Women are asked to not become pregnant while in the study.
True ☒	False ☐	9. Some participants in this study may experience side effects from VRC01 antibody infusion.
True ☒	False ☐	10. Some participants in this study may get placebos or “salt water”.
True ☐	False ☒	11. At the end of this study, I will no longer have access to antiretroviral therapy (ART).
True ☒	False ☐	12. The VRC01 antibody infusion is only administered once during the study.
True ☐	False ☒	13. The VRC01 antibody is a cure for HIV so I will not need ART anymore once I finish the study.

ATTACHMENT I: STUDY TEAM ROSTER

STUDY TEAM ROSTER

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ATTACHMENT II: ROLES AND RESPONSIBILITIES

PROTOCOL STUDY TEAM RESPONSIBILITIES

Protocol Chair:
Responsible for study design and serving as a liaison between the sites, the vaccine developers including the Sponsor, and contributing support to overall project management and the analysis and reporting of study data. Dr. Ake will support the site PIs with clinical trial guidance, protocol preparation, protocol review and response to regulatory reviews.

Protocol Co-Chair:
Responsible for study design and serving as a liaison between the sites and the vaccine developers including the Sponsor, and contributing support to overall project management and the analysis and reporting of study data. Dr. Robb will provide support with clinical trial guidance, protocol preparation, protocol review and response to regulatory reviews.

Site Principal Investigators:
Responsible for all aspects of the study at their respective site. Primary individual responsible for individual subject safety.

Associate Investigators: Assist the Protocol Chairs and Principal Investigators in performing specific study tasks and/or procedures under their supervision.

Laboratory Investigators:
Responsible for laboratory analysis. Lab investigators will not have contact with study subjects or identifiers.

Protocol Statistician:
Responsible for statistical analysis for this study. The protocol statistician will not have contact with study subjects.

Overall Study Pharmacist: qualified pharmacist with responsibility for overseeing the import, distribution, inventory, and accountability of vaccine, communication with sponsor / MHRP regarding pharmacy related issues, training and consultation with the study sites' pharmacy personnel.

Protocol Data Management:
Responsible for data management for this study. The data management team will not have contact with study subjects.

DAIDS Medical Officer:
Responsible for protocol development and liason for the submission to the DAIDS Clinical Sciences Review Committee review
The DAIDS medical officer will not have contact with study subjects or identifiers.

DAIDS Program Representative:
Responsible to all applicable regulatory authorities for conduct of the study through exercise of oversight of site PIs, chairs and other investigators. The DAIDS Program

Representative will not have contact with study subjects or identifiers.

U.S. DoD/WRAIR Research Representative:
Responsible for oversight of this study as it affects DoD interests. The US DoD/WRAIR Research Representative will not have contact with study subjects or identifiers.

VRC01 Product Representative:
Responsible for oversight of this study as it affects VRC interests. The VRC01 Product Representative will not have contact with study subjects or identifiers.

ATTACHMENT III: LIST OF COLLABORATORS

RV398 External Collaborator List

Row #	Collaborator	Institution	Work to be done	Sample types used	IRB/ Determination
1	Victor Valcour MD Professor of Medicine Division of Geriatric Medicine and Department of Neurology Memory and Aging Center Vvalcour@memory.ucsf.edu (415) 476-3746; fax: (415) 476-0213	University of California San Francisco	Data analysis	De-identified data	UCSF
2	Serena Spudich, MD Associate Professor of Neurology Yale University Department of Neurology PO Box 208018 New Haven, CT 06520 203-688-5303 serena.spudich@yale.edu	Yale University	Data analysis	De-identified data	Yale
3	Nicolas Chomont, PhD Assistant Professor Universitéde Montréal Centre de recherche du CHUM (CRCHUM) Address: 900, rue St-Denis, Tour Viger Montréal, Québec, Canada	University of Montreal	HIV quantification, microarrays, systems biology, T cell immunology TILDA	Cells/Plasma, lymph nodes	University of Montreal

	H2X 0A9				
4	Adrian McDermott, MSc. Ph.D. Director, Advanced Clinical Testing, Immunology Core Vaccine Research Center, NIAID/NIH 40 Convent Drive, MSC 3015 Building 40, Room 3508 Bethesda, MD 20814 Phone: 301 761-6963 FAX: 301 480-2779	VRC	B cell development VRC01 levels	Cells/Plasma, CSF, lymph nodes, gut biopsies, mucosal specimens	NIH
5	Bob Bailer, Ph.D. Vaccine Immunogenicity Testing Program, Director Vaccine Research Center-NIH/NIAID MDL9BG RM 165C 9 West Watkins Mill Road Gaithersburg, MD 20878 Phone: 301-594-8481 FAX: 240-276-0001	VRC/NVITAL	Cellular and Humoral Immune responses, VRC01 levels, PK	Cells/Plasma	NIH
6	Daniel Douek, M.D., MRCP, Ph.D. Vaccine Research Center Bldg 40 Room 3509 MSC	VRC / NIH	T cell reservoirs/ Pathogen Sequencing/TCR sequencing	Plasma, cells, lymph nodes	NIH

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7	Sarah Palmer, PhD University of Sydney Darcy Road, PO Box 412 Westmead NSW 2145 Australia Tel: +61 2 9845 9107 Email: sarah.palmer@sydney.edu.au	University of Sydney	HIV quantification	Plasma	University of Sydney
8	Hendrik Zetterburg, MD, PhD Institute of Neuroscience and Physiology The Sahlgrenska Academy at the University of Gothenburg S-431 80 Molndal Sweden Tel : +46 31 3430142	University of Gothenburg	Neurofilament	CSF	University of Gothenburg/ waiver
9	Timothy Schacker, MD University of Minnesota 516 Delaware Street Minneapolis, MN 55455 Tel : +1 612 624 9925	University of Minnesota	Immunohistochemistry; in-situ hybridization	Gut/Rectal biopsies and lymph nodes	University of Minnesota / waiver

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13	Claire Deleage, PhD AIDS Cancer Virus Program Frederick National Laboratory for Cancer Research (FNLCR) Leidos Biomedical Research Inc. 105 Boyles Street Bldg. 535, Rm. 424Frederick, MD 21702 Email: Claire.deleage@nih.gov	National Cancer Institute Frederick/Leidos Biomedical Research, Inc.	Immunohistochemsitry; in-situ hybridization	Gut/Rectal biopsies and lymph nodes	National Cancer Institue (NCI)

ATTACHMENT IV: DAIDS AE GRADING TABLE

Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events

Version 2.0
November 2014

Division of AIDS
National Institute of Allergy and Infectious Diseases
National Institutes of Health
US Department of Health and Human Services

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Glossary and Acronyms

AE	Adverse event; Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medical treatment or procedure regardless of whether it is considered related to the medical treatment or procedure.
ALT (SGPT)	Alanine aminotransferase (serum glutamic pyruvic transaminase)
ANC	Absolute neutrophil count
AST (SGOT)	Aspartate aminotransferase (serum glutamic-oxaloacetic transaminase)
AV	Atrioventricular
Basic Self-care Functions	Adult Activities such as bathing, dressing, toileting, transfer or movement, continence, and feeding. Young Children Activities that are age and culturally appropriate, such as feeding one's self with culturally appropriate eating implements.
BMI z-score	Body mass index z- score; A body reference norm. Specifically, the number of standard deviations a participant's BMI differs from the average BMI for their age, sex, and ethnicity.
BMD t-score	Bone mineral density t-score; The number of standard deviations above or below the mean bone mineral density of a healthy 30 year old adult of the same sex and ethnicity as the participant.
BMD z-score	Bone mineral density z-score; The number of standard deviations a participant's BMD differs from the average BMD for their age, sex, and ethnicity.
BPAP	Bilevel positive airway pressure; A mode used during noninvasive positive pressure ventilation.
Chemical Pregnancy	A pregnancy in which a positive pregnancy test is followed by a negative pregnancy test without evidence of a clinical pregnancy loss.
CNS	Central nervous system
CPAP	Continuous positive airway pressure
DAERS	DAIDS Adverse Experience Reporting System; An internet-based system developed for clinical research sites to report Expedited Adverse Events (EAEs) to DAIDS. It facilitates timely EAE report submission and serves as a centralized location for accessing and processing EAE information for reporting purposes.
Disability	A substantial disruption of a person's ability to conduct normal life functions.
ECG	Electrocardiogram
eGFR	Estimated glomerular filtration rate
Hospitalization	Does not include the following hospital admissions: under 24 hours, unrelated to an adverse event (e.g., for labor and delivery, cosmetic surgery, social or administrative for temporary placement [for lack of a place to sleep]), protocol-specified, and for diagnosis or therapy of a condition that existed before the receipt of a study agent and which has not increased in severity or frequency.
INR	International normalized ratio

Glossary and Acronyms

Intervention	Medical, surgical, or other procedures recommended or provided by a healthcare professional for the treatment of an adverse event.
IV	Intravenous
IVIG	Intravenous immune globulin
LDL	Low density lipoprotein
LLN	Lower limit of normal
Life-threatening AE	Any adverse event that places the participant, in the view of the investigator, at immediate risk of death from the reaction when it occurred (i.e., it does not include a reaction that would have caused death if it had occurred in a more severe form).
NA	Not applicable
Participant ID	The identification number assigned to a study participant which is used to track study-related documentation, including any reported AEs.
PR Interval	The interval between the beginning of the P wave and the beginning of the QRS complex of an electrocardiogram that represents the time between the beginning of the contraction of the atria and the beginning of the contraction of the ventricles.
PT	Prothrombin time
PTT	Partial thromboplastin time
QTc Interval	The measure of time between the onset of ventricular depolarization and completion of ventricular repolarization corrected for ventricular rate.
RBC	Red blood cell
SI	Standard international unit
ULN	Upper limit of normal
Usual Social & Functional Activities	Activities which adults and children perform on a routine basis and those which are part of regular activities of daily living, for example: <u>Adults</u> Adaptive tasks and desirable activities, such as going to work, shopping, cooking, use of transportation, or pursuing a hobby. <u>Young Children</u> Activities that are age and culturally appropriate, such as social interactions, play activities, or learning tasks.
WBC	White blood cell
WHO	World Health Organization
WNL	Within normal limits

Introduction

The Division of AIDS (DAIDS) oversees clinical trials throughout the world which it sponsors and supports. The clinical trials evaluate the safety and efficacy of therapeutic products, vaccines, and other preventive modalities. Adverse event (AE) data collected during these clinical trials form the basis for subsequent safety and efficacy analyses of pharmaceutical products and medical devices. Incorrect and inconsistent AE severity grading can lead to inaccurate data analyses and interpretation, which in turn can impact the safety and well-being of clinical trial participants and future patients using pharmaceutical products.

The DAIDS AE grading table is a shared tool for assessing the severity of AEs (including clinical and laboratory abnormalities) in participants enrolled in clinical trials. Over the years as scientific knowledge and experience have expanded, revisions to the DAIDS AE grading table have become necessary.

The *Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2.0* replaces the grading table published in 2004 and updated in 2009. In version 2.0, AEs not previously included, but which now are deemed medically important events, are included while other AEs have been removed. Some AE severity grading descriptions have been revised to more appropriately reflect the presentation of these events in clinical settings and their impact on clinical trials. For example, DAIDS performed an extensive literature search and reviews of select DAIDS clinical trial data in revising certain hematology parameters (i.e., hemoglobin, white cell counts, and absolute neutrophil counts). DAIDS also took into consideration the U.S. Food and Drug Administration's guidance regarding the use of local laboratory reference values and ethnic differences among certain healthy adolescent and adult populations in defining parameter limits. Finally, the revised DAIDS AE grading table also contains an updated glossary and acronyms section, an expanded instructions for use section, and an appendix that provides more age-specific information for an AE of concern to DAIDS.

DAIDS is grateful to the DAIDS Grading Table Working Group, numerous government and non-government affiliated medical subject matter experts and reviewers who were instrumental in the revision of the DAIDS AE grading table.

Instructions for Use

General Considerations

The *Division of AIDS* (*DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2.0* consists of parameters, or AEs, with severity grading guidance that are to be used in DAIDS clinical trials for safety data reporting to maintain accuracy and consistency in the evaluation of AEs. The term "severe" is not the same as the term "serious" in classifying AEs. The severity of a specific event describes its intensity, and it is the intensity which is graded. Seriousness, which is not graded, relates to an outcome of an AE and is a regulatory definition.

Clinical sites are encouraged to report parameters in the DAIDS AE grading table as they are written to maintain data consistency across clinical trials. However, since some parameters can be reported with more specificity, clinical sites are encouraged to report parameters that convey additional clinical information. For example, diarrhea could be reported as neonatal diarrhea; seizures, as febrile seizures; and pain, as jaw pain.

The DAIDS AE grading table provides an AE severity grading scale ranging from grades 1 to 5 with descriptions for each AE based on the following general guidelines:

- Grade 1 indicates a mild event
- Grade 2 indicates a moderate event
- Grade 3 indicates a severe event
- Grade 4 indicates a potentially life-threatening event
- Grade 5 indicates death (*Note*: This grade is not specifically listed on each page of the grading table).

Other points to consider include:

- Use parameters defined by age and sex values as applicable.
- Male and female sex are defined as sex at birth.
- Unless noted, laboratory values are for term neonates. Preterm neonates should be assessed using local laboratory normal ranges.
- Where applicable, Standard International (SI) units are included in italics.

Selecting and Reporting a Primary AE Term

When selecting a primary AE term to report, sites should select the term that best describes what occurred to the participant. For example, a participant may present with itching, urticaria, flushing, angioedema of the face, and dyspnea. If the underlying diagnosis is determined to be an acute allergic reaction, sites should report "Acute Allergic Reaction" as the primary AE term.

Primary AE terms should be reported using the DAIDS Adverse Experience Reporting System (DAERS) only if they meet expedited reporting criteria. However, all primary AE terms should be reported using protocol-specific case report forms (CRFs). Because the reported information is stored in different databases (i.e., safety and clinical), sites should report primary AE terms using the same terminology for data consistency.

Instructions for Use

When reporting using DAERS, other clinically significant events associated with a primary AE term that more fully describe the nature, severity, or complications of the primary AE term should be entered in the "Other Events" section. However, the severity grade for these events must be lower than or equal to the severity grade of the primary AE term. In the example above, dyspnea and angioedema of the face may be entered in the "Other Events" section, because they are more descriptive and provide additional information on the severity of the acute allergic reaction. However, their severity grades must be lower than or equal to the severity grade of the primary AE term of "Acute Allergic Reaction".

Differences exist in the reporting and recording of information (e.g., signs and symptoms, clinically significant events) in DAERS and CRFs. Therefore, sites should refer to their protocols and CRF requirements for further instructions.

Grading Adult and Pediatric AEs
When a single parameter is not appropriate for grading an AE in both adult and pediatric populations, separate parameters with specified age ranges are provided. If no distinction between adult and pediatric populations has been made, the listed parameter should be used for grading an AE in both populations.

Reporting Pregnancy Outcomes
In the *Pregnancy, Puerperium, and Perinatal* section, all parameters are pregnancy outcomes and should be reported using the mother's participant ID. If an infant is not enrolled in the same study as the mother, any identified birth defects should be reported using the mother's participant ID. However, if an infant is enrolled in the same study as the mother or in another study, any identified birth defects should be reported using the infant's participant ID. Sites should refer to the applicable network standards for reporting abnormal pregnancy outcomes on the CRFs.

Determining Severity Grade for Parameters between Grades
If the severity of an AE could fall in either one of two grades (i.e., the severity of an AE could be either grade 2 or grade 3), sites should select the higher of the two grades.

Laboratory Values
General. An asymptomatic, abnormal laboratory finding without an accompanying AE should not be reported to DAIDS in an expedited timeframe unless it meets protocol-specific reporting requirements. Sites should refer to the applicable network standards for reporting abnormal laboratory findings on the CRFs.

Values below Grade 1. Any laboratory value that is between the ULN and grade 1 (for high values) or the LLN and grade 1 (for low values) should not be graded or reported as an AE. Sites should consult the *Manual for Expedited Reporting of Adverse Events to DAIDS, Version 2.0* and their protocol when making an assessment of the need to report an AE.

Overlap of Local Laboratory Normal Values with Grading Table Ranges. When local laboratory normal values fall within grading table laboratory ranges, the severity grading is based on the ranges in the grading table unless there is a protocol-specific grading criterion for the laboratory

value. For example, “Magnesium, Low" has a grade 1 range of 1.2 to < 1.4 mEq/L, while a particular laboratory’s normal range for magnesium may be 1.3 to 2.8 mEq/L. If a study participant’s magnesium laboratory value is 1.3 mEq/L, the laboratory value should be graded as grade 1.

Appendix Usage
Appendix A takes priority over the main grading table in all assessments of total bilirubin for term and preterm neonates.

Using Addenda 1-3: Grading Tables Used in Microbicide Studies
In protocols involving topical application of products to the female and male genital tracts or rectum, strong consideration should be given to using Addenda 1-3 (see below) as the primary grading tables for these areas. Although these grading tables are used specifically in microbicide studies, they may be used in other protocols as adjuncts to the main grading table (i.e., the *Division of AIDS (AIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Version 2.0*). It should be clearly stated in a protocol which addendum is being used as the primary grading table (and thus takes precedence over the main grading table) and which addendum is being used in a complementary fashion.

- Addendum 1 – Female Genital Grading Table for Use in Microbicide Studies – PDF
- Addendum 2 – Male Genital Grading Table for Use in Microbicide Studies – PDF
- Addendum 3 – Rectal Grading Table for Use in Microbicide Studies – PDF

Estimating Severity Grade for Parameters Not Identified in the Grading Table
The functional table below should be used to grade the severity of an AE that is not specifically identified in the grading table. In addition, all deaths related to an AE are to be classified as grade 5.

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Clinical adverse event **NOT** identified elsewhere in the grading table	Mild symptoms causing no or minimal interference with usual social & functional activities with intervention not indicated	Moderate symptoms causing greater than minimal interference with usual social & functional activities with intervention indicated	Severe symptoms causing inability to perform usual social & functional activities with intervention or hospitalization indicated	Potentially life-threatening symptoms causing inability to perform basic self-care functions with intervention indicated to prevent permanent impairment, persistent disability, or death

Major Clinical Conditions
Cardiovascular

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Arrhythmia (by ECG or physical examination) *Specify type, if applicable*	No symptoms AND No intervention indicated	No symptoms AND Non-urgent intervention indicated	Non-life-threatening symptoms AND Non-urgent intervention indicated	Life-threatening arrhythmia OR Urgent intervention indicated
Blood Pressure Abnormalities[1]				
Hypertension *(with the lowest reading taken after repeat testing during a visit)* *≥ 18 years of age*	140 to < 160 mmHg systolic OR 90 to < 100 mmHg diastolic	≥ 160 to < 180 mmHg systolic OR ≥ 100 to < 110 mmHg diastolic	≥ 180 mmHg systolic OR ≥ 110 mmHg diastolic	Life-threatening consequences in a participant not previously diagnosed with hypertension (e.g., malignant hypertension) OR Hospitalization indicated
< 18 years of age	> 120/80 mmHg	≥ 95th to < 99th percentile + 5 mmHg adjusted for age, height, and gender (systolic and/or diastolic)	≥ 99th percentile + 5 mmHg adjusted for age, height, and gender (systolic and/or diastolic)	Life-threatening consequences in a participant not previously diagnosed with hypertension (e.g., malignant hypertension) OR Hospitalization indicated
Hypotension	No symptoms	Symptoms corrected with oral fluid replacement	Symptoms AND IV fluids indicated	Shock requiring use of vasopressors or mechanical assistance to maintain blood pressure
Cardiac Ischemia or Infarction *Report only one*	NA	NA	New symptoms with ischemia (stable angina) OR New testing consistent with ischemia	Unstable angina OR Acute myocardial infarction
Heart Failure	No symptoms AND Laboratory or cardiac imaging abnormalities	Symptoms with mild to moderate activity or exertion	Symptoms at rest or with minimal activity or exertion (e.g., hypoxemia) OR Intervention indicated (e.g., oxygen)	Life-threatening consequences OR Urgent intervention indicated (e.g., vasoactive medications, ventricular assist device, heart transplant)
Hemorrhage (with significant acute blood loss)	NA	Symptoms AND No transfusion indicated	Symptoms AND Transfusion of ≤ 2 units packed RBCs indicated	Life-threatening hypotension OR Transfusion of > 2 units packed RBCs (for children, packed RBCs > 10 cc/kg) indicated

[1] Blood pressure norms for children < 18 years of age can be found in: Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents. *Pediatrics* 2011;128;S213; originally published online November 14, 2011; DOI: 10.1542/peds.2009-2107C.

Cardiovascular

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Prolonged PR Interval or AV Block *Report only one* *> 16 years of age*	PR interval 0.21 to < 0.25 seconds	PR interval ≥ 0.25 seconds OR Type I 2nd degree AV block	Type II 2nd degree AV block OR Ventricular pause ≥ 3.0 seconds	Complete AV block
≤ 16 years of age	1st degree AV block (PR interval > normal for age and rate)	Type I 2nd degree AV block	Type II 2nd degree AV block OR Ventricular pause ≥ 3.0 seconds	Complete AV block
Prolonged QTc Interval[2]	0.45 to 0.47 seconds	> 0.47 to 0.50 seconds	> 0.50 seconds OR ≥ 0.06 seconds above baseline	Life-threatening consequences (e.g., Torsade de pointes, other associated serious ventricular dysrhythmia)
Thrombosis or Embolism *Report only one*	NA	Symptoms AND No intervention indicated	Symptoms AND Intervention indicated	Life-threatening embolic event (e.g., pulmonary embolism, thrombus)

[2] As per Bazett's formula.

Dermatologic

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Alopecia (scalp only)	Detectable by study participant, caregiver, or physician AND Causing no or minimal interference with usual social & functional activities	Obvious on visual inspection AND Causing greater than minimal interference with usual social & functional activities	NA	NA
Bruising	Localized to one area	Localized to more than one area	Generalized	NA
Cellulitis	NA	Non-parenteral treatment indicated (e.g., oral antibiotics, antifungals, antivirals)	IV treatment indicated (e.g., IV antibiotics, antifungals, antivirals)	Life-threatening consequences (e.g., sepsis, tissue necrosis)
Hyperpigmentation	Slight or localized causing no or minimal interference with usual social & functional activities	Marked or generalized causing greater than minimal interference with usual social & functional activities	NA	NA
Hypopigmentation	Slight or localized causing no or minimal interference with usual social & functional activities	Marked or generalized causing greater than minimal interference with usual social & functional activities	NA	NA
Petechiae	Localized to one area	Localized to more than one area	Generalized	NA
Pruritus[3] (without skin lesions)	Itching causing no or minimal interference with usual social & functional activities	Itching causing greater than minimal interference with usual social & functional activities	Itching causing inability to perform usual social & functional activities	NA
Rash *Specify type, if applicable*	Localized rash	Diffuse rash OR Target lesions	Diffuse rash AND Vesicles or limited number of bullae or superficial ulcerations of mucous membrane limited to one site	Extensive or generalized bullous lesions OR Ulceration of mucous membrane involving two or more distinct mucosal sites OR Stevens-Johnson syndrome OR Toxic epidermal necrolysis

[3] For pruritus associated with injections or infusions, see the *Site Reactions to Injections and Infusions* section (page 23).

Endocrine and Metabolic

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Diabetes Mellitus	Controlled without medication	Controlled with medication OR Modification of current medication regimen	Uncontrolled despite treatment modification OR Hospitalization for immediate glucose control indicated	Life-threatening consequences (e.g., ketoacidosis, hyperosmolar non-ketotic coma, end organ failure)
Gynecomastia	Detectable by study participant, caregiver, or physician AND Causing no or minimal interference with usual social & functional activities	Obvious on visual inspection AND Causing pain with greater than minimal interference with usual social & functional activities	Disfiguring changes AND Symptoms requiring intervention or causing inability to perform usual social & functional activities	NA
Hyperthyroidism	No symptoms AND Abnormal laboratory value	Symptoms causing greater than minimal interference with usual social & functional activities OR Thyroid suppression therapy indicated	Symptoms causing inability to perform usual social & functional activities OR Uncontrolled despite treatment modification	Life-threatening consequences (e.g., thyroid storm)
Hypothyroidism	No symptoms AND Abnormal laboratory value	Symptoms causing greater than minimal interference with usual social & functional activities OR Thyroid replacement therapy indicated	Symptoms causing inability to perform usual social & functional activities OR Uncontrolled despite treatment modification	Life-threatening consequences (e.g., myxedema coma)
Lipoatrophy[4]	Detectable by study participant, caregiver, or physician AND Causing no or minimal interference with usual social & functional activities	Obvious on visual inspection AND Causing greater than minimal interference with usual social & functional activities	Disfiguring changes	NA
Lipohypertrophy[5]	Detectable by study participant, caregiver, or physician AND Causing no or minimal interference with usual social & functional activities	Obvious on visual inspection AND Causing greater than minimal interference with usual social & functional activities	Disfiguring changes	NA

[4] Definition: A disorder characterized by fat loss in the face, extremities, and buttocks.
[5] Definition: A disorder characterized by abnormal fat accumulation on the back of the neck, breasts, and abdomen.

Gastrointestinal

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Anorexia	Loss of appetite without decreased oral intake	Loss of appetite associated with decreased oral intake without significant weight loss	Loss of appetite associated with significant weight loss	Life-threatening consequences OR Aggressive intervention indicated (e.g., tube feeding, total parenteral nutrition)
Ascites	No symptoms	Symptoms AND Intervention indicated (e.g., diuretics, therapeutic paracentesis)	Symptoms recur or persist despite intervention	Life-threatening consequences
Bloating or Distension *Report only one*	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	NA
Cholecystitis	NA	Symptoms AND Medical intervention indicated	Radiologic, endoscopic, or operative intervention indicated	Life-threatening consequences (e.g., sepsis, perforation)
Constipation	NA	Persistent constipation requiring regular use of dietary modifications, laxatives, or enemas	Obstipation with manual evacuation indicated	Life-threatening consequences (e.g., obstruction)
Diarrhea *≥ 1 year of age*	Transient or intermittent episodes of unformed stools OR Increase of ≤ 3 stools over baseline per 24-hour period	Persistent episodes of unformed to watery stools OR Increase of 4 to 6 stools over baseline per 24-hour period	Increase of ≥ 7 stools per 24-hour period OR IV fluid replacement indicated	Life-threatening consequences (e.g., hypotensive shock)
< 1 year of age	Liquid stools (more unformed than usual) but usual number of stools	Liquid stools with increased number of stools OR Mild dehydration	Liquid stools with moderate dehydration	Life-threatening consequences (e.g., liquid stools resulting in severe dehydration, hypotensive shock)
Dysphagia or Odynophagia *Report only one and specify location*	Symptoms but able to eat usual diet	Symptoms causing altered dietary intake with no intervention indicated	Symptoms causing severely altered dietary intake with intervention indicated	Life-threatening reduction in oral intake
Gastrointestinal Bleeding	Not requiring intervention other than iron supplement	Endoscopic intervention indicated	Transfusion indicated	Life-threatening consequences (e.g., hypotensive shock)

Gastrointestinal

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Mucositis or Stomatitis *Report only one and specify location*	Mucosal erythema	Patchy pseudomembranes or ulcerations	Confluent pseudomembranes or ulcerations OR Mucosal bleeding with minor trauma	Life-threatening consequences (e.g., aspiration, choking) OR Tissue necrosis OR Diffuse spontaneous mucosal bleeding
Nausea	Transient (< 24 hours) or intermittent AND No or minimal interference with oral intake	Persistent nausea resulting in decreased oral intake for 24 to 48 hours	Persistent nausea resulting in minimal oral intake for > 48 hours OR Rehydration indicated (e.g., IV fluids)	Life-threatening consequences (e.g., hypotensive shock)
Pancreatitis	NA	Symptoms with hospitalization not indicated	Symptoms with hospitalization indicated	Life-threatening consequences (e.g., circulatory failure, hemorrhage, sepsis)
Perforation (colon or rectum)	NA	NA	Intervention indicated	Life-threatening consequences
Proctitis	Rectal discomfort with no intervention indicated	Symptoms causing greater than minimal interference with usual social & functional activities OR Medical intervention indicated	Symptoms causing inability to perform usual social & functional activities OR Operative intervention indicated	Life-threatening consequences (e.g., perforation)
Rectal Discharge	Visible discharge	Discharge requiring the use of pads	NA	NA
Vomiting	Transient or intermittent AND No or minimal interference with oral intake	Frequent episodes with no or mild dehydration	Persistent vomiting resulting in orthostatic hypotension OR Aggressive rehydration indicated (e.g., IV fluids)	Life-threatening consequences (e.g., hypotensive shock)

Musculoskeletal

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Arthralgia	Joint pain causing no or minimal interference with usual social & functional activities	Joint pain causing greater than minimal interference with usual social & functional activities	Joint pain causing inability to perform usual social & functional activities	Disabling joint pain causing inability to perform basic self-care functions
Arthritis	Stiffness or joint swelling causing no or minimal interference with usual social & functional activities	Stiffness or joint swelling causing greater than minimal interference with usual social & functional activities	Stiffness or joint swelling causing inability to perform usual social & functional activities	Disabling joint stiffness or swelling causing inability to perform basic self-care functions
Myalgia (generalized)	Muscle pain causing no or minimal interference with usual social & functional activities	Muscle pain causing greater than minimal interference with usual social & functional activities	Muscle pain causing inability to perform usual social & functional activities	Disabling muscle pain causing inability to perform basic self-care functions
Osteonecrosis	NA	No symptoms but with radiographic findings AND No operative intervention indicated	Bone pain with radiographic findings OR Operative intervention indicated	Disabling bone pain with radiographic findings causing inability to perform basic self-care functions
Osteopenia[6] *≥ 30 years of age*	BMD t-score -2.5 to -1	NA	NA	NA
< 30 years of age	BMD z-score -2 to -1	NA	NA	NA
Osteoporosis[6] *≥ 30 years of age*	NA	BMD t-score < -2.5	Pathologic fracture (e.g., compression fracture causing loss of vertebral height)	Pathologic fracture causing life-threatening consequences
< 30 years of age	NA	BMD z-score < -2	Pathologic fracture (e.g., compression fracture causing loss of vertebral height)	Pathologic fracture causing life-threatening consequences

[6] BMD t and z scores can be found in: Kanis JA on behalf of the World Health Organization Scientific Group (2007). Assessment of osteoporosis at the primary health-care level. Technical Report. World Health Organization Collaborating Centre for Metabolic Bone Diseases, University of Sheffield, UK. 2007: Printed by the University of Sheffield.

Neurologic

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Acute CNS Ischemia	NA	NA	Transient ischemic attack	Cerebral vascular accident (e.g., stroke with neurological deficit)
Altered Mental Status (for Dementia, see *Cognitive, Behavioral, or Attentional Disturbance* below)	Changes causing no or minimal interference with usual social & functional activities	Mild lethargy or somnolence causing greater than minimal interference with usual social & functional activities	Confusion, memory impairment, lethargy, or somnolence causing inability to perform usual social & functional activities	Delirium OR Obtundation OR Coma
Ataxia	Symptoms causing no or minimal interference with usual social & functional activities OR No symptoms with ataxia detected on examination	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Disabling symptoms causing inability to perform basic self-care functions
Cognitive, Behavioral, or Attentional Disturbance (includes dementia and attention deficit disorder) *Specify type, if applicable*	Disability causing no or minimal interference with usual social & functional activities OR Specialized resources not indicated	Disability causing greater than minimal interference with usual social & functional activities OR Specialized resources on part-time basis indicated	Disability causing inability to perform usual social & functional activities OR Specialized resources on a full-time basis indicated	Disability causing inability to perform basic self-care functions OR Institutionalization indicated
Developmental Delay *< 18 years of age* *Specify type, if applicable*	Mild developmental delay, either motor or cognitive, as determined by comparison with a developmental screening tool appropriate for the setting	Moderate developmental delay, either motor or cognitive, as determined by comparison with a developmental screening tool appropriate for the setting	Severe developmental delay, either motor or cognitive, as determined by comparison with a developmental screening tool appropriate for the setting	Developmental regression, either motor or cognitive, as determined by comparison with a developmental screening tool appropriate for the setting
Headache	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Symptoms causing inability to perform basic self-care functions OR Hospitalization indicated OR Headache with significant impairment of alertness or other neurologic function

Neurologic

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Neuromuscular Weakness (includes myopathy and neuropathy) *Specify type, if applicable*	Minimal muscle weakness causing no or minimal interference with usual social & functional activities OR No symptoms with decreased strength on examination	Muscle weakness causing greater than minimal interference with usual social & functional activities	Muscle weakness causing inability to perform usual social & functional activities	Disabling muscle weakness causing inability to perform basic self-care functions OR Respiratory muscle weakness impairing ventilation
Neurosensory Alteration (includes paresthesia and painful neuropathy) *Specify type, if applicable*	Minimal paresthesia causing no or minimal interference with usual social & functional activities OR No symptoms with sensory alteration on examination	Sensory alteration or paresthesia causing greater than minimal interference with usual social & functional activities	Sensory alteration or paresthesia causing inability to perform usual social & functional activities	Disabling sensory alteration or paresthesia causing inability to perform basic self-care functions
Seizures ***New Onset Seizure*** *≥ 18 years of age*	NA	NA	1 to 3 seizures	Prolonged and repetitive seizures (e.g., status epilepticus) OR Difficult to control (e.g., refractory epilepsy)
< 18 years of age (includes new or pre-existing febrile seizures)	Seizure lasting < 5 minutes with < 24 hours postictal state	Seizure lasting 5 to < 20 minutes with < 24 hours postictal state	Seizure lasting ≥ 20 minutes OR > 24 hours postictal state	Prolonged and repetitive seizures (e.g., status epilepticus) OR Difficult to control (e.g., refractory epilepsy)
Pre-existing Seizure	NA	Increased frequency from previous level of control without change in seizure character	Change in seizure character either in duration or quality (e.g., severity or focality)	Prolonged and repetitive seizures (e.g., status epilepticus) OR Difficult to control (e.g., refractory epilepsy)
Syncope	Near syncope without loss of consciousness (e.g., pre-syncope)	Loss of consciousness with no intervention indicated	Loss of consciousness AND Hospitalization or intervention required	NA

Pregnancy, Puerperium, and Perinatal

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Fetal Death or Stillbirth (report using mother's participant ID) *Report only one*	NA	NA	Fetal loss occurring at ≥ 20 weeks gestation	NA
Preterm Delivery[7] (report using mother's participant ID)	Delivery at 34 to < 37 weeks gestational age	Delivery at 28 to < 34 weeks gestational age	Delivery at 24 to < 28 weeks gestational age	Delivery at < 24 weeks gestational age
Spontaneous Abortion or Miscarriage[8] (report using mother's participant ID) *Report only one*	Chemical pregnancy	Uncomplicated spontaneous abortion or miscarriage	Complicated spontaneous abortion or miscarriage	NA

[7] Definition: A delivery of a live-born neonate occurring at ≥ 20 to < 37 weeks gestational age.
[8] Definition: A clinically recognized pregnancy occurring at < 20 weeks gestational age.

Psychiatric

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Insomnia	Mild difficulty falling asleep, staying asleep, or waking up early	Moderate difficulty falling asleep, staying asleep, or waking up early	Severe difficulty falling asleep, staying asleep, or waking up early	NA
Psychiatric Disorders (includes anxiety, depression, mania, and psychosis) *Specify disorder*	Symptoms with intervention not indicated OR Behavior causing no or minimal interference with usual social & functional activities	Symptoms with intervention indicated OR Behavior causing greater than minimal interference with usual social & functional activities	Symptoms with hospitalization indicated OR Behavior causing inability to perform usual social & functional activities	Threatens harm to self or others OR Acute psychosis OR Behavior causing inability to perform basic self-care functions
Suicidal Ideation or Attempt *Report only one*	Preoccupied with thoughts of death AND No wish to kill oneself	Preoccupied with thoughts of death AND Wish to kill oneself with no specific plan or intent	Thoughts of killing oneself with partial or complete plans but no attempt to do so OR Hospitalization indicated	Suicide attempted

Respiratory

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Acute Bronchospasm	Forced expiratory volume in 1 second or peak flow reduced to ≥ 70 to < 80% OR Mild symptoms with intervention not indicated	Forced expiratory volume in 1 second or peak flow 50 to < 70% OR Symptoms with intervention indicated OR Symptoms causing greater than minimal interference with usual social & functional activities	Forced expiratory volume in 1 second or peak flow 25 to < 50% OR Symptoms causing inability to perform usual social & functional activities	Forced expiratory volume in 1 second or peak flow < 25% OR Life-threatening respiratory or hemodynamic compromise OR Intubation
Dyspnea or Respiratory Distress *Report only one*	Dyspnea on exertion with no or minimal interference with usual social & functional activities OR Wheezing OR Minimal increase in respiratory rate for age	Dyspnea on exertion causing greater than minimal interference with usual social & functional activities OR Nasal flaring OR Intercostal retractions OR Pulse oximetry 90 to < 95%	Dyspnea at rest causing inability to perform usual social & functional activities OR Pulse oximetry < 90%	Respiratory failure with ventilator support indicated (e.g., CPAP, BPAP, intubation)

Sensory

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Hearing Loss *≥ 12 years of age*	NA	Hearing aid or intervention not indicated	Hearing aid or intervention indicated	Profound bilateral hearing loss (> 80 dB at 2 kHz and above) OR Non-serviceable hearing (i.e., >50 dB audiogram and <50% speech discrimination)
< 12 years of age (based on a 1, 2, 3, 4, 6 and 8 kHz audiogram)	> 20 dB hearing loss at ≤ 4 kHz	> 20 dB hearing loss at > 4 kHz	> 20 dB hearing loss at ≥ 3 kHz in one ear with additional speech language related services indicated (where available) OR Hearing loss sufficient to indicate therapeutic intervention, including hearing aids	Audiologic indication for cochlear implant and additional speech-language related services indicated (where available)
Tinnitus	Symptoms causing no or minimal interference with usual social & functional activities with intervention not indicated	Symptoms causing greater than minimal interference with usual social & functional activities with intervention indicated	Symptoms causing inability to perform usual social & functional activities	NA
Uveitis	No symptoms AND Detectable on examination	Anterior uveitis with symptoms OR Medicamylasal intervention indicated	Posterior or pan-uveitis OR Operative intervention indicated	Disabling visual loss in affected eye(s)
Vertigo	Vertigo causing no or minimal interference with usual social & functional activities	Vertigo causing greater than minimal interference with usual social & functional activities	Vertigo causing inability to perform usual social & functional activities	Disabling vertigo causing inability to perform basic self-care functions
Visual Changes (assessed from baseline)	Visual changes causing no or minimal interference with usual social & functional activities	Visual changes causing greater than minimal interference with usual social & functional activities	Visual changes causing inability to perform usual social & functional activities	Disabling visual loss in affected eye(s)

Systemic

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Acute Allergic Reaction	Localized urticaria (wheals) with no medical intervention indicated	Localized urticaria with intervention indicated OR Mild angioedema with no intervention indicated	Generalized urticaria OR Angioedema with intervention indicated OR Symptoms of mild bronchospasm	Acute anaphylaxis OR Life-threatening bronchospasm OR Laryngeal edema
Chills	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	NA
Cytokine Release Syndrome[9]	Mild signs and symptoms AND Therapy (i.e., antibody infusion) interruption not indicated	Therapy (i.e., antibody infusion) interruption indicated AND Responds promptly to symptomatic treatment OR Prophylactic medications indicated for ≤ 24 hours	Prolonged severe signs and symptoms OR Recurrence of symptoms following initial improvement	Life-threatening consequences (e.g., requiring pressor or ventilator support)
Fatigue or Malaise *Report only one*	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Incapacitating symptoms of fatigue or malaise causing inability to perform basic self-care functions
Fever (non-axillary temperatures only)	38.0 to < 38.6°C or 100.4 to < 101.5°F	≥ 38.6 to < 39.3°C or ≥ 101.5 to < 102.7°F	≥ 39.3 to < 40.0°C or ≥ 102.7 to < 104.0°F	≥ 40.0°C or ≥ 104.0°F
Pain[10] (not associated with study agent injections and not specified elsewhere) *Specify location*	Pain causing no or minimal interference with usual social & functional activities	Pain causing greater than minimal interference with usual social & functional activities	Pain causing inability to perform usual social & functional activities	Disabling pain causing inability to perform basic self-care functions OR Hospitalization indicated
Serum Sickness[11]	Mild signs and symptoms	Moderate signs and symptoms AND Intervention indicated (e.g., antihistamines)	Severe signs and symptoms AND Higher level intervention indicated (e.g., steroids or IV fluids)	Life-threatening consequences (e.g., requiring pressor or ventilator support)

[9] Definition: A disorder characterized by nausea, headache, tachycardia, hypotension, rash, and/or shortness of breath.
[10] For pain associated with injections or infusions, see the *Site Reactions to Injections and Infusions* section (page 23).
[11] Definition: A disorder characterized by fever, arthralgia, myalgia, skin eruptions, lymphadenopathy, marked discomfort, and/or dyspnea.

Systemic

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Underweight[12] *> 5 to 19 years of age*	NA	WHO BMI z-score < -2 to ≤ -3	WHO BMI z-score < -3	WHO BMI z-score < -3 with life-threatening consequences
2 to 5 years of age	NA	WHO Weight-for-height z-score < -2 to ≤ -3	WHO Weight-for-height z-score < -3	WHO Weight-for-height z-score < -3 with life-threatening consequences
< 2 years of age	NA	WHO Weight-for-length z-score < -2 to ≤ -3	WHO Weight-for-length z-score < -3	WHO Weight-for-length z-score < -3 with life-threatening consequences
Weight Loss (excludes postpartum weight loss)	NA	5 to < 9% loss in body weight from baseline	≥ 9 to < 20% loss in body weight from baseline	≥ 20% loss in body weight from baseline OR Aggressive intervention indicated (e.g., tube feeding, total parenteral nutrition)

[12] WHO reference tables may be accesed by clicking the desired age range or by accessing the following URLs: http://www.who.int/growthref/who2007_bmi_for_age/en/ for participants > 5 to 19 years of age and http://www.who.int/childgrowth/standards/chart_catalogue/en/ for those ≤ 5 years of age.

Urinary

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Urinary Tract Obstruction	NA	Signs or symptoms of urinary tract obstruction without hydronephrosis or renal dysfunction	Signs or symptoms of urinary tract obstruction with hydronephrosis or renal dysfunction	Obstruction causing life-threatening consequences

Site Reactions to Injections and Infusions

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Injection Site Pain or Tenderness *Report only one*	Pain or tenderness causing no or minimal limitation of use of limb	Pain or tenderness causing greater than minimal limitation of use of limb	Pain or tenderness causing inability to perform usual social & functional activities	Pain or tenderness causing inability to perform basic self-care function OR Hospitalization indicated
Injection Site Erythema or Redness[13] *Report only one* *> 15 years of age*	2.5 to < 5 cm in diameter OR 6.25 to < 25 cm^2 surface area AND Symptoms causing no or minimal interference with usual social & functional activities	≥ 5 to < 10 cm in diameter OR ≥ 25 to < 100 cm^2 surface area OR Symptoms causing greater than minimal interference with usual social & functional activities	≥ 10 cm in diameter OR ≥ 100 cm^2 surface area OR Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR Drainage OR Symptoms causing inability to perform usual social & functional activities	Potentially life-threatening consequences (e.g., abscess, exfoliative dermatitis, necrosis involving dermis or deeper tissue)
≤ 15 years of age	≤ 2.5 cm in diameter	> 2.5 cm in diameter with < 50% surface area of the extremity segment involved (e.g., upper arm or thigh)	≥ 50% surface area of the extremity segment involved (e.g., upper arm or thigh) OR Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR Drainage	Potentially life-threatening consequences (e.g., abscess, exfoliative dermatitis, necrosis involving dermis or deeper tissue)
Injection Site Induration or Swelling *Report only one* *> 15 years of age*	Same as for **Injection Site Erythema or Redness,** *> 15 years of age*	Same as for **Injection Site Erythema or Redness,** *> 15 years of age*	Same as for **Injection Site Erythema or Redness,** *> 15 years of age*	Same as for **Injection Site Erythema or Redness,** *> 15 years of age*
≤ 15 years of age	Same as for **Injection Site Erythema or Redness,** *≤ 15 years of age*	Same as for **Injection Site Erythema or Redness,** *≤ 15 years of age*	Same as for **Injection Site Erythema or Redness,** *≤ 15 years of age*	Same as for **Injection Site Erythema or Redness,** *≤ 15 years of age*
Injection Site Pruritus	Itching localized to the injection site that is relieved spontaneously or in < 48 hours of treatment	Itching beyond the injection site that is not generalized OR Itching localized to the injection site requiring ≥ 48 hours treatment	Generalized itching causing inability to perform usual social & functional activities	NA

[13] Injection Site Erythema or Redness should be evaluated and graded using the greatest single diameter or measured surface area.

Laboratory Values
Chemistries

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Acidosis	NA	pH ≥ 7.3 to < LLN	pH < 7.3 without life-threatening consequences	pH < 7.3 with life-threatening consequences
Albumin, Low (g/dL; *g/L*)	3.0 to < LLN *30 to < LLN*	≥ 2.0 to < 3.0 *≥ 20 to < 30*	< 2.0 *< 20*	NA
Alkaline Phosphatase, High	1.25 to < 2.5 x ULN	2.5 to < 5.0 x ULN	5.0 to < 10.0 x ULN	≥ 10.0 x ULN
Alkalosis	NA	pH > ULN to ≤ 7.5	pH > 7.5 without life-threatening consequences	pH > 7.5 with life-threatening consequences
ALT or SGPT, High *Report only one*	1.25 to < 2.5 x ULN	2.5 to < 5.0 x ULN	5.0 to < 10.0 x ULN	≥ 10.0 x ULN
Amylase (Pancreatic) or Amylase (Total), High *Report only one*	1.1 to < 1.5 x ULN	1.5 to < 3.0 x ULN	3.0 to < 5.0 x ULN	≥ 5.0 x ULN
AST or SGOT, High *Report only one*	1.25 to < 2.5 x ULN	2.5 to < 5.0 x ULN	5.0 to < 10.0 x ULN	≥ 10.0 x ULN
Bicarbonate, Low (mEq/L; *mmol/L*)	16.0 to < LLN *16.0 to < LLN*	11.0 to < 16.0 *11.0 to < 16.0*	8.0 to < 11.0 *8.0 to < 11.0*	< 8.0 *< 8.0*
Bilirubin ***Direct Bilirubin[14], High*** *> 28 days of age*	NA	NA	> ULN	> ULN with life-threatening consequences (e.g., signs and symptoms of liver failure)
≤ 28 days of age	ULN to ≤ 1 mg/dL	> 1 to ≤ 1.5 mg/dL	> 1.5 to ≤ 2 mg/dL	> 2 mg/dL
Total Bilirubin, High *> 28 days of age*	1.1 to < 1.6 x ULN	1.6 to < 2.6 x ULN	2.6 to < 5.0 x ULN	≥ 5.0 x ULN
≤ 28 days of age	See Appendix A. Total Bilirubin for Term and Preterm Neonates	See Appendix A. Total Bilirubin for Term and Preterm Neonates	See Appendix A. Total Bilirubin for Term and Preterm Neonates	See Appendix A. Total Bilirubin for Term and Preterm Neonates
Calcium, High (mg/dL; *mmol/L*) *≥ 7 days of age*	10.6 to < 11.5 *2.65 to < 2.88*	11.5 to < 12.5 *2.88 to < 3.13*	12.5 to < 13.5 *3.13 to < 3.38*	≥ 13.5 *≥ 3.38*
< 7 days of age	11.5 to < 12.4 *2.88 to < 3.10*	12.4 to < 12.9 *3.10 to < 3.23*	12.9 to < 13.5 *3.23 to < 3.38*	≥ 13.5 *≥ 3.38*

[14] Direct bilirubin > 1.5 mg/dL in a participant < 28 days of age should be graded as grade 2, if < 10% of the total bilirubin.

Chemistries

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Calcium (Ionized), High (mg/dL; *mmol/L*)	> ULN to < 6.0 *> ULN to < 1.5*	6.0 to < 6.4 *1.5 to < 1.6*	6.4 to < 7.2 *1.6 to < 1.8*	≥ 7.2 *≥ 1.8*
Calcium, Low (mg/dL; *mmol/L*) *≥ 7 days of age*	7.8 to < 8.4 *1.95 to < 2.10*	7.0 to < 7.8 *1.75 to < 1.95*	6.1 to < 7.0 *1.53 to < 1.75*	< 6.1 *< 1.53*
< 7 days of age	6.5 to < 7.5 *1.63 to < 1.88*	6.0 to < 6.5 *1.50 to < 1.63*	5.50 to < 6.0 *1.38 to < 1.50*	< 5.50 *< 1.38*
Calcium (Ionized), Low (mg/dL; *mmol/L*)	< LLN to 4.0 *< LLN to 1.0*	3.6 to < 4.0 *0.9 to < 1.0*	3.2 to < 3.6 *0.8 to < 0.9*	< 3.2 *< 0.8*
Cardiac Troponin I, High	NA	NA	NA	Levels consistent with myocardial infarction or unstable angina as defined by the local laboratory
Creatine Kinase, High	3 to < 6 x ULN	6 to < 10 x ULN	10 to < 20 x ULN	≥ 20 x ULN
Creatinine, High	1.1 to 1.3 x ULN	> 1.3 to 1.8 x ULN OR Increase of > 0.3 mg/dL above baseline	> 1.8 to < 3.5 x ULN OR Increase of 1.5 to < 2.0 x above baseline	≥ 3.5 x ULN OR Increase of ≥ 2.0 x above baseline
Creatinine Clearance[15] **or eGFR, Low** *Report only one*	NA	< 90 to 60 ml/min or ml/min/1.73 m^2 OR 10 to < 30% decrease from baseline	< 60 to 30 ml/min or ml/min/1.73 m^2 OR ≥ 30 to < 50% decrease from baseline	< 30 ml/min or ml/min/1.73 m^2 OR ≥ 50% decrease from baseline or dialysis needed
Glucose (mg/dL; *mmol/L*) ***Fasting, High***	110 to 125 *6.11 to < 6.95*	> 125 to 250 *6.95 to < 13.89*	> 250 to 500 *13.89 to < 27.75*	> 500 *≥ 27.75*
Nonfasting, High	116 to 160 *6.44 to < 8.89*	> 160 to 250 *8.89 to < 13.89*	> 250 to 500 *13.89 to < 27.75*	> 500 *≥ 27.75*
Glucose, Low (mg/dL; *mmol/L*) *≥ 1 month of age*	55 to 64 *3.05 to 3.55*	40 to < 55 *2.22 to < 3.05*	30 to < 40 *1.67 to < 2.22*	< 30 *< 1.67*
< 1 month of age	50 to 54 *2.78 to 3.00*	40 to < 50 *2.22 to < 2.78*	30 to < 40 *1.67 to < 2.22*	< 30 *< 1.67*
Lactate, High	ULN to < 2.0 x ULN without acidosis	≥ 2.0 x ULN without acidosis	Increased lactate with pH < 7.3 without life-threatening consequences	Increased lactate with pH < 7.3 with life-threatening consequences

[15] Use the applicable formula (i.e., Cockroft-Gault in mL/min or Schwatrz in mL/min/1.73m^2).

Chemistries

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Lipase, High	1.1 to < 1.5 x ULN	1.5 to < 3.0 x ULN	3.0 to < 5.0 x ULN	≥ 5.0 x ULN
Lipid Disorders (mg/dL; *mmol/L*)				
Cholesterol, Fasting, High				
≥ 18 years of age	200 to < 240 *5.18 to < 6.19*	240 to < 300 *6.19 to < 7.77*	≥ 300 *≥ 7.77*	NA
< 18 years of age	170 to < 200 *4.40 to < 5.15*	200 to < 300 *5.15 to < 7.77*	≥ 300 *≥ 7.77*	NA
LDL, Fasting, High				
≥ 18 years of age	130 to < 160 *3.37 to < 4.12*	160 to < 190 *4.12 to < 4.90*	≥ 190 *≥ 4.90*	NA
> 2 to < 18 years of age	110 to < 130 *2.85 to < 3.34*	130 to < 190 *3.34 to < 4.90*	≥ 190 *≥ 4.90*	NA
Triglycerides, Fasting, High	150 to 300 *1.71 to 3.42*	>300 to 500 *>3.42 to 5.7*	>500 to < 1,000 *>5.7 to 11.4*	> 1,000 *> 11.4*
Magnesium[16], Low (mEq/L; *mmol/L*)	1.2 to < 1.4 *0.60 to < 0.70*	0.9 to < 1.2 *0.45 to < 0.60*	0.6 to < 0.9 *0.30 to < 0.45*	< 0.6 *< 0.30*
Phosphate, Low (mg/dL; *mmol/L*)				
> 14 years of age	2.0 to < LLN *0.81 to < LLN*	1.4 to < 2.0 *0.65 to < 0.81*	1.0 to < 1.4 *0.32 to < 0.65*	< 1.0 *< 0.32*
1 to 14 years of age	3.0 to < 3.5 *0.97 to < 1.13*	2.5 to < 3.0 *0.81 to < 0.97*	1.5 to < 2.5 *0.48 to < 0.81*	< 1.5 *< 0.48*
< 1 year of age	3.5 to < 4.5 *1.13 to < 1.45*	2.5 to < 3.5 *0.81 to < 1.13*	1.5 to < 2.5 *0.48 to < 0.81*	< 1.5 *< 0.48*
Potassium, High (mEq/L; *mmol/L*)	5.6 to < 6.0 *5.6 to < 6.0*	6.0 to < 6.5 *6.0 to < 6.5*	6.5 to < 7.0 *6.5 to < 7.0*	≥ 7.0 *≥ 7.0*
Potassium, Low (mEq/L; *mmol/L*)	3.0 to < 3.4 *3.0 to < 3.4*	2.5 to < 3.0 *2.5 to < 3.0*	2.0 to < 2.5 *2.0 to < 2.5*	< 2.0 *< 2.0*
Sodium, High (mEq/L; *mmol/L*)	146 to < 150 *146 to < 150*	150 to < 154 *150 to < 154*	154 to < 160 *154 to < 160*	≥ 160 *≥ 160*
Sodium, Low (mEq/L; *mmol/L*)	130 to < 135 *130 to < 135*	125 to < 130 *125 to < 135*	121 to < 125 *121 to < 125*	≤ 120 *≤ 120*
Uric Acid, High (mg/dL; *mmol/L*)	7.5 to < 10.0 *0.45 to < 0.59*	10.0 to < 12.0 *0.59 to < 0.71*	12.0 to < 15.0 *0.71 to < 0.89*	≥ 15.0 *≥ 0.89*

[16] To convert a magnesium value from mg/dL to mmol/L, laboratories should multiply by 0.4114.

Hematology

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Absolute CD4+ Count, Low (cell/mm^3; *cells/L*) *> 5 years of age (not HIV infected)*	300 to < 400 *300 to < 400*	200 to < 300 *200 to < 300*	100 to < 200 *100 to < 200*	< 100 *< 100*
Absolute Lymphocyte Count, Low (cell/mm^3; *cells/L*) *> 5 years of age (not HIV infected)*	600 to < 650 *0.600 x 10^9 to < 0.650 x 10^9*	500 to < 600 *0.500 x 10^9 to < 0.600 x 10^9*	350 to < 500 *0.350 x 10^9 to < 0.500 x 10^9*	< 350 *< 0.350 x 10^9*
Absolute Neutrophil Count (ANC), Low (cells/mm^3; *cells/L*) *> 7 days of age*	800 to 1,000 *0.800 x 10^9 to 1.000 x 10^9*	600 to 799 *0.600 x 10^9 to 0.799 x 10^9*	400 to 599 *0.400 x 10^9 to 0.599 x 10^9*	< 400 *< 0.400 x 10^9*
2 to 7 days of age	1,250 to 1,500 *1.250 x 10^9 to 1.500 x 10^9*	1,000 to 1,249 *1.000 x 10^9 to 1.249 x 10^9*	750 to 999 *0.750 x 10^9 to 0.999 x 10^9*	< 750 *< 0.750 x 10^9*
≤ 1 day of age	4,000 to 5,000 *4.000 x 10^9 to 5.000 x 10^9*	3,000 to 3,999 *3.000 x 10^9 to 3.999 x 10^9*	1,500 to 2,999 *1.500 x 10^9 to 2.999 x 10^9*	< 1,500 *< 1.500 x 10^9*
Fibrinogen, Decreased (mg/dL; *g/L*)	100 to < 200 *1.00 to < 2.00* OR 0.75 to < 1.00 x LLN	75 to < 100 *0.75 to < 1.00* OR ≥ 0.50 to < 0.75 x LLN	50 to < 75 *0.50 to < 0.75* OR 0.25 to < 0.50 x LLN	< 50 *< 0.50* OR < 0.25 x LLN OR Associated with gross bleeding
Hemoglobin[17], Low (g/dL; *mmol/L*)[18] *≥ 13 years of age (male only)*	10.0 to 10.9 *6.19 to 6.76*	9.0 to < 10.0 *5.57 to < 6.19*	7.0 to < 9.0 *4.34 to < 5.57*	< 7.0 *< 4.34*
≥ 13 years of age (female only)	9.5 to 10.4 *5.88 to 6.48*	8.5 to < 9.5 *5.25 to < 5.88*	6.5 to < 8.5 *4.03 to < 5.25*	< 6.5 *< 4.03*

[17] Male and female sex are defined as sex at birth.
[18] The conversion factor used to convert g/dL to mmol/L is 0.6206 and is the most commonly used conversion factor. For grading hemoglobin results obtained by an analytic method with a conversion factor other than 0.6206, the result must be converted to g/dL using the appropriate conversion factor for the particular laboratory.

Hematology

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
57 days of age to < 13 years of age (male and female)	9.5 to 10.4 *5.88 to 6.48*	8.5 to < 9.5 *5.25 to < 5.88*	6.5 to < 8.5 *4.03 to < 5.25*	< 6.5 *< 4.03*
36 to 56 days of age (male and female)	8.5 to 9.6 *5.26 to 5.99*	7.0 to < 8.5 *4.32 to < 5.26*	6.0 to < 7.0 *3.72 to < 4.32*	< 6.0 *< 3.72*
22 to 35 days of age (male and female)	9.5 to 11.0 *5.88 to 6.86*	8.0 to < 9.5 *4.94 to < 5.88*	6.7 to < 8.0 *4.15 to < 4.94*	< 6.7 *< 4.15*
8 to ≤ 21 days of age (male and female)	11.0 to 13.0 *6.81 to 8.10*	9.0 to < 11.0 *5.57 to < 6.81*	8.0 to < 9.0 *4.96 to < 5.57*	< 8.0 *< 4.96*
≤ 7 days of age (male and female)	13.0 to 14.0 *8.05 to 8.72*	10.0 to < 13.0 *6.19 to < 8.05*	9.0 to < 10.0 *5.59 to < 6.19*	< 9.0 *< 5.59*
INR, High (not on anticoagulation therapy)	1.1 to < 1.5 x ULN	1.5 to < 2.0 x ULN	2.0 to < 3.0 x ULN	≥ 3.0 x ULN
Methemoglobin (% hemoglobin)	5.0 to < 10.0%	10.0 to < 15.0%	15.0 to < 20.0%	≥ 20.0%
PTT, High (not on anticoagulation therapy)	1.1 to < 1.66 x ULN	1.66 to < 2.33 x ULN	2.33 to < 3.00 x ULN	≥ 3.00 x ULN
Platelets, Decreased (cells/mm^3; *cells/L*)	100,000 to < 124,999 *100.000 x 10^9 to < 124.999 x 10^9*	50,000 to < 100,000 *50.000 x 10^9 to < 100.000 x 10^9*	25,000 to < 50,000 *25.000 x 10^9 to < 50.000 x 10^9*	< 25,000 *< 25.000 x 10^9*
PT, High (not on anticoagulation therapy	1.1 to < 1.25 x ULN	1.25 to < 1.50 x ULN	1.50 to < 3.00 x ULN	≥ 3.00 x ULN
WBC, Decreased (cells/mm^3; *cells/L*)				
> 7 days of age	2,000 to 2,499 *2.000 x 10^9 to 2.499 x 10^9*	1,500 to 1,999 *1.500 x 10^9 to 1.999 x 10^9*	1,000 to 1,499 *1.000 x 10^9 to 1.499 x 10^9*	< 1,000 *< 1.000 x 10^9*
≤ 7 days of age	5,500 to 6,999 *5.500 x 10^9 to 6.999 x 10^9*	4,000 to 5,499 *4.000 x 10^9 to 5.499 x 10^9*	2,500 to 3,999 *2.500 x 10^9 to 3.999 x 10^9*	< 2,500 *< 2.500 x 10^9*

Urinalysis

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Glycosuria (random collection tested by dipstick)	Trace to 1+ or ≤ 250 mg	2+ or > 250 to ≤ 500 mg	> 2+ or > 500 mg	NA
Hematuria (not to be reported based on dipstick findings or on blood believed to be of menstrual origin)	6 to < 10 RBCs per high power field	≥ 10 RBCs per high power field	Gross, with or without clots OR With RBC casts OR Intervention indicated	Life-threatening consequences
Proteinuria (random collection tested by dipstick)	1+	2+	3+ or higher	NA

Appendix A.
Total Bilirubin Table for Term and Preterm Neonates

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Total Bilirubin[19]**, High** (mg/dL; *µmol/L*)[20]				
Term Neonate[21] *< 24 hours of age*	4 to < 7 *68.4 to < 119.7*	7 to < 10 *119.7 to < 171*	10 to < 17 *171 to < 290.7*	≥ 17 *≥ 290.7*
24 to < 48 hours of age	5 to < 8 *85.5 to < 136.8*	8 to < 12 *136.8 to < 205.2*	12 to < 19 *205.2 to < 324.9*	≥ 19 *≥ 324.9*
48 to < 72 hours of age	8.5 to < 13 *145.35 to < 222.3*	13 to < 15 *222.3 to < 256.5*	15 to < 22 *256.5 to < 376.2*	≥ 22 *≥ 376.2*
72 hours to < 7 days of age	11 to < 16 *188.1 to < 273.6*	16 to < 18 *273.6 to < 307.8*	18 to < 24 *307.8 to < 410.4*	≥ 24 *≥ 410.4*
7 to 28 days of age (breast feeding)	5 to < 10 *85.5 to < 171*	10 to < 20 *171 to < 342*	20 to < 25 *342 to < 427.5*	≥ 25 *≥ 427.5*
7 to 28 days of age (not breast feeding)	1.1 to < 1.6 x ULN	1.6 to < 2.6 x ULN	2.6 to < 5.0 x ULN	≥ 5.0 x ULN
Preterm Neonate[20] *35 to < 37 weeks gestational age*	Same as for ***Total Bilirubin, High, Term Neonate*** (based on days of age).	Same as for ***Total Bilirubin, High, Term Neonate*** (based on days of age).	Same as for ***Total Bilirubin, High, Term Neonate*** (based on days of age).	Same as for ***Total Bilirubin, High, Term Neonate*** (based on days of age).
32 to < 35 weeks gestational age and < 7 days of age	NA	NA	10 to < 14 *171 to < 239.4*	≥ 14 *≥ 239.4*
28 to < 32 weeks gestational age and < 7 days of age	NA	NA	6 to < 10 *102.6 to < 171*	≥ 10 *≥ 171*
< 28 weeks gestational age and < 7 days of age	NA	NA	5 to < 8 *85.5 to < 136.8*	≥ 8 *≥ 136.8*
7 to 28 days of age (breast feeding)	5 to < 10 *85.5 to < 171*	10 to < 20 *171 to < 342*	20 to < 25 *342 to < 427.5*	≥ 25 *≥ 427.5*
7 to 28 days of age (not breast feeding)	1.1 to < 1.6 x ULN	1.6 to < 2.6 x ULN	2.6 to < 5.0 x ULN	≥ 5.0 x ULN

[19] Severity grading for total bilirubin in neonates is complex because of rapidly changing total bilirubin normal ranges in the first week of life followed by the benign phenomenon of breast milk jaundice after the first week of life. Severity grading in this appendix corresponds approximately to cut-offs for indications for phototherapy at grade 3 and for exchange transfusion at grade 4.
[20] A laboratory value of 1 mg/dL is equivalent to 17.1 µmol/L.
[21] Definitions: Term is defined as ≥ 37 weeks gestational age; near-term, as ≥ 35 weeks gestational age; preterm, as < 35 weeks gestational age; and neonate, as 0 to 28 days of age.