Protocol Title: VRC 603: A Phase I Dose-Escalation Study of the Safety of AAV8-VRC07 (VRC-HIVAAV070-00-GT) Recombinant AAV vector Expressing VRC07 HIV-1 Neutralizing Antibody in Antiretroviral-Treated, HIV-1 Infected Adults with Controlled Viremia

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- IRB-approved Protocol (v8.0 09MAR2023) - Statistical Analysis Considerations located in Section 6 of the Protocol
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VACCINE RESEARCH CENTER

Protocol VRC 603
(NIH 18-I-0030)

A PHASE 1 DOSE-ESCALATION STUDY OF THE SAFETY OF AAV8-VRC07 (VRC-HIVAAV070-00-GT) RECOMBINANT AAV VECTOR EXPRESSING VRC07 HIV-1 NEUTRALIZING ANTIBODY IN ANTIRETROVIRAL-TREATED, HIV-1 INFECTED ADULTS WITH CONTROLLED VIREMIA

Study Agent Provided by
Vaccine Research Center/NIAID/NIH, Bethesda, MD

Clinical Trial Sponsored by:
National Institute of Allergy and Infectious Diseases (NIAID)
Vaccine Research Center (VRC)
Bethesda, Maryland

IND 16979 - held by VRC, NIAID, NIH

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ABBREVIATIONS

Abbreviation	Term
AAV	adeno-associated virus
ADA	anti-drug antibody
AE	adverse event
AIDS	Acquired Immunodeficiency Syndrome
ALT	alanine aminotransferase
AoU	Assessment of Understanding
ARV	antiretroviral
ART	antiretroviral therapy
AST	aspartate aminotransferase
AUC	area under the curve
β-HCG	human chorionic gonadotropin
CL	clearance
Cmax	maximum concentration
COVID-19	coronavirus disease 2019
cGMP	current Good Manufacturing Practice
DNA	deoxyribonucleic acid
dL	deciliter
EAE	expedited adverse event
EDTA	Ethylenediaminetetraacetate
ELISA	enzyme-linked immunosorbent assay
EOI	end of injection
F	bioavailability
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HRPP	Human Research Protections Program
IB	Investigator's Brochure
IBC	Institutional Biosafety Committee
IC	informed consent
ICH	International Council on Harmonization
IgG1	Immunoglobulin G1
IM	intramuscular
IND	investigational new drug application
IRB	Institutional Review Board
ITR	inverted terminal repeats
IV	intravenous
kg	kilogram
L	liter
LIMS	Laboratory Information Management System
LPLD	lipoprotein lipase deficiency

Abbreviation	Term
λz	terminal slope of concentration vs time profile
MAb	monoclonal antibody
mcg	microgram
mcL	microliter
mg	milligram
mL	milliliter
mM	millimolar
MSD	Meso Scale Discovery
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NIH CC	National Institutes of Health Clinical Center
NHP	Non-human primate
OSP	Office of Science Policy
OHRP	Office for Human Research Protections
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
PI	Principal Investigator
PK	pharmacokinetic
PSRT	Protocol Safety Review Team
Q	Inter-compartmental clearance
QA	quality assurance
qPCR	quantitative PCR
rAAV	recombinant adeno-associated virus
RPE65	retinal pigment epithelial 65 kDa protein
SAE	serious adverse event
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SC	subcutaneous
SHIV	simian-human immunodeficiency virus
SMN	survival motor neuron
SoE	schedule of evaluations
TCR	tissue cross reactivity
$T_{½}$	half-life
Tmax	time of maximal concentration (Cmax)
UNAIDS	Joint United Nations Programme on HIV/AIDS
UP	unanticipated problem
ULN	upper limit of normal
USP	United States Pharmacopeia
vg	viral genome
VIP	Vaccine Immunology Program, formerly VITL/NVITAL
VL	virus load
VRC	Vaccine Research Center
WBC	white blood cell

PRINCIPAL INVESTIGATOR PROTOCOL SIGNATURE PAGE

VRC 603: A Phase I Dose-Escalation Study of the Safety of AAV8-VRC07 (VRC-HIVAAV070-00-GT) Recombinant AAV vector Expressing VRC07 HIV-1 Neutralizing Antibody in Antiretroviral-Treated, HIV-1 Infected Adults with Controlled Viremia.

I, the Principal Investigator for the study site indicated above, agree to conduct the study in full accordance with the provisions of this protocol and all applicable protocol-related documents. I agree to conduct the study in compliance with United States (US) Health and Human Services (HHS) regulations (45CFR 46); applicable US Food and Drug Administration (FDA) regulations; standards of the International Council on Harmonization Guidelines for Good Clinical Practice (E6); Institutional Review Board/Ethics Committee (IRB/EC) determinations; all applicable in-country, state, and local laws and regulations; and other applicable requirements (e.g., US National Institutes of Health) and institutional policies. I will comply with all requirements regarding the obligations of investigators as outlined in the Statement of Investigator (Form FDA 1572), which I have also signed. The protocol signature page will be signed for subsequent protocol approvals.

I agree to maintain all study documentation pertaining to the conduct of this study, including but not limited to, case report forms, source documents, laboratory test results, and medication inventory records, per FDA regulation (21 CFR 312.62) and all applicable requirements. No study records will be destroyed without prior authorization from VRC/NIAID.

Publication of the results of this study will be governed by the VRC/NIAID policies. Any presentation, abstract, or manuscript will be made available by the investigators to VRC Leadership for review prior to submission.

I have read and understand the information in this protocol and will ensure that all associates, colleagues, and employees assisting in the conduct of the study are informed about the obligations incurred by their contribution to the study.

Name/Title of Principal Investigator	Study Site Name
Signature of Principal Investigator	Date

PRÉCIS

VRC 603: A Phase I Dose-Escalation Study of the Safety of AAV8-VRC07 (VRC-HIVAAV070-00-GT) Recombinant AAV vector Expressing VRC07 HIV-1 Neutralizing Antibody in Antiretroviral-Treated, HIV-1 Infected Adults with Controlled Viremia.

Study Design: This is a Phase I study of the safety and tolerability of AAV8-VRC07 (VRC-HIVAAV070-00-GT) expressing VRC07 human monoclonal antibody with broad HIV-1 neutralizing activity in HIV-1 infected adults. It is a dose-escalation study to examine pharmacokinetics of VRC07 expression following intramuscular (IM) administration of AAV8-VRC07 in subjects on anti-retroviral therapy (ARV). The hypotheses are: 1) AAV8-VRC07 will be safe for human administration and will not elicit hypersensitivity or anti-drug antibody (ADA) to VRC07; and 2) intramuscular delivery of AAV8-VRC07 will result in production of biologically active VRC07 antibody at a concentration in serum that is measurable and safe.

Product Description: AAV8-VRC07 was developed by VRC, NIAID, NIH, and manufactured by the Clinical Vector Core, Center for Cellular and Molecular Therapeutics, The Children's Hospital of Philadelphia (CHOP), Philadelphia, PA. It is composed of an AAV8 recombinant vector expressing genes encoding the heavy and light chains of the VRC07 monoclonal antibody. AAV8-VRC07 will be supplied at $2.84x10^{13}$ vg/mL.

Subjects: HIV-1 infected adult volunteers (18 to 65 years old) on a stable antiretroviral regimen for ≥ 3 months, with controlled viremia, under the care of a physician, and without additional clinically significant medical conditions.

Study Plan: There are 3 dose escalation groups. Sequentially enrolled subjects will be assigned to the dosage level being evaluated at the time of enrollment. All injections will be administered intramuscularly (IM) by needle and syringe. Cumulative safety data will be reviewed weekly by a Protocol Safety Review Team (PSRT) that includes an Independent Safety Monitor (ISM) while injections are being administered. Safety, including reactogenicity and unsolicited AEs, laboratory findings, pharmacokinetics, and VRC07 antibody levels in blood will be assessed after the injection and summarized for an interim analysis at 4 weeks post injection. The second subject in each dose group will be injected after the 4 weeks safety assessment for the first subject. Decisions regarding dose escalation and subject enrollments will be based on safety data and the VRC07 concentration in blood at 4 weeks after product administration (Section 4.3). The pharmacokinetics of VRC07 at each dose level will be evaluated to determine the dose that would result in antibody production that achieves at least 50 mcg/mL VRC07 concentration in serum at 4 weeks post injection with a target set point of ≥ 5 mcg/mL at 12 weeks post injection.

VRC 603 Study Schema

Group	Dose	Subjects	Total product volume based on product concentration of $2.84x10^{13}$ vg/mL and a subject weight of 100 kg
1	$5x10^{10}$ vg/kg	2-5	0.18 mL
2	$5x10^{11}$ vg/kg	2-5	1.76 mL
3	$2.5x10^{12}$ vg/kg	2-5	8.80 mL
Total	2 to 5 subjects per group to enrollment of 15 subjects. Enrollment up to a total of 25 subjects is permitted in case there are subjects who do not complete the schedule, if additional PK evaluations are needed, or if an enrollment of additional subjects is recommended for safety evaluations.		

Study Duration: Each participant will be asked to complete 5 years of clinical follow up from the day of study injection. The study dose escalation portion of the study is projected to take about a year.

STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with the International Council on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1. INTRODUCTION

The global incidence of new human immunodeficiency virus (HIV) infection peaked in the mid-1990s. As reported by Joint United Nations Programme on HIV/AIDS (UNAIDS), about 2.1 million people became newly infected with HIV in 2015, down from 2.9 million in 2005, with an estimated global total of 36.7 million people living with HIV. The decrease in incidence is an encouraging trend, but the scope and cost of the epidemic remain of great global concern. The reduction of HIV incidence is attributed to multiple factors that include prevention and treatment programs. The wider availability of antiretroviral (ARV) therapy, mother to child transmission prevention programs, and a diverse array of other prevention programs have all contributed to turning the tide of the epidemic [1]. Each effective form of prevention and treatment is a welcome public health measure.

The National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH) is committed to the development of safe and effective methods to prevent and treat HIV infection and AIDS worldwide. In this regard, the Vaccine Research Center (VRC), NIAID, NIH, plans to evaluate the potential clinical uses of the investigational AAV8-VRC07 gene transfer product that expresses an HIV-specific broadly neutralizing human monoclonal antibody (MAb), VRC07.

The VRC, NIAID, NIH recently discovered highly potent and broadly neutralizing HIV-1 human monoclonal antibodies (MAb) that target the HIV-1 CD4 binding site on the HIV envelope protein [2-5]. VRC01 neutralizes 88% of viral envelope (Env) pseudoviruses at concentration <50mcg/mL and 74% at <1mcg/mL [2]. The VRC01 MAb, currently in clinical trials under IND 113611 for prevention indication and INDs 126001 and 12664 for therapeutic indication, was originally discovered in a subject infected with HIV-1 for more than 15 years and whose immune system controlled the virus without ARV[5].

VRC07 is a clonal relative of VRC01 isolated from the same chronically HIV-1 infected person who had sera with broad cross neutralizing activity. VRC07 IgG heavy chain shares 90% nucleotide sequence identity with VRC01, but differs at 15 amino acid positions and contains a 4 amino acid insertion in the third complementarity determining region (CDRH3) that provides more extensive contacts with the envelope protein [3, 4]. The natural light chain of VRC07 is unknown, as heavy and light chains were sequenced *en masse* from peripheral blood mononuclear cells (PBMCs). Thus, the VRC07 heavy chain was paired with the VRC01 light chain to generate a more potent and broadly reactive anti-HIV antibody than VRC01 [3, 4].

HIV-infected individuals who develop this class of highly neutralizing antibodies against HIV-1 naturally have shown good immune control of the virus [2, 3]. However, potent antibodies that exert strong control of HIV-1 do not naturally develop in most HIV-infected individuals and, thus far, investigational vaccines have not induced such antibodies. Although ARV medications have improved the control of virus in HIV-infected individuals, additional methods of viremia control that have a long durability of action would be potentially beneficial to individuals and to the overall slowing the epidemic. Gene transfer for a potent neutralizing antibody, such as VRC07, may lead to sustained production of these antibodies *in vivo* and contribute to sustained control of viremia.

The adeno-associated virus (AAV) vector-based investigational product, AAV8-VRC07, was therefore designed with the goal to achieve production of a highly neutralizing MAb against the HIV-1 virus *in vivo*. The clinical development plan for AAV8-VRC07 is based on two potential indications of the product:

- Therapeutic application in HIV-1-infected individuals (vectored immunotherapy), and,

- Prophylactic application in healthy subjects at high risk for HIV infection (vectored immunoprophylaxis).

1.1. Study Rationale

Phase 1 clinical trials of VRC01 in both HIV-infected and healthy adults have been completed [6, 7]. Doses up to 40 mg/kg IV have been well tolerated (Section 1.3.1). VRC01 is currently being further evaluated for potential therapeutic and preventive uses in healthy and HIV infected adults and in infants at risk of mother-to-child HIV transmission.

Data accumulated in HIV-1 infected subjects demonstrate the VRC01 mediated anti-viral effect and support the hypothesis that a schedule with sustained antibody expression may have a beneficial clinical effect. In VRC 601 study, 6 of 8 viremic HIV-infected subjects infused with a single 40 mg/kg dose of VRC01 experienced a 1.1 to 1.8 $\log_{10}$ reduction in plasma viremia [7]. VRC07 is about 2-fold more potent than VRC01 and neutralizes 93% of viral envelope (Env) pseudoviruses at concentrations of <50 mcg/mL, and 83% at <1 mcg/mL. Therefore it could be expected that VRC07 may have the same or even more prominent anti-viral effect [3].

The AAV-mediated antibody expression has been studied in several animal models [8-12]. The proof-of-concept animal studies (Section 2.3) confirmed that AAV8-VRC07 induces the sustained VRC07 expression that is protective against HIV/SHIV infection [8-10].

AAV8-VRC07 is an investigational product which has not been administered to humans. It is intended to provide a long-term expression of VRC07 upon intramuscular (IM) administration. In this first in human phase 1 study, safety and tolerability of AAV8-VRC07 will be evaluated in HIV-1 infected subjects.

1.1.1. Characteristics and Rationale for AAV vectors

AAV is a nonpathogenic parvovirus with a single-stranded DNA genome encapsulated within a non-enveloped capsid. AAV has an ability to infect both dividing and non-dividing cells; there are 12 described serotypes of AAV with different tissue specificity. Wild type AAV has not been associated with any human disease [13-15]. Efficient replication of AAV requires helper function from an adenovirus or herpesvirus. Following infection in the absence of helper virus, wild type AAV enters a latent phase and DNA is retained in extrachromosomal episomal form or integrated selectively into the genomic DNA at a specific site on the human chromosome 19 [15-17].

Simple AAV genome organization that facilitates engineering of recombinant vector DNA, easy manipulation of the AAV cap gene which encodes the viral shell proteins essential for genome packaging, and absence of known pathogenicity have made recombinant versions of AAV (rAAV) good candidates for therapeutic gene delivery [14, 15]. In the last 50 years, multiple rAAV vectors have been developed with the goal of using these for gene transfer [18]. In rAAV vectors, genes encoding for viral proteins are deleted and a gene of interest is inserted between the inverted terminal repeats (ITR) that are sufficient for viral packaging. The non-replicating rAAV vector genomes persist as extra-chromosomal circular forms in non-dividing tissues such as normal skeletal muscle and are capable of mediating long-term gene expression following administration to muscle [17, 19].

Preexisting immunity to AAV is a limitation of rAAV vectors as gene delivery vehicles, with host immune responses being a concern in clinical studies [20-22]. Published reports estimate that up to 60-80% of the human population have some level of preexisting immunity to AAV [14, 23].

Measured by antibody level, AAV2 and AAV1 have the highest prevalence of preexisting immune responses ranging 50-60% of the population, and the lowest prevalence was observed for AAV8 (19%) and AAV5 (3%). In addition, about 70-100% of individuals seropositive for AAV5, AAV8, and AAV9 have low antibody titers [23].

In a non-human primate (NHP) model developed by VRC to inform clinical AAV-based approaches to passive immunization, AAV8-mediated SIV antibody expression was confirmed in serum and mucosal surfaces [24]. Antibody expression increased proportionally with the number of administered injections, and development of anti-vector and anti-transgene immune responses was evaluated. It was demonstrated that animals with low/intermediate pre-existing anti-vector immunity (AAV8 antibody titers 1:100 and 1:1000) can be successfully transducted with the level of transgene expression being similar to animals who have not had pre-existing immunity to the vector [24].

Several studies have noted that the rAAV-mediated gene transfer results in dose-dependent activation of capsid-specific T cells [21, 25]. Immune-mediated elimination of transduced cells has been thought to limit efficacy of gene transfer and account for a loss of transgene expression with time [21, 25, 26]. To overcome immune responses and prolong the transgene expression, immunosuppressive agents have been used in clinical studies in subjects with lipoprotein lipase deficiency (LPL) and in gene therapy studies for hemophilia [27, 28]. In addition, using immunosuppressants such as cyclosporine or mycophenolate mofetil starting 3 days before and for 12 weeks after IM administration is recommended as a part of the administration regimen for Glybera® (alipogene tiparvovec), the first rAAV1-based gene therapy product authorized for distribution in Europe [29].

The FDA guidance "Gene Therapy Clinical Trials – Observing Subjects for Delayed Adverse Events" classifies rAAV vectors as "non-integrating" [30]. There is no evidence that these vectors reactivate following latency and, therefore, subjects may be at low risk of gene-therapy-related delayed adverse events [30].

1.1.2. Dose Selection Rationale

The proposed dosages of AAV8-VRC07 are based on publicly available data from human clinical trials, experience with other rAAV vectors developed for use in gene transfer studies for human diseases, and preclinical animal studies with AAV8-VRC07 and related simian constructs.

Two primate studies of passive immunotherapy with other highly potent neutralizing antibodies in SHIV-infected macaques (3BNC117 and 10-1074, [31]) and rhesus monkeys (PGT121, [32]) also indicate that the immunotherapy approach may have a therapeutic effect in HIV-infected individuals. The intravenous antibody dosage in these NHP studies was in the 5-20 mg/kg range; and the therapeutic plasma concentration of antibodies that induced a rapid decline in viral load was found to be in the range of 5-10 mcg/mL. In NHP studies with VRC01, sustained passive transfer of a simian version of VRC01 antibody to macaques maintained therapeutic levels of antibody and provided complete protection against SHIV infection. These findings support the feasibility and potential of long-term passive antibody for protection against HIV in humans [33].

Studies in mice demonstrated that after a single intramuscular injection with $1x10^{10}$-$1x10^{11}$ viral genomes (vg) of the AAV8-VRC01 construct, a level up to 100 mcg/mL of VRC01 in serum was observed. On average, a level of 10 mcg/mL was achieved after IM injection of about a $1.3x10^{10}$ vg dose of rAAV8-VRC01 [8]. In the mouse challenge model, the protective VRC07 concentration was defined in the range of 3 to 6 mcg/mL. This antibody concentration was achieved with a vector

similar to AAV8-VRC07 dose between $0.63x10^{10}$ vg and $1.25x10^{10}$ vg per mouse. Assuming that a mouse weighs 0.02 kg, the protective dose of rAVV8-VRC07 was about $3x10^{11}$ to $6x10^{11}$ vg/kg in the mouse model.

A serum level of 50 mcg/mL at 4 weeks post injection has been selected for the study design dose escalation threshold based on the PK data for AAV8-VRC07 and similar constructs in mice and NHP that showed a peak level of at least 50 mcg/mL achieved at 3-5 weeks post injection, and then transgene concentration stabilized at a trough in the range of 5-10 mcg/mL.

Refer to the Investigator's Brochure (IB) for more information on these proof-of-concept preclinical studies.

Based on pre-clinical animal data and projected product concentration, dose escalation in the proposed Phase 1 study was designed in the $5x10^{10}$ to $2.5x10^{12}$ vg/kg range. This dose range is similar to that used in the clinical trials for the AAV8-mediated human factor IX (FIX) gene transfer by IV ($2x10^{11}$ to $2x10^{12}$ vg/kg) [34] and in earlier studies for the AAV2-mediated FIX transfer by IM injection ($2x10^{11}$-$1.8x10^{12}$ vg/kg) [35, 36]. Similarly, the AAV1 vector expressing lipoprotein lipase (Glybera®, alipogene tiparvovec) is authorized for marketing in Europe at $1x10^{12}$ vg /kg administered IM.

1.2. Specific Research Laboratory Assessments

Research assays designed to characterize the investigational product rather than assess the health of the subjects are described below.

Laboratory assessments in the Phase 1 study of AAV8-VRC07 include VRC07 pharmacokinetic (PK) analysis, testing for the AAV8-VRC07 presence in plasma, evaluation of pre-existing antibody responses to the AAV8, assessment for the development of anti-VRC07 antibodies post dosing, assessment for the development of anti-vector (anti-AAV8) immune responses, and functional capacity of the MAb in the collected research specimens for HIV neutralization.

For the PK analysis, VRC07 concentration may be measured by ELISA using the VRC01 anti-idiotype, Fab-specific 5C9 monoclonal antibody, as previously described [6] or by other similar and more sensitive assay.

Testing to detect the AAV8-VRC07 in blood will be done by a quantitative PCR (qPCR) similar to previously described [37, 38]. Plasma samples will be collected monthly and tested in batches. Testing will continue for each subject until 2 consecutive VRC07-AAV8 negative samples are identified.

Assessment of pre- and post-exposure anti-AAV8 antibodies will be done by ELISA using a vector-matched AAV8 capsid as the capture agent. Serial dilutions of sera will be analyzed to determine an end point antibody titer at specified time-points as described in the Schedule of Evaluations (SoE).

Assessment of anti-VRC07 antibodies in subject sera will be performed using the Meso Scale Discovery (MSD) platform based on electrochemiluminescence. The developed anti-drug antibody (ADA) assay uses the biotin-labeled VRC07 immobilized on a streptavidin-coated MSD plate as the capture molecule, and the SULFO-TAG labeled VRC07 as the reporter molecule. This assay is independent of the anti-VRC07 antibody isotype, and permits the detection of both high and low affinity antibodies.

Depending upon the VRC07 concentrations measured in collected specimens, samples may be assessed for their functional capacity to neutralize HIV by an *in vitro* cell-based virus neutralization assay using the pseudotyped viruses [39-41].

In addition to plasma viral load evaluation by a CLIA-certified laboratory assay, ultrasensitive single-copy research assay may be used to assess for a potential effect of VRC07 expression on a viral load as described previously [42].

Also subjects may be evaluated for their IgG1 allotypes to determine the potential for theoretical allotype-specific impacts on the VRC07 pharmacokinetics [43-45]. Coded stored samples will be used for evaluation of the genetic sequence of the immunoglobulin heavy chain constant region allotype.

In a potential event of tumor development reported by a subject during the long-term follow-up, tumor tissue, if available, may be analyzed for AAV vector integration by methods similar to those previously described [46].

Sample testing will be conducted by the Vaccine Immunology Program (VIP, formerly VITL / NVITAL), Gaithersburg, MD, by VRC Laboratories in Building 40 and by approved scientific collaborators.

1.3. Previous Human Experience

There is a limited human experience with AAV8-VRC07 administration accumulated in this study. Data accumulated in studies with VRC01, a clonal relative of VRC07, and an engineered variant of VRC07 of the same binding specificity designated VRC07-523LS, as well as with other rAAV vectors, can be used to inform the safety and potential efficacy of AAV8-mediated VRC07 expression in adults.

1.3.1. Human Experience with AAV8-VRC07

As of May 9, 2022, 10 subjects have been enrolled in VRC 603 study and received injections of AAV8-VRC07 at the $5x10^{10}$ vg/kg (3 subjects, Group 1), the $5x10^{11}$ vg/kg (3 subjects, Group 2), and the $2.5x10^{12}$ vg/kg (4 subjects, Group 3) dose.

The first subject enrollment and study product administration occurred on January 11, 2018. The last subject enrollment and study product administration occurred on August 4, 2021. Nine (9) out of 10 subjects are being followed for safety; one subject in Group 2 ($5x10^{11}$ vg/kg dose) is off study due to an unrelated SAE of methamphetamine overdose and subsequent death. Subjects are to be followed for 5 years after product administration.

No study pause rules have been met. Two dose escalation reviews have been completed as per protocol. The VRC07 concentration in serum was evaluated as less than 1μg/ml (the lower limit of detection for the assay used for testing) for subjects in Groups 1 and 2 at 4 weeks post product administration, supporting the dose escalation decisions.

The AAV8-VRC07 administrations were well tolerated at all doses, and no safety signals or dose-limiting toxicity have been detected.

No solicited local reactogenicity symptoms were reported during 7 days after product administration at doses $5x10^{10}$ vg/kg and $5x10^{11}$ vg/kg. Four out of 4 (100.0%) Group 3 subjects ($2.5x10^{12}$ vg/kg dose) reported mild pain/tenderness that began during the 7-day period after product administration and resolved after the solicited period. One subject reported mild pain/tenderness on Day 1 and Day

6 post product administration that resolved on Day 14, 7 days after the solicited period. One subject reported mild pain/tenderness on Day 5 that resolved on Day 9, 2 days after the solicited reporting period. One subject reported mild pain/tenderness on Day 1 and Day 5 post product administration that resolved on Day 10, 3 days after the solicited reporting period. One subject reported mild pain/tenderness on Day 6 post product administration that resolved on Day 11, 4 days after the solicited reporting period. No other solicited local reactions were reported.

As to systemic reactogenicity symptoms, three (3) out of 10 (30.0%) subjects reported one or more solicited systemic reactogenicity symptoms. One Group 2 subject ($5x10^{11}$ vg/kg dose) reported mild myalgia, one Group 3 subject ($2.5x10^{12}$ vg/kg dose) reported mild myalgia and mild headache, and one Group 3 subject ($2.5x10^{12}$ vg/kg dose) reported mild headache. These symptoms were reported on Day 1 and resolved the same day for all subjects. No fever or other solicited systemic reactogenicity symptoms were reported.

As of May 9, 2022, 6 out of 10 (60.0%) subjects had one or more unsolicited AEs. Mild serum creatinine elevation was the most frequently reported event, reported for 3 out of 10 (30.0%) subjects.

One SAE of a fatal illicit drug overdose was reported and assessed as unrelated to study product: a Group 2 subject ($5x10^{11}$ vg/kg dose) died because of a methamphetamine overdose 716 days post product administration.

One severe AE of diarrhea in a Group 1 subject ($5x10^{10}$ vg/kg dose) occurred at 44 days post product administration, evaluated as not related to study product, and resolved in 4 days with no residual effects.

Two AEs of mild aspartate aminotransferase (AST) elevation were reported for two Group 1 subjects ($5x10^{10}$ vg/kg dose). For one subject, grade 1 AST elevation was detected at 7 days post product administration, evaluated as not related to study product and resolved with no residual effects. The grade 1 AST elevation (52U/L) in another subject was detected at 49 days post product administration and was initially attributed to alcohol consumption. This attribution was later changed to the product related because of the identification of the concurrent AAV8 capsid-specific cytotoxic CD8 T cells by a research laboratory.

Mild upper respiratory tract infection (URTI) was reported for one Group 2 subject ($5x10^{11}$ vg/kg dose) at 54 days post product administration, evaluated as not related to study product, and resolved with no residual effects.

Four episodes of mild serum creatinine elevation were reported for three subjects, with one event reported for a Group 3 subject ($2.5x10^{12}$ vg/kg dose) at 7 days post product administration evaluated as related to study product.

Study results from the time of first enrollment in VRC 603 through a period of 104-198 weeks after product administration for 8 of the 10 subjects are included in a publication by Casazza, J.P. et al. [47].

1.3.2. VRC01 and VRC07-523LS Safety Data

Evaluations of the highly neutralizing antibody VRC01 started in September 2013. VRC01 has been tested in HIV-infected adults in VRC 601 study [6], in healthy adults in VRC 602 [7] and HVTN 104 studies, and in infants born from HIV-1 infected mothers in P1112 study (IND 113611). Further evaluation of safety, tolerability, PK and effect of VRC01 in HIV-1 infected adults, healthy adults

and infants is currently ongoing in several clinical studies in the United States (U.S.) and internationally.

As of March 2019, VRC01 administered in the dose ranges from 1 to 40 mg/kg by the IV route and 5 to 40 mg/kg by the SC route has been assessed as well-tolerated in adults and infants and safe for further evaluation. Cumulatively, VRC01 has been administered either IV or SC to over 3370 HIV-uninfected and HIV-infected adults, and 45 HIV-uninfected and HIV-Infected infants. There have been no serious adverse events (SAE) related to VRC01, as assessed by the Sponsor, and no study pauses for any adverse events.

The majority of human experience is being accumulated in two ongoing phase 2 trials, HVTN 704/HPTN 085 and HVTN 703/HPTN 081, collectively known as AMP trials (Antibody Mediated Prevention). 4,625 HIV-uninfected healthy participants were enrolled and received either VRC01 (two-thirds of participants) or placebo (one-third of participants) by IV route. The most common adverse events in the two AMP trials assessed as related to study product administration have been infusion related reactions. In both phase 2 trials combined, a total of 136 (2.9 %) participants experienced infusion reactions. Of the 136 participants with infusion reactions, 64 experienced urticarial reactions, most of which occurred during the first infusion. Both trials are still blinded with product administration ongoing as per protocols. The infusion reaction symptoms are typical of infusion reactions observed with other monoclonal antibodies administered IV, and these are not expected to occur upon vector-mediated production of VRC07 in muscle cells.

VRC01 IV administration to adults has been well tolerated. Observed local reactogenicity events included pain/tenderness, bruising, swelling, and localized erythema at the site of infusion that resolved within a few minutes to a few hours after the administration. Most participants reported no systemic reactogenicity symptoms with VRC01 IV administration; when observed, systemic solicited symptoms were of mild or moderate severity, started 1-3 days after administration of study product and lasted 1-2 days, with malaise/fatigue, myalgias, headaches and mild nausea being most common. There have been no severe solicited reactogenicity symptoms related to VRC01 IV injections.

VRC01 SC administration has been well tolerated in adults and infants. Most study participants had either no local reactogenicity or only mild local symptoms limited to injection site pain and/or tenderness, redness, and mild localized pruritus (itchiness), which resolved within a few minutes to a few hours after the administration was completed. There have been no severe solicited reactogenicity symptoms related to VRC01 SC injections.

Overall, VRC01 administration in the dose range from 1 to 40 mg/kg IV and at 5 mg/kg SC has been assessed as well-tolerated and safe for further evaluation.

The VRC 605 study designed to assess the safety and PK of VRC07-523LS in healthy adults has been recently completed [48]. Overall, 25 subjects have received at least one product administration by IV (17 subjects) or SC (8 subjects) route, for a total of 25 IV and 16 SC product administrations during the study. There were no serious adverse events (SAEs), and no study pauses for adverse events (AEs). A total of 6 AEs were assessed as related to study product mild dizziness, mild abdominal pain, and 4 infusion reactions (1 mild and 3 moderate, reported for 2 subjects). One 40 mg/kg IV recipient developed moderate infusion reaction within one hour of infusion, and one 20 mg/kg IV recipient experienced mild to moderate infusion reactions with 3 infusions, with symptoms complete resolution within 12 hours. Symptoms were typical of infusion reactions observed with other monoclonal antibodies in terms of characteristics and temporal onset [49]. No participant required epinephrine or steroid administration. All AEs assessed as related to the study product resolved without residual effects. Other reported unsolicited AEs have been mild or moderate in

severity.

Within this limited dataset for solicited local reactogenicity, 2 of 17 (11.8%) subjects in IV groups reported mild pain and bruising at the injection site, and 4 of 8 (50.0%) subjects in SC groups reported mild symptoms of pain (n=4) and moderate redness (n=1) at the injection site.

For solicited systemic adverse events reported 3 days after product administration, 4 of 17 subjects (23.5%) receiving VRC07-523LS IV reported mild or moderate systemic reactogenicity symptoms of malaise (n=3); myalgia (n=3); joint pain (n=2); headache (n=2), chills (n=2), nausea (n=1), and fever (n=1). Five (5) of 8 subjects (62.5%) receiving VRC07-523LS SC reported mild systemic reactogenicity symptoms: malaise (n=3), headache (n=3), myalgia (n=2), joint pain (n=2), chills (n=1), and nausea (n=1).

Overall, VRC07-523LS administrations have been generally well tolerated with no unexpected reactions or SAEs reported; no safety signals or dose limited toxicity were detected.

1.3.3. *In Vivo* Anti-Viral Effect of VRC01 and VRC07-523LS

Analysis of the VRC 601 viral load data obtained from 8 viremic adults shows that VRC01 has a statistically significant *in vivo* virological effect on HIV viral load when administered as a single 40 mg/kg IV dose. None of these adults were taking ARV therapy when enrolled into the study and had not started on ARV during the time period when the viral load data were collected. Six of the eight adult subjects had ≥1 $\log_{10}$ copies/mL decrease in viral load and two subjects had a viral load drop of 0.26 and 0.18 $\log_{10}$ copies/mL respectively [7].

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 $\log_{10}$ decrease in viral load is 5 days; and,
- The median time to greatest decrease in viral load is 7 days.

A 0.5 $\log_{10}$ copies/mL or greater decrease in viral load is considered to be a positive response to ART [50]. To have clinical benefit, such a change would need to be sustained. In VRC 601, subjects were administered only one dose of VRC01 at 40 mg/kg and, thus, a sustained effect on viral load was not expected. However, the data demonstrate a VRC01 mediated anti-viral effect and that the established benchmarks can be obtained, and support the hypothesis that a schedule with repetitive dosing or with sustained antibody expression may have a beneficial clinical effect.

In protocol A5340, of the 13 evaluable participants who received VRC01 and underwent an analytic treatment interruption, 38% remained suppressed (HIV-1 RNA <200 copies/mL) at the week 4 visit, compared to 13% in historic controls (p=0.04). By week 8 only one of 13 (8%) participants maintained viral suppression, compared to 3% in historic controls (p=0.44) [51].

In protocol NIH 15-I-0140, all 10 participants with chronic HIV infection undergoing analytical treatment interruption experienced plasma viral rebound (>40 copies/ml) with a median time to rebound of 39 days (interquartile range, 29 to 39) or 5.6 weeks (interquartile range, 4.1 to 5.6) following cessation of ART [51].

In individuals with chronic HIV-1 infection on ART (protocol A5342), VRC01 infusions were safe and well tolerated but did not affect plasma viremia, cellular HIV-1 RNA/DNA levels, or stimulated virus production from CD4+ T cells [52].

In protocol RV397, after ART interruption, only one participant who received VRC01 achieved viral suppression for 24 weeks. The other 17 participants who underwent treatment interruption restarted ART because of confirmed recording of 1000 or more HIV-1 RNA copies per mL before 24 weeks [53].

In a limited experience with VRC07-523LS in HIV-infected, viremic adults enrolled in protocol VRC 607/A5378, preliminary interim viral load data showed that at 7 days post infusion, 8 of 9 participants had a $\geq$1.2 $\log_{10}$ decrease in viral copies/mL and 2 of 9 participants had a $\geq$ 2 $\log_{10}$ decrease. By day 14 post infusion, 6 of 9 participants had a > 1.5 $\log_{10}$ decrease in viral load [54].

1.3.4. Human Experience with Other rAAV Vectors

Evaluation of rAAV vectors in human clinical trials thus far demonstrated that these vectors are safe and well tolerated in humans [20, 22].

One serious adverse event resulting in a patient death was reported in 2007 and was evaluated as unlikely to be related to study product. In this study for rheumatoid arthritis, a TNF-α inhibitor was expressed using the rAAV2 vector. The event was reviewed by the Recombinant DNA Advisory Committee (RAC) and a panel of experts. A combination of factors including concurrent anti-TNF-α therapy, other immunosuppressive therapy, and residence in an area endemic for histoplasmosis was thought to be the likely explanation for the participant's fatal disseminated histoplasmosis [55].

Overall, investigational rAAV vectors have been used in clinical trials since the mid-1990s, with a strong safety record established upon local or systemic administration in over 300 subjects treated by 2011 [20]. An indication for potential clinical efficacy has been observed in the treatment against a number of diseases including congenital blindness [56], Parkinson disease [57, 58], and hemophilia [34, 35, 59]. Notably, a clinical trial of the rAAV8 vector expressing human factor IX (FIX) used for treatment of severe hemophilia B has been reported [34]. This study used a single intravenous infusion of AAV8-FIX; the treatment was well tolerated up to the highest dose of $2x10^{12}$ vg/kg body weight and the transgene was expressed in the range of 3-11% of normal levels. The study reported long-term follow up data for 10 patients along with a conclusion that the infusion of a single dose of rAAV8 vector resulted in long-term therapeutic factor IX expression associated with clinical improvement. No late toxic effects from the therapy were reported within a follow-up period up to 3 years [60].

An IM delivery of the rAAV1 vector expressing lipoprotein lipase (Glybera®, alipogene tiparvovec) at doses up to $1x10^{12}$ vg /kg has been demonstrated to be safe and well-tolerated in subjects with lipoprotein lipase deficiency (LPLD) [27]. On November 2, 2012, the European Commission approved the marketing authorization for Glybera®, as a treatment for LPLD, under exceptional circumstances, in all EU member states.

Two gene therapy products based on AAV vectors have been recently approved by the FDA for treatment of genetic diseases. Luxturna® was approved for treatment of the Leber's congenital amaurosis type 2, a retinal dystrophy in persons with reduced or absent levels of biologically active human retinal pigment epithelial 65 kDa protein, RPE65 (1.5×10^{11} vg of the AAV2 vector carrying a functional copy of the RPE65 gene to cells of the retina, administered by subretinal injection [61, 62]). Zolgensma® was approved for treatment of a spinal muscular atrophy in children less than 2 years of age with bi-allelic mutations in the survival motor neuron (SMN) gene (2.0×10^{14} vg/kg of the AAV9 vector carrying a functional copy of the SMN gene, administered IV [63, 64]).

rAAV vectors have been tested for HIV-1 antibody expression in animal studies [8-10]. In one study completed in 21 healthy volunteers (NCT01937455) with a gp 120 V1/V2-specific PG9 antibody, delivered IM using the rAAV1 vector to mediate antibody expression, no safety concerns were identified [65]. Reactogenicity was generally mild or moderate and resolved without intervention. No serious adverse events or adverse events evaluated as related to product were recorded. The PG9 antibodies were detected by HIV neutralization in the serum of four subjects and not detected by ELISA. PG9 anti-drug antibody was present in ten volunteers in the higher dose groups. Both anti-AAV1 antibodies and AAV1-specific T-cell responses were detected [65].

Recent reports indicated that three deaths occurred in a clinical study of the AAV8-based gene therapy product for a rare genetic neuromuscular disorder, x-linked myotubular myopathy (XLMTM) [66-68]. Per a brief report from the July 3, 2020 issue of Science [66], Audentes announced on June 23, 2020 that 2 patients in its phase III AT132 XLMTM trial had died from sepsis related to liver dysfunction. These patients received 3 x 10^{14} vg/kg of the AT123 product by IV infusion. At least one of these two patients had pre-existing liver disease. Earlier dosing of the product resulted in improved muscle function in other participants in this trial. A new press release from Audentes confirmed death of the third subject in the same study [69]. By preliminary report, the third subject died of gastrointestinal bleeding; 3 of 17 subjects treated with the high IV dose of the AT123 product have now died [70].

The only similarity of the vector used in the Audentes study to that used in VRC 603 is the AAV8 coat protein; the promoter sequence and the gene expressed are different. The gene therapy product dose administered by IV infusion to subjects in the Audentes XLMTM trial is more than 100 times greater than our highest VRC 603 injection dose administered IM. No hepatotoxicity has been observed on the VRC 603 study so far, and this new information may not be applicable to the VRC 603 study since the product in Audentes study has different indication, different route of administration, and was administered at much higher dose. The 8 subjects that are currently enrolled in VRC 603 already received the study product without safety concerns identified and are currently being followed for safety.

1.4. Pharmacokinetic Parameters

PK parameters of the passively administered MAbs VRC01 and VRC07-523LS have been evaluated in a limited number of healthy and HIV-infected adults [6, 7, 71]. Studies are ongoing to further describe the PK of VRC01 and VRC07-523LS after a single dose and a repeat dosing by IV and SC routes.

In animal studies in humanized mice, rAAV8 vector encoding HIV-specific MAb 4E10 was administered IM and induced a detectable level of antibody in circulation after a week. Expression continued to rise to maximum levels at 10-16 weeks, and then decreased 2 or 3 folds before stabilizing for the 64-week study [8]. Similar PK parameters were observed with AAV8-VRC01 and AAV8-VRC07 constructs in humanized mice, and protection from HIV infection was demonstrated [8, 9].

In NHPs, rAAV8 vector encoding a simianized form of VRC07 provided for detectable antibody in blood within a week after IM administration at 1x10^{13} vg, with a peak VRC07 concentration in plasma in the range 2.5-7.7 mcg/mL between weeks 2 and 4. Strong immune response to the product was noted, and VRC07 became undetectable in the blood of all macaques by week 9. With immune suppression by cyclosporine, VRC07 circulated in macaques for 16 weeks at levels up to 66 mcg/mL [10].

PK parameters of VRC07 delivered by episomal gene expression using the AAV8 vector in humans will be evaluated in this study.

2. STUDY AGENT

The study agent, AAV8-VRC07 (VRC-HIVAAV070-00-GT) was produced under current Good Manufacturing Practice (cGMP) regulations by the Clinical Vector Core, Center for Cellular and Molecular Therapeutics, The Children's Hospital of Philadelphia (CHOP), Philadelphia, PA, under contract to VRC. Refer to the IB for manufacturing information. Quality Assurance (QA) lot release testing and ongoing stability studies verify conformance to product specifications.

2.1. AAV8-VRC07 (VRC-HIVAAV070-00-GT)

AAV8-VRC07, developed by VRC/NIAID/NIH, is a gene transfer product intended for long-term expression of VRC07, a broadly neutralizing human MAb targeted against the HIV-1 CD4 binding site. The product is composed of a serotype 8 adeno-associated viral capsid containing a DNA expression cassette for genes encoding the heavy and light chains of the VRC07 monoclonal antibody. AAV8-VRC07 is provided at a concentration of $2.84x10^{13}$ vg/mL in a volume of 1 mL/vial.

The construct is non-replicating, and both heavy and light chain genes are expressed in the same reading frame and then processed post-translationally to produce the heavy and light chain proteins. Flanking each end of the construct are ITRs from the AAV serotype 2 that allow for vector genome packaging into AAV particles. The ITRs are the only AAV sequences remaining in the vector genome. The open reading frames for the AAV Rep and Cap gene products have been removed from the vector and therefore, there are no AAV proteins encoded by the construct. The AAV8 capsid protein was provided *in trans* during manufacturing.

The expressed VRC07 is a natural antibody and a clonal relative of VRC01. The variable region of the VRC07 heavy chain has 15 amino acid differences from VRC01 and a 4 amino acid insertion in the complementarity-determining region (CDR) H3. The VRC07 heavy chain (IgG1) was paired with the VRC01 light chain. Using a panel of 179 Env pseudoviruses, VRC07 was able to neutralize 93% of primary HIV-1 viruses with a half-maximal inhibitory concentration (IC_{50}) less than 50 mcg/mL and 83% of viruses with an IC_{50} less than 1 mcg/mL [3, 4].

Refer to the IB for more details on AAV8-VRC07 composition and manufacturing.

2.2. Preclinical Safety Studies

The *in vitro* preclinical safety studies performed to assess potential off target binding of VRC07 are summarized in **Table 1**.

Table 1: *In Vitro* Preclinical Safety Studies with VRC07

	Study Purpose	Study Outcome
1	Assessment of anti-phospholipid reactivity by binding to cardiolipin.	VRC07 does not react to phospholipids [3]
2	Assessment of anti-nuclear antigen reactivity	VRC07 does not react with nuclear antigens
3	Assessment of anti-phospholipid reactivity by impact on activated partial thromboplastin time (aPTT)	VRC07 does not react to phospholipids
4	Assessment of binding to a human epithelial cell line (HEp-2) by Immunohistochemistry	VRC07 does not bind to HEp-2 cells [3]
5	Assessment of potential "off target" binding in a human Tissue Cross-Reactivity study with VRC07	VRC07 staining of the cytoplasmic compartment was judged of no toxicologic relevance as staining was observed in the cytoplasmic compartment [72, 73]. No binding to membranous targets was observed. Extracellular granules/globules often observed in connective tissue or in vascular walls and/or perivascular connective tissue were stained in several human tissues.

A single dose safety and biodistribution GLP study of AAV8-VRC07 (VRC-HIVAAV070-00-GT) administered by intramuscular injection was conducted in male and female BALB/c mice. No toxicologically meaningful findings or changes were seen in clinical observations, body weight, food consumption, body temperature, dose site irritation, ophthalmology, or clinical chemistry that could be attributed to AAV8-VRC07 administration, and no target organs of toxicity were identified upon histopathologic examination.

Biodistribution analysis revealed that all high-dose ($2.4x10^{13}$ vg/kg) treated mice were positive for AAV8-VRC07 in each of the 15 examined tissues on Days 7, 30, and 90. Levels of AAV8-VRC07 in blood were below the lower limit of quantitation (LLQ, <40 copies/mcg gDNA) in half of the animals on Day 90. In general, the levels of AAV8-VRC07 did not differ meaningfully between males and females.

Refer to the IB for more information on these and other preclinical studies with AAV8-VRC07.

2.3. Proof-of-Concept Studies with AAV8-VRC07

VRC conducted several proof-of-concept studies with the proposed clinical construct and with laboratory modifications of AAV8-VRC07 vectors (1835-rAAV8-VRC07 and 1830-rAAV8-VRC07) (Table 2). More information on these research grade vectors and on proof-of-concept studies can be found in the IB. Murine and non-human primate studies conducted with research-grade AAV8-VRC07 vectors provide a foundation for understanding the activity of VRC07 vectored immunoprophylaxis and vectored immunotherapy.

Table 2: Pre-clinical Proof-of-concept Studies of AAV8-VRC07 and Other Vectors

	Study Purpose	Study Outcome
1	Kinetics of expression of VRC07 by AAV8-VRC07, 1830- and 1835–rAAV8-VRC07 vectors in NSG immunodeficient mice	When administered IM at 1 x 10^{11} vg to NSG immunodeficient mice, the 1830-rAAV8-VRC07 vector expressed approximately 200 mcg/mL of VRC07 within 8 weeks of administration. 1835-rAAV8-VRC07 and AAV8-VRC07 exhibited somewhat lower levels of expression at >100 mcg/mL within 8 weeks.
2	Dose-dependent VRC07 expression in immunocompetent BALB/c mice mediated by AAV8-VRC07	When AAV8-VRC07 was administered IM to BALB/c mice at doses of 5 x 10^{9} vg, 5 x 10^{10} vg, 5 x 10^{11} vg and 1 x 10^{11} vg, the VRC07 expression was dose dependent and resulted in high-level expression for at least 12 weeks in immunocompetent mice despite the presumed ability of the mice to generate an anti-human immunoglobulin and anti-AAV8 response.
3	Dose response efficacy of the 1830 - rAAV8-VRC07 vector in a NSG mouse HIV challenge model	When 1830-rAAV8-VRC07, a vector similar to AAV8-VRC07, was administered IM at two-fold decreasing doses of the vector, starting at 1 x 10^{11} vg, the minimal protective serum concentration of VRC07 was determined to be 3 to 6 mcg/mL in NSG mice.
4	Ability of VRC07 expressed by 1835-rAAV8-VRC07 vector to control HIV-1 infection in BLT humanized mice	BLT humanized mice with an established HIV-1 infection were able to control viral replication after a single IM administration of 1835-rAAV8-VRC07 at 5x10^{11} vg, as viral load approached the assay lower limit of detection (LOD) in animals even after discontinuation of Highly Active Antiretroviral Therapy (HAART).
5	Efficacy of AAV8-simVRC07 in rhesus macaques immunosuppressed by transient cyclosporine administration	One dose of AAV8-simVRC07 at 1 x 10^{13} vg administered IM 5.5 weeks prior to a single intrarectal challenge with SHIV, prevented infection in 4/6 rhesus macaques. Of the 2 infected macaques, one had no detectable simVRC07 in circulation, and the other showed anti-simVRC07 plasma activity and had the lowest sim-VRC07 concentration on the day of challenge [10].

3. STUDY OBJECTIVES

3.1. Primary Objectives

- To evaluate the safety and tolerability of AAV8-VRC07 administered IM at 5x10^{10} vg/kg, 5x10^{11} vg/kg, or 2.5x10^{12} vg/kg to HIV-1 infected adults on effective ARV therapy.
- To evaluate the pharmacokinetics of VRC07 at each dose level through 24 weeks after injection and to determine the AAV8-VRC07 dose that achieves at least 50 mcg/mL VRC07 concentration in serum at 4 weeks post injection with a target set point of ≥5 mcg/mL at 12 weeks post injection.
- To describe immune responses to the AAV8-VRC07 product.

3.2. Secondary Objectives

- To assess for potential clinical effects of the pAAV8-VRC07 administration on CD4 cell count and viral load in study participants.
- To determine the serum concentration of VRC07 at specified time intervals for 1 year after injection, and if persistent, then every 6 months as long as there is detectable antibody in serum.

3.3. Exploratory Objectives

- To determine if measurable levels of VRC07 can be found in oral mucosal secretions after AAV8-VRC07 administration.
- To evaluate for evidence of functional activity of VRC07 in samples collected at representative timepoints throughout the study.
- To test subjects for the IgG1 allotypes in order to evaluate allotype-specific effects on VRC07 pharmacokinetics.
- To investigate immune responses to the AAV8-VRC07 product.

4. STUDY DESIGN

This is a Phase I dose-escalation study of the safety and tolerability of AAV8-VRC07 expressing a HIV-1 CD4 binding site-specific neutralizing antibody, VRC07, in HIV-1 infected adults on ARV therapy. The study will also examine the pharmacokinetics of VRC07 expression following IM administration of AAV8-VRC07. The hypotheses are: 1) AAV8-VRC07 will be safe for human administration and will not elicit hypersensitivity or anti-drug antibody (ADA) to VRC07; and 2) intramuscular (IM) delivery of AAV8-VRC07 will result in VRC07 antibody production at a concentration in serum that is measurable and safe.

The study schema is shown in Table 3**.**

Table 3: VRC 603 Study Schema

Group	Dose	Subjects	Total product volume based on product concentration of $2.84x10^{13}$ vg/mL and a subject weight of 100 kg
1	$5x10^{10}$ vg/kg	2-5	0.18 mL
2	$5x10^{11}$ vg/kg	2-5	1.76 mL
3	$2.5x10^{12}$ vg/kg	2-5	8.80 mL
Total	2 to 5 subjects per group to enrollment of 15 subjects. Enrollment up to a total of 25 subjects is permitted in case there are subjects who do not complete the schedule, if additional PK evaluations are needed, or if an enrollment of additional subjects is recommended for safety evaluations.		

Subjects will be enrolled sequentially and assigned to the dosage level being evaluated at the time of enrollment. Cumulative safety data will be reviewed weekly by a Protocol Safety Review Team (PSRT) while injections are being administered.

Each subject will receive a single dose of study product, as one or more IM injections. Safety, including reactogenicity and unsolicited AEs, laboratory findings, and VRC07 levels in blood will be assessed after the injection and summarized for an interim analysis at 4 weeks post injection. **The**

second subject in each dose group will be injected after the 4 weeks safety assessment for the first enrolled subject.

Decisions on dose escalation and subject enrollments will be based on safety data and the VRC07 concentration in blood achieved at 4 weeks after product administration (Section 4.3). The pharmacokinetics of VRC07 at each dose level will be evaluated to determine the dose that achieves at least 50 mcg/mL VRC07 concentration in serum at 4 weeks post injection with a target set point of ≥ 5 mcg/mL at 12 weeks post injection.

Safety lab samples will be collected per Schedule of Evaluations (**Error! Reference source not found.**). Subjects will keep a daily diary of solicited systemic symptoms for 7 days after product administration. PK samples will be collected at specified intervals through 52 weeks after product administration, and if persistent, every 6 months through 5 years after injection.

In subjects who agree to the optional mucosal sample collection, oral fluid samples will be obtained at specified time-points (Section 4.2.6).

The study will be conducted by the VRC Clinic at the NIH Clinical Center (NIH CC).

4.1. Study Population

All inclusion and exclusion criteria must be met for eligibility.

4.1.1. Inclusion Criteria

A volunteer must meet all of the following criteria:

1. Able and willing to complete the informed consent process.
2. 18 to 65 years of age.
3. HIV-1 infected.
4. On a stable antiretroviral regimen for ≥ 3 months.
5. Available for clinical follow-up through the last study visit.
6. Based on history and examination, must be in general good health with no evidence of clinically significant lab abnormalities and without additional clinically significant medical conditions as per exclusion criteria.
7. Willing to maintain or establish a relationship with a primary health care provider for medical management of HIV infection while participating in the study.
8. Willing to have blood samples collected, stored indefinitely, and used for research purposes.
9. Able to provide proof of identity to the satisfaction of the study clinician completing the enrollment process.
10. Laboratory tests assessing subject health will be conducted within 84 days prior to enrollment and values must meet the following criteria:
    a. White blood cell count (WBC) 2,500-12,000/mm^3;
    b. WBC differential either within institutional normal range or accompanied by approval of the Principal Investigator (PI) or designee;
    c. Platelets = 125,000 – 400,000/mm^3;
    d. Hemoglobin ≥ 10.0 gm/dL;

e. Creatinine ≤ 1.25 x upper limit of normal (ULN);
f. ALT ≤ 1.1 x ULN;
g. AST≤ 1.1 x ULN; and,
h. VL ≤ 50 copies/mL and a CD4 count ≥ 300/mcL.

Male-Specific Criteria:

1. Males must agree to use condoms for all sexual activity of any reproductive potential for 52 weeks after receiving the study product.

Female-Specific Criteria:

1. If a woman is sexually active with a male partner and has no history of hysterectomy, tubal ligation or menopause, she must agree to use either a prescription birth control method or a barrier birth control method from the time of study enrollment through study Week 52, or to be monogamous with a partner who has had a vasectomy.
2. Negative β-HCG (human chorionic gonadotropin) pregnancy test (urine or serum) on day of enrollment for women presumed to be of reproductive potential.

4.1.2. Exclusion Criteria

A volunteer will be excluded if one or more of the following conditions apply:

1. Previous receipt of monoclonal antibody whether licensed or investigational.
2. Previous receipt of gene therapy product.
3. Ongoing AIDS-related opportunistic infection (including oral thrush).
4. Active injection drug use or active drug or alcohol use or dependence that, in the opinion of the site investigator, would interfere with adherence to study requirements.
5. A titer of pre-existing antibodies to AAV8 capsid is > 1:90.
6. Weight > 115 kg for Group 3 subjects only.
7. History of a severe allergic reaction with generalized urticaria, angioedema or anaphylaxis within the 2 years prior to enrollment that has a reasonable risk of recurrence.
8. Bleeding disorder diagnosed by a doctor (e.g. factor deficiency, coagulopathy, or platelet disorder requiring special precautions) or significant bruising or bleeding difficulties with IM injections or blood draws.
9. Active liver disease such as chronic hepatitis.
10. Hypertension that is not well controlled by medication.
11. Woman who is breast-feeding or planning to become pregnant during the study participation.
12. Receipt of any investigational study agent within 28 days prior to enrollment.
13. Current infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).
14. 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; OR clinically significant forms of asthma, autoimmune disease, psychiatric disorders, heart disease, or cancer.

4.2. Clinical Procedures and Laboratory Assays

Evaluation of safety for this study will include clinical laboratory assessments, medical history, and physical examination by clinicians. In response to the coronavirus disease 2019 (COVID-19) pandemic and changing information related to testing, all NIH CC epidemiologic and testing guidelines will be followed in the study conduct.

The study schedule is provided in **Error! Reference source not found.**. Total blood volume drawn from each subject will comply with the NIH Clinical Center Guidelines, which is available on the NIH intranet at the following link: http://cc-internal.cc.nih.gov/policies/PDF/M95-9.pdf.

4.2.1. Screening

Screening for this study will be completed through the Vaccine Research Center's screening protocol, VRC 500 (NIH 11-I-0164). Volunteers will be recruited through Institutional Review Board (IRB)-approved advertising. The evaluations and sample collection that will be included in screening are a medical history, physical exam, laboratory tests and procedures listed in APPENDIX I**,** any laboratory tests needed to confirm eligibility, and pregnancy test (for females of reproductive potential). Additional clinical and laboratory assessments of health may be conducted at screening based on clinical judgment. Screening evaluations must be completed within 84 days prior to enrollment and may be repeated as needed to confirm eligibility within this time interval.

A nasopharyngeal swab to evaluate for current SARS-CoV-2 infection must be collected no more than 5 days prior to enrollment and study product administration on Day 0. The testing result must be negative for a subject to participate in the study.

Research samples of PBMCs, plasma and serum will also be collected during the screening period. VRC 603 informed consent documents will be reviewed. Counseling related to potential risks of study agent and pregnancy prevention will be performed. An Assessment of Understanding (AoU) will be completed in association with enrollment into VRC 603.

4.2.2. Enrollment, Study Days and Visit Numbers

In this study, Day 0 is defined as the day of protocol enrollment and the day of AAV8-VRC07 product administration. Informed consent is obtained on Day 0 prior to enrollment. The study identification number and study group schedule will be assigned in the clinical database on Day 0. For all subjects, a clinician will discuss the timing of the study product administration and PK sample collection before completing an enrollment to help ensure that the subject can comply with the projected schedule.

Medical history and Day 0 evaluations prior to the study product administration are the baseline for subsequent safety assessments. For calculating elapsed days, each subsequent calendar date is labeled by the next sequential "Study Day" as shown in **Error! Reference source not found.**. The visit schedule is the same for all groups, and it is based on intervals of time after study injection (**Error! Reference source not found.**).

The schedule of study visits, permitted windows for completing the visits, and evaluations performed at each visit are shown in (**Error! Reference source not found.**).

4.2.3. Administration of AAV8-VRC07

All study agent administrations will be completed according to the assigned group. For women of childbearing potential, study agent administration may not proceed unless a negative pregnancy test

has been obtained within the previous 24 hours. Prior to administration, temperature, blood pressure, heart rate (pulse) and weight will be collected and a targeted physical examination (based on signs, reported symptoms or interim medical history) will be conducted. A subject who arrives at the clinic with fever or evidence of an acute illness that would preclude administration of the product may be rescheduled for a later date.

Injections will be administered into the deltoid muscle and/or in a thigh. The administration site(s) to be used will be discussed with the subject and must be assessed as acceptable by the clinician and the subject. All injections will be administered intramuscularly (IM) by needle and syringe in 1 to 11 sites depending on the volume. Not more than 1 mL will be administered at a single injection site.

Refer to Section 7 for AAV8-VRC07 preparation for administration procedures. For administration of the prepared product in the clinical setting, the clinical staff should practice universal precautions and dispose of the used needles and syringes in keeping with the required practices for handling sharps in the medical facility.

In all study groups, the subject will be observed for at least 30 minutes following each product administration.

4.2.4. Solicited Adverse Events and Clinical Follow-up

Subjects will be given a "Diary Card" to use as a memory aid for solicited adverse events, on which to record temperature and systemic symptoms daily for 7 days after study product administration. Subjects will be trained and encouraged to use the secure database but may complete the paper diary if necessary. When the 7-day diary card parameters are recorded directly by the subject through a password-protected secure database, the subject's electronic record will be the source for these data. The written (paper) diary card may be used as a source document. When neither a written nor electronic diary card is available from the subject, the study clinician will note the source of reactogenicity information recorded in the study database.

For this study, solicited adverse events occurring during the 7 days after receipt of study agent will include: unusually tired/feeling unwell, muscles ache, headache, chills, nausea, and joint pain. Subjects will also record the highest measured temperature daily. Local reactogenicity parameters will include pain/tenderness, swelling, and redness at the injection site(s).

The diary cards will be reviewed for accuracy and completeness at follow-up visits. Clinicians will follow and collect resolution information for any reactogenicity symptoms that are not resolved within 7 days.

Events that may require a clinic visit include rash, urticaria, fever of 38.6˚C (Grade 2) or higher lasting greater than 24 hours or significant impairment in the activities of daily living. Clinical concerns may prompt an unscheduled study visit based on the judgment of a study clinician. Clinical laboratory assays and clinical evaluations will assess safety and tolerability at specified intervals after product administration.

4.2.5. Pharmacokinetics Procedures

Pharmacokinetic samples will be collected as close as reasonably possible to the target timepoint. Actual time of collection will be recorded for all samples. PK timepoints are shown in **Error! Reference source not found.**.

4.2.6. Mucosal Samples

In order to begin to understand the tissue distribution and duration of VRC07 expression, this protocol includes exploratory collection of oral mucosal samples. Subject will be offered the option of participating in the mucosal sampling schedule, but it will not be required. If subjects agree to the procedures, an oral fluid sample will be obtained before the product administration. All subjects will have an option to participate in collection of oral mucosal samples at specified intervals through the study (APPENDIX I). Samples will be collected using small ophthalmic sponges designed for clinical use.

Oral fluid samples may be collected at enrollment and on Days 42, 84, 168, 252, and 336 after product administration.

4.2.7. Schedule of Evaluations

Refer to **Error! Reference source not found.** for details on the schedule of evaluations and the windows permitted for completion of each visit. After Day 0, deviations from the visit windows in completing study visits are discouraged and will be recorded as protocol deviations but are permitted at the discretion of the PI (or designee) in the interest of completing the follow-up schedule and obtaining subject safety and VRC07 PK evaluations.

Additional visits and blood drawing may be scheduled during the study if needed to assess subject safety or for sample collection for immunological testing.

Any evaluation for an adverse event or possible exacerbation of a pre-existing condition may be evaluated at study team discretion as a "protocol related" evaluation.

4.2.8. Concomitant Medications

Only routine prescription medications will be entered in the database at the time of enrollment. After administration of AAV8-VRC07, only concomitant medications associated with a change in the ARV regimen, an adverse event that requires expedited reporting, or the development of a new chronic condition requiring ongoing medical management will be recorded in the database. Otherwise, concomitant medications taken throughout the study will be recorded in the subject's chart as needed for general medical records but will not be recorded in the study database.

4.3. Criteria for Dose Escalation

The Protocol Safety Review Team (PSRT, Section 8.8) will conduct an interim safety data review before dose escalation may occur. The PSRT must assess the data as showing no significant safety concerns before proceeding with enrollment of the next dose level.

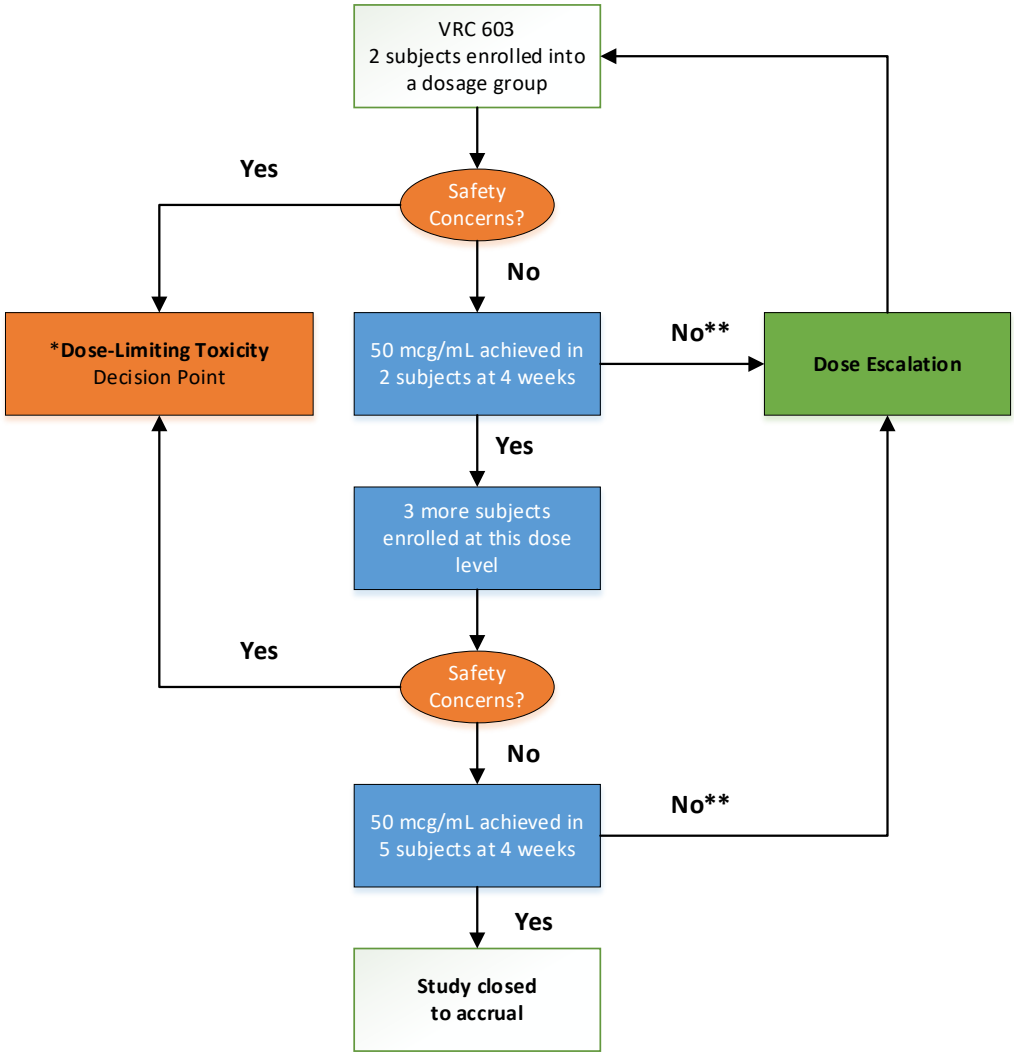
Safety, including reactogenicity and unsolicited AEs, laboratory findings, pharmacokinetics and VRC07 levels in blood will be assessed after the injection and summarized for an interim analysis at 4 weeks post injection for each subject.

The following algorithm will be used for enrollments and dose escalation:

- If serum levels of 50 mcg/mL VRC07 are not achieved at 4 weeks post-injection in the first 2 subjects in the dose group, and if there are no safety concerns, the dose escalation will occur and the same procedure will be followed for the next higher dose group as shown in the decision tree schema (Figure 1).

- If both of the first two subjects in a dose group achieve a serum VRC07 level of at least 50 mcg/mL at 4 weeks post injection with an acceptable safety profile, then 3 more subjects will receive this dose at no more than 1 subject per day.
- If all 5 subjects within a dose group reach the 50 mcg/mL concentration of VRC07 in serum at 4 weeks post-injection, escalation to the next dose level will not be conducted and the study will be closed for accrual.
- If less than 5 subjects achieve serum levels of 50 mcg/mL of VRC07 AND
  - There are no safety concerns, then accrual will begin at the next dosage level.
  - There are safety concerns, the PSRT may recommend evaluation of additional subjects at the current dose level and reassess for safety and VRC07 concentration before proceeding to a higher dose level **(**Figure 1: VRC 603 Decision Tree for Dose Escalation Based on Safety and VRC07 Concentration in Figure 1).
- If a dose-limiting toxicity is detected as per **Section 4.5**, the safety and dose escalation review will include a decision on accrual continuation at a lower product dose that was evaluated to be safe.

Figure 1: VRC 603 Decision Tree for Dose Escalation Based on Safety and VRC07 Concentration in Serum



* As defined in Section 4.5.

** Before proceeding to a higher dose level, the PSRT may recommend evaluation of additional subjects at the current dose level.

If there are discontinuations from the study before there are sufficient data to conduct the dose escalation review for a group, then extra subjects may be enrolled into that group in order to have the requisite data.

The IRB, the IND sponsor, and the FDA will be provided with documentation of the safety review process and notification of the dose escalation.

4.4. Protocol Criteria for Pausing the Study and Resuming the Study

Administration of the study agent and new enrollments will be paused by the study PI according to the criteria noted below.

Pause criteria are as follows:

- **One** (or more) subject experiences a **Serious Adverse Event** (SAE) that is assessed as related to study agent, or
- **Two** (or more) subjects experience the same **Grade 3 or higher** adverse events (AE) assessed as related to study agent.

4.4.1. Plan for Review of Pauses and Resuming Rules:

Administration of the study agent and enrollments would resume only if review of the adverse events that caused the pause resulted in a recommendation to permit further study product administrations and study enrollments. The reviews to make this decision will occur as follows:

Pauses for related SAEs: The IND Sponsor, with participation by the PI, will consult with the FDA to conduct the review and make the decision to resume, amend, or close the study. The IRB will be notified accordingly.

Pauses for Grade 3 or higher related AEs: The IND Sponsor and the study PI will conduct the review and make the decision to resume, amend or close the study for the Grade 3 or higher events that meet the criteria for pausing the study. As part of the pause review, the reviewers will also advise on whether the study needs to be paused again for any subsequent events of the same type. The FDA and the IRB will be notified of pause reviews triggered by Grade 3 or higher AEs and the IND sponsor decisions.

4.5. Dose-Limiting Toxicity

Dose-limiting toxicity is defined in this protocol as an AE that prevents a dose escalation of the study product or prevents continuation of study injections at a given dosage level. If the study is paused as per criteria in Section 4.4 and a decision is made to not resume the study injections at the current dose, then the safety review will include a decision as to whether or not to continue accrual at a lower product dose that was evaluated to be safe in order to achieve the target study accrual of 15 subjects.

5. SAFETY AND ADVERSE EVENT REPORTING

5.1. Adverse Events

An adverse event (AE) is any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (e.g., abnormal physical exam or laboratory finding), symptom, or disease temporally associated with the subject's participation in research, whether or not considered related to the study treatment. In the context of FDA-required reporting, an AE means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Severity of AEs will be assessed using the *DAIDS Table for Grading the Severity of Adult and Pediatric Adverse Events*. Additional information can be found in **Error! Reference source not found.**I.

Injection site bruising is an expected adverse event and will not be generally recorded as an AE. Clinically significant hematomas will be recorded as an AE.

Reporting of all AEs will occur during the period from the study agent administration through a visit completed at 56 days after product administration (Visit 10, week 8). After this through completion of study participation only serious adverse events (SAE) and new chronic medical conditions that require ongoing medical management will be recorded as AEs in the study database.

5.1.1. Attribution Categories

Attribution categories used (i.e., terms used for assessment of relationship of AE to study agent) for this study will be as follows:

- **Related** – There is a reasonable possibility that the AE may be related to the study agent(s).
- **Not Related** – There is not a reasonable possibility that the AE is related to the study agent(s).

If circumstances arise where other attribution categories are used in describing an adverse event, the attribution terms "Definitely," "Probably," and "Possibly" related will be mapped to the "Related" category while the terms "Unlikely," "Probably Not Related," and "Not Related" will be mapped to the "Not Related" category.

5.2. Serious Adverse Events (SAE)

The term "Serious Adverse Event" (SAE) is defined in 21 CFR 312.32 as follows: "An adverse event or suspected adverse reaction is considered serious if, in the view of either the investigator or the 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."

"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 a Serious Adverse Event. In Section 5.3 the term "Expedited Adverse Event" (EAE) encompasses the events that would be considered an SAE by the 21 CFR 312.32 definition.

5.3. Adverse Event Reporting to the IND Sponsor

Adverse events that meet SAE reporting requirements per the FDA definition (Section 5.2) must be reported and submitted by the clinical site on an expedited basis to the IND Sponsor, VRC/NIAID/NIH, according to sponsor guidelines as follows:

- results in death
- is life threatening

- results in persistent or significant disability/incapacity
- requires unplanned inpatient hospitalization or prolongation of existing hospitalization
- is a congenital anomaly/birth defect in the offspring of a study subject
- is an important medical event that may jeopardize the subject or may require intervention to prevent one of the other outcomes listed above.

In addition, any event, regardless of severity, which in the judgment of an investigator represents a serious adverse event, may be reported on an expedited basis.

An investigator will communicate the initial SAE report within 24 hours of site awareness of occurrence to the IND sponsor.

A written report by investigator should be submitted to the IND Sponsor within 3 working days. In order for the IND Sponsor to comply with regulations mandating sponsor notification of specified SAEs to the FDA within 7 or 15 calendar days, the investigator must submit additional information as soon as it is available.

5.3.1. IND Sponsor Reporting to the FDA

The IND Sponsor is responsible for making the determination of which SAEs are suspected unexpected serious adverse reactions (SUSARs) that meet criteria for expedited reporting as defined in 21 CFR 312.32.

- *Suspected adverse reaction* means any adverse event for which there is a reasonable possibility that the drug caused the adverse event.
- *Unexpected Adverse Event* means an AE that is not listed in the Investigator's Brochure or is not listed at the specificity or severity that has been observed.

All SUSARs (as determined by the IND Sponsor) will be reported to the FDA as IND Safety Reports as soon as possible but not exceeding 7 calendar days for unexpected fatal or life-threatening events, and not exceeding 15 calendar days for other qualifying events.

The IND Sponsor will also submit an IND Annual Report of the progress of the investigation to the FDA as defined in 21 CFR 312.33.

The IND Sponsor is responsible for providing copies of IND Safety Reports to the Clinical Site for submission to the IRB.

5.4. Reporting to the Institutional Review Board

The following information is consistent with NIH IRB Policy 801: Reporting Research Events, Version 1, effective July 1, 2019.

Reportable Event - An event that occurs during the course of human subjects research that requires notification to the IRB.

For the purposes of this policy, reportable events include the following:

- Unanticipated problems involving risks to subjects or others (UPs)
- Non-compliance (including major protocol deviations and noncompliance that is not related to a protocol deviation)
- Deaths related or possibly related to research activities

- New information that might affect the willingness of subjects to enroll or continue participation in the study

5.4.1. Unanticipated Problem (UP)

An "Unanticipated Problem (UP)" is defined as any incident, experience, or outcome that meets all three of the following criteria:

- **Unexpected** (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB approved research protocol and informed consent document: and (b), the characteristics of the subject population being studied; **and**,
- **Related or possibly related to** participation in the **research** (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); **and**,
- Suggests that the research **places subjects, or others** (which may include research staff, family members or other individuals not directly participating in the research) **at a greater risk of harm** (including physical, psychological, economic, or social harm) related to the research than was previously known or expected.

An UP must be reported to the NIH IRB within 7 calendar days of an investigator becoming aware of the actual or suspected UP.

5.4.2. Non-Compliance Definition

Non-compliance is the failure of investigator(s) to follow the applicable laws, regulations, or institutional policies governing the protection of human subjects in research, or the requirements or determinations of the IRB, whether intentional or not.

Non-compliance may be unintentional (e.g. due to lack of understanding, knowledge, or commitment), or intentional (e.g. due to deliberate choice to ignore or compromise the requirements of any applicable regulation, organizational policy, or determination of the IRB).

Non-compliance is further characterized as serious or continuing as follows:

- "Serious non-compliance" - Non-compliance, whether intentional or not, that results in harm or otherwise materially compromises the rights, welfare and/or safety of the subject. Non-compliance that materially effects the scientific integrity or validity of the research may be considered serious non-compliance, even if it does not result in direct harm to research subjects.
- "Continuing non-compliance" - A pattern of recurring non-compliance that either has resulted, or, if continued, may result in harm to subjects or otherwise materially compromise the rights, welfare and/or safety of subjects, affect the scientific integrity of the study or validity of the results. The pattern may comprise repetition of the same non-compliant action(s), or different noncompliant events. .

Any actual or suspected non-compliance by any investigator or entity associated with the protocol must be reported to the IRB by the PI/designee within 7 calendar days of any investigator or individual associated with the protocol first becoming aware

5.4.3. Protocol Deviation

A Protocol Deviation is a non-compliance defined as any change, divergence, or departure from the IRB-approved research protocol and is further characterized as major and minor as follows:

- Major Deviations – Deviations from the IRB approved protocol that have, or may have the potential to, negatively impact, the rights, welfare or safety of the subject, or to substantially negatively impact the scientific integrity or validity of the study.
- Minor Deviations – Deviations that do not have the potential to negatively impact the rights, safety, or welfare of subjects or others, or the scientific integrity or validity of the study.

For the reporting purposes, failure of subjects to comply with the research protocol does not represent non-compliance unless that failure is due to an action or omission of a member of the research team, for example, the failure to give adequate instruction to the subject.

A major deviation must be reported to the NIH IRB within 7 calendar days of an investigator becoming aware of an actual or suspected deviation. Although protocol deviations are also non-compliance, these should only be reported once as deviations. Major deviations resulting in death must be reported within 24 hours of the occurrence of the event or of any member of the study team becoming aware of the death.

A series of minor deviations pointing toward a more global issue that could affect the rights, safety or welfare of the participant or affect the validity of the study should be reported as a major deviation. In all other instances, a summary of minor deviations should be provided to the IRB at the time of continuing review.

5.4.4. Death

Any death of a research subject that is possibly, probably or definitely related to the research must be reported within 24 hours of an investigator becoming aware of the death.

5.4.5. New Information

New information that might affect the willingness of a subject to enroll or remain in the study should be reported within 7 calendar days of an investigator first becoming aware.

5.4.6. Suspension or Termination of Research Activities

Any suspension or termination of research activities, including holds on new enrollment, placed upon the research by the study sponsor, NIH leadership, or any regulatory agency must be reported to the NIH IRB within 7 calendar days of an investigator becoming aware.

5.4.7. Expedited Reporting to the NIH IRB

Death related to research must be reported within **24 hours**.

The following will be reported within **7 calendar days** of investigator awareness:

- Actual or suspected UPs;
- Actual or suspected non-compliance;
- Actual or suspected major protocol deviations;
- SAEs that are actual or suspected UPs;

- New information that might affect the willingness of a subject to enroll or remain in the study;
- Suspension or termination of research activities, including holds on new enrollment, placed upon the research by the study sponsor, NIH leadership, or any regulatory agency.

5.4.8. Annual Reporting to the NIH IRB

The following will be reported to the NIH IRB in summary at the time of Continuing Review:

- Summary of UPs and non-compliance;
- Adverse Events (AEs), including Serious Adverse Events (SAEs), that are not UPs, as a narrative summary statement indicating whether these events were within the expected range;
- Minor Protocol Deviations (aggregate summary); and,
- Any trends or events which in the opinion of the investigator should be reported.

5.5. Reporting to the Institutional Biosafety Committee

The NIH Institutional Biosafety Committee (IBC) (Bethesda, MD) reviews research using recombinant DNA for compliance with NIH Guidelines. In keeping with IBC requirements, any SAE report sent to the IRB will be subsequently provided to the IBC.

6. STATISTICAL CONSIDERATIONS

6.1. Overview

This is a Phase I study of the safety, tolerability and pharmacokinetics of AAV8-VR07 (VRC-HIVAAV070-00-GT) expressing VRC07 human monoclonal antibody with broad HIV-1 neutralizing activity in HIV-1 infected adults. It is a dose-escalation study to examine safety, tolerability, dose and pharmacokinetics of VRC07 expression following IM administration of AAV8-VRC07 in subjects on ARV therapy.

6.2. Objectives

The primary objectives of the study are to evaluate the safety and tolerability of AAV8-VRC07, to evaluate the pharmacokinetics of VRC07 expression, and to estimate the dose of AAV8-VRC07 that results in antibody production that achieves VRC07 concentration of at least 50 mcg/mL in serum at 4 weeks after product administration. Immune responses to the product will also be evaluated.

6.3. Size and Accrual

6.3.1. Sample Size Considerations

This study is primarily descriptive. For safety analysis, the goal is to identify safety concerns associated with different AAV8-VRC07 dosages. There may be as few as 2 to 5 subjects in a group at the time of a dose escalation; therefore, this section considers group sizes of both n=2 and n=5.

The ability to identify serious adverse experiences is best expressed by the maximum true rate of SAE that would unlikely be observed and the minimum true SAE rate that would very likely be observed. Within a group of size n=2, there is an 80% chance of observing at least 1 event if the true rate is no less than 0.55 and a 90% chance of observing no event if the true rate is no bigger than 0.05. Within a group of size n=5, there is an 80% chance of observing at least 1 event if the true rate is no less than 0.28 and a 90% chance of observing no event if the true rate is no bigger than 0.02.

Probabilities of observing 0 or more than 1 event are presented in Table 4 for a range of possible true event rates. These calculations provide a complete picture of the sensitivity of this study design to identify potential safety problems with the study agent. For example, within the group of size n=2, if the true event rate is 0.01, then there is a probability of 0.81 to observe no event and a probability of 0.01 to observe more than 1 event; while, within the group of size n=5, if the true event rate is 0.1, then there is a probability of 0.590 to observe no event and a probability of 0.081 to observe more than 1 event.

Table 4: Probability of observing event for different scenarios

	Within a group (n=2)		Within a group (n=5)	
True event rate	Pr(0 event observed)	Pr(more than 1 event observed)	Pr(0 event observed)	Pr(more than 1 event observed)
0.05	0.903	0.003	0.774	0.023
0.1	0.810	0.010	0.590	0.081
0.2	0.640	0.040	0.328	0.263
0.3	0.490	0.090	0.168	0.472
0.4	0.360	0.160	0.078	0.663

Table 5 gives the upper and lower bounds for 95% exact binomial confidence intervals for all possible number of observed events within a group. Within the group of size n=2, if no subjects experience the event, the 95% exact 2-sided confidence interval for the true rate has upper bound as 0.84; if all subjects experience the event, the 95% exact 2-sided confidence interval for the true rate has lower bound as 0.16. Within the group of size n=5, if no subjects experience the event, the 95% exact 2-sided confidence interval for the true rate has upper bound as 0.522; if all subjects experience the event, the 95% exact 2-sided confidence interval for the true rate has lower bound as 0.478.

Table 5: 95% confidence intervals of the true rate for all possible number of observed events within a group

Within a group (n=2) 95% confidence interval			Within a group (n=5) 95% confidence interval		
Observed rate	Lower bound	Upper bound	Observed rate	Lower bound	Upper Bound
0/2	0	0.842	0/5	0	0.522
1/2	0.013	0.987	1/5	0.005	0.716
2/2	0.158	1	2/5	0.053	0.853
			3/5	0.147	0.947
			4/5	0.284	0.995
			5/5	0.478	1

Tables 4 and 5 also apply to the secondary and exploratory endpoints.

6.4. Statistical Analysis

6.4.1. Analysis Variables

Analysis variables consist of baseline variables, pharmacokinetics and safety variables for primary and secondary objective analyses.

6.4.2. Baseline Demographics

Baseline characteristics including demographics and laboratory measurements will be summarized using descriptive statistics.

6.4.3. Safety Analysis

Summaries of the number and percentage of subjects experiencing any AE or reactogenicity will be tallied by subgroup, and presented along with exact 95% confidence intervals for the proportion.

Solicited Adverse Events:

Solicited adverse event data is collected after the product is administered in this study. The 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.

Adverse Experiences:

Unsolicited AEs are coded into Medical Dictionary for Regulatory Activities (MedDRA) preferred terms. The number and percentages of participants experiencing each specific AE 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, relationship to treatment type, onset, duration and outcome.

Local laboratory values:

Boxplots of local laboratory values will be generated for baseline values and for values measured during the course of the study. Each boxplot will show the 1st quartile, the median, and the 3rd quartile. Outliers, or values outside the boxplot, will also be plotted. If appropriate, horizontal lines representing boundaries for normal values will be plotted.

6.4.4. Tolerability Evaluation

The tolerability of the medical product represents the degree to which overt adverse effects can be tolerated by the subject [74]. VRC 603 is the first trial of AAV8-VRC07 in HIV-1 infected adults. The tolerability evaluation will be mostly descriptive by nature and consist of solicited adverse events that occur during the 7 days following each AAV8-VRC07 administration and reasons for any withdrawal or discontinuation based upon subject discomfort. This early assessment of tolerability of AAV8-VRC07 will inform which parameters should be solicited or routinely assessed to further characterize the tolerability profile in larger number of subjects.

6.4.5. Pharmacokinetics Analysis

Blood samples for PK evaluations will be collected at timepoints defined in the Schedule of Evaluations (**Error! Reference source not found.**).

Individual Subject Pharmacokinetic Analysis: A non-compartmental pharmacokinetic analysis will be performed using Phoenix (Centara) or a similar program on the VRC07 concentration data generated from each subject. Calculated pharmacokinetic parameters for all groups will include: area-under-the-curve (AUC), maximum concentration (Cmax), time to Cmax (Tmax), terminal rate constant (λz) and the terminal half-life ($T_{1/2}$). Cmax and Tmax will be taken directly from the observed concentration-time data. The terminal slope, λz, will be determined from the log-linear portion of the curve and the $T_{1/2}$ calculated as $0.693/\lambda z$. $AUC_{0\text{-}Clast}$ will be determined using the linear trapezoidal method, where C_{last} is the concentration at 24 weeks after the dose. If the final sample (C_{last}) has measurable VRC07 concentrations, the remaining AUC after the final concentration ($AUC_{Clast\text{-}inf}$) will be estimated as $C_{last}/\lambda z$. Data will be summarized by dose group.

Population Pharmacokinetic Analyses: Population pharmacokinetic analyses will be attempted on the VRC07 pharmacokinetic data to determine compartmental PK parameters using the program NONMEM. Based on prior PK studies of VRC01 and the preclinical PK studies of AAV8 -VRC07, VRC07 concentrations will initially be assessed using a one compartment PK model as initial generation of VRC07 from AAV8-VRC07 is expected to be much slower than the PK distribution of VRC07. Generation of VRC07 from AAV8-VRC07 will be modeled using zero and first order processes including a generation plateau and potential decline in production over time. The impact of AAV8-VRC07 dose will be assessed as a potential fixed effect on the input and decline of VRC07 generation. The population analysis will generate estimates for initial and final volumes of distribution (Vd_1 and Vd_{ss}), inter-compartmental clearance (Q), clearance (CL) and bioavailability (F). Given the small subject numbers, the population PK analysis will not include an exploratory covariate analysis to assess clinical factors as fixed effects associated with VRC07 PK parameters with the exception of dose level ($5x10^{10}$ vg/kg vs. $5x10^{11}$ vg/kg vs. $2.5x10^{12}$ vg/kg). The terminal half-life, $t_{1/2,}$ will be determined from CL/F, Vd_{ss}/F and the estimated VRC07 generation. Final model selection will be based on changes in the objective function and graphically by goodness of fit plots. The final population model will be assessed using bootstrap analysis. The AAV8-VRC07 dosing strategies and their ability to achieve and maintain of target VRC07 concentrations will be performed using the final population PK model and Monte Carlo simulations with at least 5000 replicates.

6.4.6. Interim Analyses

Preliminary PK analyses may be done once per dose level as the data for each dose level is obtained. This will be used to inform decisions about the dose levels to be administered in studies that may begin while the study is still in progress.

7. PHARMACY PROCEDURES

The study groups and study agent dosing schedule are shown in **Table 3**. Refer to the IB for further information about the investigational study agent. The Investigational Drug Control Unit (CC-PHAR IDCU) at the Clinical Center Pharmacy (Building 10/1N257, 301-496-1031) will prepare the product for administration as described below.

7.1. Study Product and Administration Regimen

The study includes investigational products described as follows:

- AAV8-VRC07 (VRC-HIVAAV070-00-GT)
- Excipient (AAV8-VRC07 Diluent)

AAV8-VRC07 is a sterile, aqueous buffered solution filled into single dose 1.5 mL cryovials. Each cryovial contains 1 mL (with 10% overage) of AAV8-VRC07 at a concentration of $2.84x10^{13}$ vg/mL in formulation buffer composed of 180mM sodium chloride, 10mM sodium phosphate plus 0.001% Pluronic F68 at pH 7.3. Vials are intended for single use only and thus do not contain a preservative.

In calculating the dose to administer and number of vials to thaw, it should be assumed that the concentration is $2.84x10^{13}$ vg/mL and that a volume of at least 1 mL can be withdrawn from a vial. Preparation of AAV8-VRC07 may require a dilution to deliver a required dose relative to the subject's weight.

Excipient (formulation buffer, AAV8-VRC07 diluent) is composed of 180mM sodium chloride, 10mM sodium phosphate plus 0.001% Pluronic F68 at pH 7.3, and will be provided in vials that contain 1 mL of diluent with 10% average.

7.2. Study Product Storage

The AAV8-VRC07 product label designates the long-term storage temperature as <-60°C.

The diluent product label designates storage temperature as <-10°C.

Clinical site storage in a qualified, continuously monitored, temperature-controlled freezer is acceptable.

The site pharmacist must promptly report any storage temperature excursions to the Study PI and the IND sponsor's authorized representative. The effected product must be quarantined in a separate area. The IND Sponsor's authorized representative will notify the site pharmacist if continued clinical use of the product is acceptable.

7.3. Preparation of Study Product for Administration

This section describes how the site pharmacist will prepare the study product for administration and how the clinician will administer the product. Clinician instructions on how to select an administration site are in Section 4.2.3.

Preparation is to be done in a clean preparation unit with limited access. Preparation should be done using aseptic technique, in a laminar flow biosafety cabinet. Standard universal precaution practices should be followed for handling of AAV8-VRC07 in the pharmacy. Personal protection including laboratory coat, gloves, and safety glasses are recommended. Assure that only the required vials are present in the preparation unit during dilution, and medication labels are strictly segregated to avoid errors.

Thaw the vial(s) containing AAV8-VRC07 and vials containing diluent (if needed) at ambient temperature, 15 to 27°C, immediately prior to use. Gently mix the vials by swirling.

It is expected that each product vial will be used for no more than 1 mL withdrawal volume ($2.84x10^{13}$ vg/mL), however, more may be withdrawn if it is possible to do so.

No more than 1 mL will be administered per single injection site. Total volume for a dose will be calculated based on subject weight using formulas provided below.

Following initial thaw, the product may be stored at room temperature and should be used within 4 hours. The prepared product may be stored at room temperature and should be administered within 4 hours after the initial thaw.

It is expected that up to 11 injections may need to be administered at the highest dose. Product for each injection will be filled in a separate syringe at no more than 1 mL per injection. For example, if a volume of 1.1 mL is needed as calculated using the formula below, two injections, 0.5 mL and 0.6 mL, can be administered.

7.3.1. Preparation of the $5x10^{10}$ vg/kg dose

Diluent will be provided for product dilution at 1 mL/vial. For each $5x10^{10}$ vg/kg dose preparation, thaw one vial of the product and 3 vials of diluent.

Step 1: Dilute the product with diluent at a 1:10 ratio by aspirating 0.25 mL of the product from a vial and mixing it with 2.25 mL of a diluent.

Step 2: Calculate volume of the diluted product needed for injection, based on subject's weight:

$$V(mL) = \frac{[\boldsymbol{weight}](kg) * [\boldsymbol{dose}](vg/kg)}{2.84 * 10^{13}\ (vg/mL)} * 10$$

As an example for a 115 kg subject, V= ((115 kg * $5x10^{10}$ vg/kg) / $2.84x10^{13}$ vg/mL) *10 = 2.03 mL, and therefore 2.03 mL of the 1:10 diluted product is needed for injection.

7.3.2. Preparation of the $5x10^{11}$ vg/kg dose

The following formula will be used for calculations of the product volume needed for injection, based on subject's weight:

$$V(mL) = \frac{[\boldsymbol{weight}](kg) * [\boldsymbol{dose}](vg/kg)}{2.84 * 10^{13}\ (vg/mL)}$$

An example of product volume calculations for the $5x10^{11}$ vg/kg dose administered to a 115 kg subject:

V= (115 kg * $5x10^{11}$ vg/kg) / $2.84x10^{13}$ vg/mL = 2.03 mL of undiluted product.

7.3.3. Preparation of the $2.5x10^{12}$ vg/kg dose

The formula provided in Section 7.3.2 will be used for calculations of the product volume needed for injection, based on subject's weight.

An example of product volume calculations for the $2.5x10^{12}$ vg/kg dose administered to a 115 kg subject:

V= (115 kg * $2.5x10^{12}$ vg/kg) / $2.84x10^{13}$ vg/mL = 10.12 mL of undiluted product.

7.4. Labeling of Study Agents

Vials of study agents will be individually labeled with the name of the material, volume, lot number, concentration, storage instructions, Investigational Use Statement ("Limited by Federal Law to Investigational Use"), and manufacturer information.

7.5. Study Agent Accountability

The study pharmacist is responsible for maintaining an accurate record of the codes, inventory, and an accountability record of study agent supplies. Electronic documentation as well as paper copies may be used.

7.6. Study Agent 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 at the end of the study will be returned to the production facility or discarded at the discretion of the sponsor 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. These vials be disposed of in accordance with institutional or pharmacy policy.

8. HUMAN SUBJECT PROTECTIONS AND ETHICAL OBLIGATIONS

This research study will be conducted in compliance with the protocol, Good Clinical Practices (GCP), and all applicable regulatory requirements.

8.1. Institutional Review Board

A copy of the protocol, informed consent form, other written subject information, and any advertising material will be submitted to the IRB for written approval.

The investigator must submit and, where necessary, obtain approval from the IRB for all subsequent protocol amendments and changes to the informed consent document. The investigator will notify the IRB of unanticipated problems, non-compliance, deviations from the protocol, and serious SAEs as described in Section 5.4.

The investigator will be responsible for obtaining IRB approval of the annual Continuing Review throughout the duration of the study.

8.2. Subject Identification and Enrollment of Study Participants

All study activities will be carried out at the NIH CC. Study subjects will be recruited through on-site and off-site advertising done for the screening protocol, VRC 500 (NCT 01375530). Effort will be made to include women and minorities in proportions similar to that of the community from which they are recruited and will be limited to persons at least 18 years of age and no older than 65 years of age at enrollment.

8.2.1. Participation of Children

Children are not eligible to participate in this clinical trial because the study agent has not been previously evaluated in adults. If the product is assessed as safe for further study other protocols specifically designed for children may be conducted.

8.2.2. Participation of NIH Employees

NIH employees and members of their immediate families may participate in this protocol. The Guidelines for the Inclusion of Employees in NIH Research Studies will be followed. Each NIH

employee considering participation in this trial will receive a copy of the "NIH Information Sheet on Employee Research Participation" and a copy of the "Leave Policy for NIH Employees Participating in NIH Medical Research Studies." Neither participation nor refusal to participate will have an effect, either beneficial or adverse, on the participant's employment or work situation. The employee subject's privacy and confidentiality will be preserved in accordance with NIH CC and NIAID policies. For NIH employee subjects, consent will be obtained by an individual who is independent of the employee's team. If the individual obtaining consent is a co-worker to the subject, independent monitoring of the consent process will be included through the Bioethics Consultation Service. Protocol study staff will be trained on obtaining potentially sensitive and private information from co-workers or subordinates.

8.2.3. Participation of Decisionally Impaired Subjects

Per protocol inclusion criterion #1 (Section 4.1.1), volunteers must be able and willing to complete the informed consent process to participate in the study. Decisionally impaired adults are not eligible to enroll in the study, and a probability of already enrolled subjects to become decisionally impaired is not anticipated to be higher than in general population.

Subjects will get a dose of AAV8-VRC07 on a day of study enrollment and will be encouraged to keep follow-up visits to monitor their health. Collection of samples that are for research purposes only may be terminated based on clinical judgement and subject willingness/ability to participate.

8.3. Informed Consent

The study informed consent is provided as a stand-alone document. It describes the investigational product and all aspects involved in protocol participation.

Before a subject's participation in the study, it is the investigator's responsibility to obtain 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 are conducted or study agent is administered. The Assessment of Understanding quiz will be completed before the study consent is signed.

The acquisition of informed consent will be documented in the subject's medical records, as required by 21 CFR 312.62. The informed consent form will be signed and personally dated by the subject and the person who conducted the informed consent discussion. The original signed informed consent form will be retained in the medical chart and a copy will be provided to the subject.

8.4. Subject Confidentiality

The investigator must ensure that no information identifying the subject will be released to any unauthorized party. Individual identifying information will not be included in any reports. Subjects will be identified only by coded numbers. All records will be kept confidential to the extent provided by federal, state and local law. Medical records are made available for review when required by the FDA or other authorized users, such as the study agent manufacturer, only under the guidelines set by the Federal Privacy Act. Direct access includes examining, analyzing, verifying, and reproducing any records and reports that are important to the evaluation of the study. The investigator is obligated to inform the subjects that the above-named representatives will review their study-related records without violating the confidentiality of the subjects.

8.5. Risks and Benefits

8.5.1. Risks of AAV8-VRC07

AAV8-VRC07: There is limited human experience with administration of AAV8-VRC07 as described in Section 1.3.1. The AAV8-VRC07 injections were well tolerated in 8 subjects, and no safety signals or dose-limiting toxicity have been detected. The most frequently experienced reactogenicity was mild pain/tenderness at the injection site that resolved in about 8-14 days. Since the product is targeted for a long-term expression of VRC07 HIV-1 neutralizing antibody delivered by episomal gene expression using rAAV8 vector, potential risks of monoclonal antibodies and risks of vector administration are described below.

Risks of Monoclonal Antibodies: There is no prior human experience with administration of the VRC07 antibody. First human clinical trials of the clonal relative of VRC07, VRC01 MAb, administered by passive transfer determined that VRC01 is safe for further evaluation in HIV-infected and healthy adults. Solicited local and systemic signs and symptoms following administration of VRC01 were generally none to mild. The SC administrations were infrequently associated with mild local transient reactions (Section 1.3.2).

In the VRC 605 study, 25 healthy adult subjects have received at least one VRC07-523LS administration. Product administrations were well tolerated. Two subjects, one in the 20 mg/kg and one in the 40 mg/mL IV groups, experienced an infusion reaction that began within an hour after infusion and was manifested by chills, fever, myalgia, malaise, and headache, and had all symptoms resolved within 1 day (Section 1.3.2).

Administration of some MAbs may cause immune reactions such as acute anaphylaxis, serum sickness and the generation of anti-drug antibodies. However, these reactions are rare and more often associated with MAbs targeted to human proteins or with the use of murine monoclonal antibodies which would have a risk of human anti-mouse antibodies [75]. In this regard, VRC07 is targeted to a viral antigen and is a human monoclonal antibody and, therefore, expected to have a low risk of such side effects.

Typically, the side effects of other 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 MAbs that are targeted to cytokines or antigens associated with human cells may be associated with an increased risk of these side effects and in some cases, infections [75]; however, this is not expected to be a risk for a MAb like VRC07 that is targeted to a viral antigen.

Delayed allergic reactions to other MAbs may include a serum sickness type of reaction, which may include rash, fever, lymph node enlargement, and joint pains. These symptoms may not appear until several days after the exposure to the MAb and are noted to be more common with chimeric types of MAb rather than with human MAb such as VRC07 [75].

Participation in this study may limit a subject's eligibility for other future MAb clinical trials.

Risks of the AAV8 Vector: rAAV vectors have been evaluated in human clinical studies for testing of recombinant investigational products as having good safety profiles. One of these products, Glybera®, was granted marketing authorization in Europe (Section 1.3.4). Another 2 products, Luxturna® and Zolgensma® have received approval from the FDA for marketing in the U.S. (Section 1.3.4).

The FDA guidance "Gene Therapy Clinical Trials – Observing Subjects for Delayed Adverse Events", classifies rAAV vectors as "non-integrating" and at low risk of gene-therapy-related delayed adverse events [30].

Theoretical risks of a viral vector may include potential integration into the human genome and an integration-mediated tumorogenicity. Wild type AAV can integrate in the genome at a preferred site on human chromosome 19 without causing any known human disease. In one recent report, wild type AAV2 integration in known cancer driver genes was implicated in hepatocellular carcinoma development in humans [76]. There is no evidence that this rAAV vector would integrate in the human genome, and it was not designed to integrate. The risk of insertional mutagenesis is considered to be low with these vectors [46, 77, 78]. There has been no carcinogenesis reported in human studies with rAAV vectors [20].

An even more remote theoretical risk of rAAV vectors is germline transmission. In GLP biodistribution studies in mice, AAV8-VRC07 was detected in all 15 tested tissues including testes and ovaries. However, it has been noted that motile germ cells may be resistant to AAV infection, suggesting that insertion of new genetic material in germ line is extremely unlikely [78]. In human studies with AAV2 vector, there were no evidence of vector DNA in semen at any time after IM product administration [36].

Another risk of the AAV8-VRC07 product is development of immune responses to AAV8 and/or to VRC07. These immune responses may decrease efficacy of future treatments with AAV vectors or effect eligibility for treatment with other monoclonal antibodies in the future. There may be other unknown risks of AAV8-VRC07.

8.5.2. Other Risks

Risks of Intramuscular Injection: IM injections may cause stinging, discomfort, redness of the skin, or mild bruising at injection sites.

Risks of Blood Drawing: Blood drawing may cause pain and bruising and may, infrequently, cause a feeling of lightheadedness or fainting. Rarely, it may cause infection at the site where the blood is taken.

Risks of Mucosal Sample Collection: Collection of samples by swabs and wicks by rubbing them over the mucosal surfaces can cause momentary discomfort and, in some cases, minor bleeding.

8.5.3. Study Benefits

There are no direct benefits to study subjects from study participation. Others may benefit from knowledge gained in this study that may aid in the development of HIV prevention or therapeutic methods.

8.6. Plan for Use and Storage of Biological Samples

The plan for use and storage of biological samples from this protocol is as outlined in the following sections.

8.6.1. Use of Samples, Specimens and Data

Samples, specimens and data collected under this protocol may be used to conduct protocol-related safety and immune response evaluations, exploratory laboratory evaluations related to the type of

infection the study agent was designed to prevent, exploratory laboratory evaluations related to vaccine or infectious disease research in general and for research assay validation.

8.6.2. Samples for Genetic Testing

Some of the blood research samples collected in this study may be used for genetic testing related to immune system investigation and PK analyses. Blood samples used in these genetic tests will be coded and the results will not be in subjects' medical records. Genetic testing will be performed in accordance with the genetic testing information that was included in the study informed consent.

When HLA typing is done through the NIH Clinical Center medical laboratory, the HLA type results will be in subjects' medical records. Any genetic testing, including HLA testing, will be done for research purposes only and the results will not be communicated to subjects. All genetic test results are confidential, will be kept securely and will not be released without subject's permission.

8.6.3. Storage and Tracking of Blood Samples and Other Specimens

All of the stored study research samples are labeled by a code (such as a number) that only the VRC Clinic can link to the subject. Samples are stored at the Vaccine Immunology Program (VIP, formerly VITL / NVITAL), Gaithersburg, MD, or VRC Laboratories in Building 40, which are both secure facilities with limited access. Data will be kept in password-protected computers. Only investigators or their designees will have access to the samples and data. Samples will be tracked in the Laboratory Information Management System (LIMS) database or using another software designed for this purpose (e.g., Freezerworks).

8.6.4. Disposition of Samples, Specimens and Data at Completion of the Protocol

In the future, other investigators (both at NIH and outside) may wish to study these samples and/or data. IRB approval must be sought prior to any sharing of samples. Any clinical information shared about those samples would similarly require prior IRB approval. The research use of stored, unlinked or unidentified samples may be exempt from the need for prospective IRB review and approval. Exemption requests will be submitted in writing to the NIH Office of Human Subjects Research, which is authorized to determine whether a research activity is exempt.

At the time of protocol termination, samples will remain in the VIP facility or VRC laboratories or, after IRB approval, transferred to another repository. Regulatory oversight of the stored samples and data may be transferred to a stored samples protocol as part of the IRB-approved termination plan. Data will be archived by the VRC in compliance with requirements for retention of research records, or after IRB and study sponsor approval, it may be either destroyed or transferred to another repository.

8.6.5. Loss or Destruction of Samples, Specimens or Data

The NIH Intramural Protocol Deviation definition related to loss of or destruction of samples or data will be followed. Any loss or unanticipated destruction of samples (for example, due to freezer malfunction) or data (for example, misplacing a printout of data with identifiers) that compromises the scientific integrity of the study will be reported to the IRB in accordance with institutional policies. The PI will also notify the IRB if the decision is made to destroy the remaining samples.

8.7. Compensation

Subjects will be compensated for time and inconvenience in accordance with the standards for compensation of the Clinical Research Volunteer Program. Compensation will be $200 for outpatient scheduled visits that include oral swabs and/or blood drawing. Compensation for the study agent administration visit will depend on the number of injection sites used for administration: $315 for 1-2 sites, $450 for 3-5 sites, $600 for 6-8 sites, and $750 for 9-12 sites. Compensation is $100 for clinic visits that do not include a blood draw or procedure, and $25 for timely completion of electronic diary card.

8.8. Safety Monitoring, Protocol Safety Review Team

Close cooperation between the designated members of the Protocol Team will occur to evaluate and respond to individual AEs in a timely manner. The VRC designated Safety Officer for the day conducts a daily safety review of clinical data per VRC Standard Operating Procedures. The Protocol Safety Review Team (PSRT), comprised of the PI, Associate Investigators, Study Coordinator, Protocol Specialists, other Study Clinicians, and an Independent Safety Monitor (ISM), will review the summary study safety data reports on a weekly basis while injections are being administered and through 8 weeks after the last subject receives the last product administration in order to be certain that the study agent has an acceptable safety profile, and will continue to monitor the study safety data reports on a monthly basis through completion of the last study visit.

9. ADMINISTRATIVE AND LEGAL OBLIGATIONS

9.1. Protocol Amendments and Study Termination

Protocol Amendments must be made only with the prior approval of the IND sponsor, VRC, NIAID. Agreement from the investigator must be obtained for all protocol amendments and amendments to the informed consent document. All study amendments will be submitted to the IRB for approval.

The VRC, the Principal Investigator, the NIAID IRB, the Office of Human Research Protections, and FDA reserve the right to terminate the study. The PI will notify the IRB in writing of the study's completion or early termination.

9.2. Study Documentation and Storage

The PI will maintain a list of appropriately qualified persons to whom trial duties have been delegated.

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, microfiches, radiographs, and correspondence.

The PI and staff are responsible for maintaining a comprehensive and centralized filing system of all study-related (essential) documentation, suitable for inspection at any time by representatives from the VRC, IRB, FDA, and/or applicable regulatory authorities. Elements include:

- Subject files containing completed informed consent forms, and supporting copies of source documentation (if kept)

- Study files containing the protocol with all amendments, Investigator Brochures, copies of all correspondence with the IRB and the Vaccine Research Center

In addition, all original source documentation must be maintained and be readily available.

All essential documentation should be retained by the institution for the same period of time required for medical records retention. The FDA requires study records to be retained for up to two years after marketing approval or refusal (21 CFR 312.62). No study document should be destroyed without prior written agreement between the Vaccine Research Center and the Investigator. Should the investigator wish to assign the study records to another party or move them to another location, they must notify the NIAID and VRC in writing of the new responsible person and/or the new location.

9.3. Study Monitoring, Data Collection and Data Sharing

9.3.1. Study Monitoring

The VRC regulatory authority inspectors or their authorized representatives are responsible for contacting and visiting the investigator for the purpose of inspecting the facilities and, upon request, inspecting the various records of the trial, provided that subject confidentiality is respected.

Site visits by study monitors will be made in accordance with the IND Sponsor 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, the IRB, and FDA to inspect study documents (e.g., consent forms, drug distribution forms, and case report forms) and pertinent hospital or clinic records for confirmation of the study data.

9.3.2. Data Collection

Clinical research data will be collected in a secure electronic data management system through a contract research organization, the Emmes Company, LLC, Rockville, MD. Extracted data without patient identifiers will be sent to the PSRT for safety review and to the protocol statistician for statistical analysis.

9.3.3. Data Sharing

Data generated in this study will be shared as de-identified data in the government-funded public repository, www.ClinicalTrials.gov. Data may be shared prior to publication at approved public presentations or for collaborative development and will be shared at the time of publication or within 1 year of the primary study completion date (data collection completed for primary outcome).

9.4. Language

All written information and other material to be used by subjects and investigative staff must use vocabulary and language that are clearly understood.

9.5. Policy Regarding Research-Related Injuries

The NIH CC will provide short-term medical care for any injury resulting from participation in this research. In general, the NIH, the Clinical Center, or the U.S. Government will provide no long-term medical care or financial compensation for research-related injuries.

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APPENDIX I
SCHEDULE OF EVALUATIONS

VRC 603 Schedule of Evaluations																			
Visit			02	02A	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18
Study Week			Wk0	Wk1	Wk1	Wk2	Wk3	Wk4	Wk5	Wk6	Wk7	Wk8	Wk9	Wk10	Wk11	Wk12	Wk14	Wk16	Wk18	Wk20
[1]Day of Study		**0 to -84**	D0	D1	D7	D14	D21	D28	D35	D42	D49	D56	D63	D70	D77	D84	D98	D112	D126	D140
Clinical	**Tube***	**Screen**																		
VRC 500 Screening Consent		X																		
VRC 603 AoU; Consent			X																	
[2]Physical exam		X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
[3]Medical history		X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Product Administration			X																	
Phone contact; clinic visit if needed				X																
CBC / differential	EDTA	3	3		3	3	3	3	3	3	3	3	3	3	3	3		3		3
ALT, AST, creatinine	GLT	4	4		4	4	4	4	4	4	4	4		4		4		4		4
Alpha-fetoprotein (AFP)	SST	4	4																	
Drug screen	Urine	X																		
[4]Pregnancy test: urine or serum		X	X							X						X				
[4]Pregnancy prevention counseling		X	X							X						X				
HIV positive prevention counseling		X	X							X						X				
[5]HIV 1/2 Ab/Ag Combo	EDTA	3																		
Hepatitis BsAg	SST	4																		
Hep C viral load	SST	4																		
HIV-1 RNA (viral load)	EDTA	6	6		6	6	6	6	6	6	6	6	6	6	6	6		6		6
CD4/CD8	EDTA	3	3		3	3	3	3	3	3	3	3	3	3	3	3		3		3
[6]SARS-CoV-2 PCR, nasopharyngeal swab		X																		
[7]ARV medication record		X	X																	
[8]HLA type	EDTA														20					
Research Samples / Research Test Schedule																				
[9]PK samples	SST		4		4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
[10]Oral sample			[X]							[X]						[X]				
[11]Plasma for qPCR (2x spin)	EDTA		6					6				6				6		6		6
Plasma for ultrasensitive VL (2x spin)	EDTA	12	12							12								12		
PBMC and plasma (2x spin)	EDTA	20	20			40		40		40		20				20		20		20
Serum	SST	24	16			16		16		16		16				16		16		16
AAV8 antibody test /ADA test		X	X			X		X		X		X				X		X		X
Daily Volume (mL)		**87**	**77**	**0**	**20**	**76**	**20**	**82**	**20**	**88**	**20**	**62**	**16**	**20**	**36**	**62**	**4**	**74**	**4**	**62**
Cumulative Volume (mL)		**87**	**165**	**165**	**185**	**261**	**281**	**363**	**383**	**471**	**491**	**553**	**569**	**589**	**625**	**687**	**691**	**765**	**769**	**831**

Visit windows: Visit 02A (+ 1 day), Visits 03-06 (± 1 day), Visits 07-14 (± 2 days), and Visits 15-18 (± 3 days).

*Tube types for clinical labs are according to institutional requirements and are shown to estimate blood volumes. Different tubes for clinical evaluations may be used to meet site requirements. Research sample tube types and blood volumes must be used as shown or as otherwise instructed by the IND sponsor.

VRC 603 Schedule of Evaluations Continued											Long Term Follow-up								
Visit		19	20	21	22	23	24	25	26	27	**Visit**	28	29	30	31	32	33	34	35
Study Week		Wk22	Wk24	Wk28	Wk32	Wk36	Wk40	Wk44	Wk48	Wk52	**Study year**	2	2	3	3	4	4	5	5
[1]Day of Study		D154	D168	D196	D224	D252	D280	D308	D336	D364	**Study Week**	78	104	130	156	182	208	234	260
Clinical	**Tube***																		
[2] Physical exam		X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X
[3]Medical history		X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X
CBC / differential	EDTA		3	3		3		3		3		3	3	3	3	3	3	3	3
ALT, AST, creatinine	GLT		4	4		4		4		4		4	4	4	4	4	4	4	4
Alpha-fetoprotein (AFP)	SST		4						4										
[4]Pregnancy test: urine or serum			X			X			X	X									
[4]Pregnancy prevention counseling			X			X			X										
HIV positive prevention counseling			X			X			X	X		X	X	X	X	X	X	X	X
HIV PCR (viral load)	EDTA		6	6		6		6		6		6	6	6	6	6	6	6	6
CD4/CD8	EDTA		3	3		3		3		3		3	3	3	3	3	3	3	3
Research Samples / Research Test Schedule																			
[9]PK samples	SST	4	4	4	4	4	4	4	4	4		[4]	[4]	[4]	[4]	[4]	[4]	[4]	[4]
[10]Oral sample			[X]			[X]			[X]										
[11]Plasma for qPCR(2x spin)	EDTA		6																
Plasma for ultrasensitive viral load (2x spin)	EDTA		12							12									
PBMC and plasma (2x spin)	EDTA		20	20		20		20		20		20	20	20	20	20	20	20	20
Serum	SST		16	16		16		16		16		16	16	16	16	16	16	16	16
AAV8 antibody test /ADA test			X	X		X		X		X		X	X	X	X	X	X	X	X
Daily Volume (mL)		**4**	**78**	**56**	**4**	**56**	**4**	**56**	**8**	**68**		**56**	**56**	**56**	**56**	**56**	**56**	**56**	**56**
Cumulative Volume (mL)		**835**	**913**	**969**	**973**	**1029**	**1033**	**1089**	**1097**	**1165**		**1221**	**1277**	**1333**	**1389**	**1445**	**1501**	**1557**	**1613**

Visit windows: Visits 19-27 (±3 days), Visits 28-35 (±14 days).

*Tube types for clinical labs are according to institutional requirements and are shown to estimate blood volumes. Different tubes for clinical evaluations may be used to meet site requirements. Research sample tube types and blood volumes must be used as shown or as otherwise instructed by the IND sponsor.

[1] Day of Study: Day 0=day of product administration. Medical history and Day 0 evaluations prior to AAV8-VRC07 administration are the baseline for assessing subsequent AEs.

[2] Physical Exam: A complete physical exam is performed at screening, including height (ht) and weight (wt). At other visits, if medically indicated, a targeted exam is performed. Otherwise physical exam includes only vital signs (blood pressure (BP), pulse, and temperature), except at Visit 02 when the current weight is also obtained to use for ordering the study agent dosed on a "vg/kg" basis. For the long term follow-up visits, vital signs will be collected and kept in subject's chart, but will not be recorded in the study database.

[3] Medical History: A complete medical history is collected at screening. After that only an interim medical history is required.

[4] Pregnancy test and pregnancy prevention counseling are required at visits as indicated for women of reproductive potential. Negative urine or serum pregnancy test result must be confirmed for women of reproductive potential prior to study agent administration.

[5] The HIV confirmatory testing by HIV 1/2 Ab/Ag Combo and/or detectable HIV-1 RNA does not need to be repeated if previously performed at the NIH Clinical Center and results are available in the NIH medical record (outside lab test results are not acceptable). HIV-1 RNA testing must be performed for all subjects at screening.

[6]A nasopharyngeal swab for the SARS-CoV-2 PCR should be collected no more than 5 days prior to product administration.

[7]ARV medications will be documented in the medical history at screening and recorded in the database at Visit 02. Only subsequent changes to the ARV regimen will be recorded in the database.

[8] HLA type blood sample is collected once at any timepoint in the study and is shown as a Visit 13 evaluation for convenience; however, if HLA type is already available in the medical record it does not need to be repeated. HLA type may also be obtained from a stored frozen sample: blood sample for HLA testing may not be collected when a frozen stored sample is used for testing.

[9] PK samples are collected relatively to the exact time of the end of injection (EOI). The exact time of EOI and the time of PK blood draw are recorded to ensure accurate PK analysis. PK samples are collected at the long term follow up Visits 28-34 only if VRC07 persisted at a prior visit.

[10] Oral mucosal sample collection is encouraged for all subjects, but it is not mandatory.

[11] Plasma for qPCR: Samples will be collected every 4 weeks and tested in batches until two consecutive serum samples show no detectable AAV8-VRC07.

APPENDIX II
TABLE FOR GRADING SEVERITY OF ADVERSE EVENTS

The U.S. Department of Health and Human Services, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Division of AIDS. Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1. [July 2017] will be used in this study. The table is available at the following link: https://rsc.tech-res.com/docs/default-source/safety/daidsgradingcorrecetedv21.pdf

The grading table and supplementary tutorial can be found on the Division of AIDS Regulatory Support Center (RSC) website:

http://rsc.tech-res.com/safetyandpharmacovigilance/

The Table will be used as posted at the link above with the following exemption:

- Weight loss will be recorded as an adverse event only if it is considered deleterious to the participant's health.
- Injection site bruising is an expected adverse event and will not be generally recorded as an AE. Clinically significant hematomas will be recorded as an AE.

PRINCIPAL INVESTIGATOR: Joseph Casazza, M.D., Ph.D.

STUDY TITLE: VRC 603 (18-I-0030): A Phase I, Dose-Escalation Study of the Safety of AAV8-VRC07 (VRC-HIVAAV070-00-GT) Recombinant AAV Vector Expressing VRC07 HIV-1 Neutralizing Antibody in Antiretroviral –Treated, HIV-1 Infected Adults with Controlled Viremia

STUDY SITE: NIH / NIAID / VRC / Vaccine Evaluation Clinic (VEC)

Cohort: VRC 603 HIV-1 infected subjects

Consent Version: Version 7; February 11, 2025

WHO DO YOU CONTACT ABOUT THIS STUDY?

Principal Investigator: Joseph Casazza, M.D., Ph.D., [redacted] at [redacted]
Study Coordinator: Laura Novik, RN, [redacted] at [redacted]

This consent form describes a research study and is designed to help you decide if you would like to be a part of the research study.

You are being asked to take part in a research study at the National Institutes of Health (NIH). Members of the study team will talk with you about the information described in this document. Some people have personal, religious, or ethical beliefs that may limit the kinds of medical or research treatments they would want to receive (such as blood transfusions). Take the time needed to ask any questions and discuss this study with NIH staff, and with your family, friends, and personal health care providers. Taking part in research at the NIH is your choice.

IT IS YOUR CHOICE TO TAKE PART IN THE STUDY

You may choose not to take part in this study for any reason. If you join this study, you may change your mind and stop participating in the study at any time and for any reason. In either case, you will not lose any benefits to which you are otherwise entitled. However, to be seen at the NIH, you must be taking part in a study or are being considered for a study. If you do choose to leave the study, please inform your study team to ensure a safe withdrawal from the research.

WHY IS THIS STUDY BEING DONE?

This is the study of an experimental gene transfer treatment called "AAV8-VRC07." Scientists have created a gene in the laboratory that could be transferred to the cells in the body. This gene carries information to tell the cells how to make an antibody called VRC07. VRC07 is an antibody against HIV. There is no HIV virus in this experimental treatment, and this treatment is not a vaccine. The VRC07 gene is 'packaged' into a man-made version of a virus called AAV8. The AAV8 cannot make more AAV8 viruses in your body. It is only being used because its

outer shell helps to carry the VRC07 gene into the muscle of the body where the VRC07 antibody can be made. The AAV8 is very similar to other man-made viruses (viral vectors) that have been used in other gene transfer studies.

The main purpose of this study is to see if the administration of AAV8-VRC07 is safe. We will also study if AAV8-VRC07 causes cells to produce VRC07 antibody. We will study the amount of VRC07 made in the body and how long it lasts.

About 15 to 25 people will take part in this study at the NIH Clinical Center in Bethesda, Maryland. The study will include a total of 34 clinic visits per person over 5 years.

STUDY PRODUCT

The Vaccine Research Center (VRC) at the NIH developed AAV8-VRC07 and made it in a drug manufacturing facility. The U.S. Food and Drug Administration (FDA) allows this experimental product to be used for research only. Sometimes we refer to AAV8-VRC07 simply as the "study product."

There are many research gene transfer products in development for different diseases including hemophilia, inherited blindness, and Parkinson's disease. As of 2011, over 300 people received experimental gene transfer with AAV vectors. There are two AAV-based products currently approved by the FDA. Luxturna®, for treatment of a form of inherited blindness and Zolgensma®, for treatment of an inherited spinal muscular atrophy in children.

Antibodies are part of the immune system and help fight infection. Everyone who becomes infected with HIV makes antibodies against the virus, but most of these antibodies are not able to stop infection. VRC07 is a newly found HIV antibody that binds to a part of the HIV virus needed for infection and blocks it from doing further harm. It was discovered in a person with HIV.

Over 3370 adults with and without HIV infection received antibodies similar to VRC07 in other research studies. These antibodies were found to be safe and well tolerated. This study was the first study to give AAV8-VRC07 to people.

You cannot get re-infected with HIV from the study product. It will not protect against or cure HIV infection. There is currently no cure for HIV. We do not expect the study product to control HIV. You should not change or stop your HIV medications before discussing it with your primary health care provider.

We do not know how long the study product will stay in the muscle. We also do not know if and how long it will make VRC07. In laboratory and animal studies, the study product made VRC07 antibodies that attach to and inactivate many types of HIV viruses. In mice and in monkeys, this study product prevented infection with HIV. We do not know if the study product will act the same way in people. It will take many studies to learn if the study product will be useful for preventing or treating HIV. This study alone will not answer this question.

STUDY PROCEDURES

The study will have 3 groups of 2-5 people each. Participants will get a different amount (dose) of study product in each group by one or more injection(s) in the upper arm(s) or a thigh(s) using

a needle. The study product administration visit will last about 4-6 hours. Other clinic visits will take 1-2 hours.

If you agree to take part in this study, you will get the study product only on the first day of the study (Day 0). The dose depends on the group. You will know what dose you will get. Each person gets an amount of study product based on how much they weigh. We will measure your weight on Day 0 to calculate the dose. You may get up to 11 injections on Day 0, depending on the group and your weight. After the injection(s) we will watch you in the clinic for at least 30 minutes.

The Study Groups are shown in the following table:

VRC 603			
Group	**Dose of AAV8-VRC07**	**Participants**	**Estimated Number of Injections**
1	$5x10^{10}$ vg/kg	2-5	1-2
2	$5x10^{11}$ vg/kg	2-5	1-3
3	$2.5x10^{12}$ vg/kg	2-5	5-11
Total	A total of 6 to 15 subjects may take part in the study. This number can increase to 25 subjects if needed to achieve the study goals.		

The study started by enrolling people in Group 1 at the lowest dose. People are only being enrolled in a higher dose group if there are no safety concerns in the dose group before it.

Women who can become pregnant will have a pregnancy test on Day 0. The test result must be negative to get an injection.

For 7 days after you get the study product, we will ask you to check your temperature with a thermometer that we give. We will ask you to make note of any symptoms you may have. We will give you access to a secure electronic "diary" to enter the information. You may choose to use a paper form instead. If you have any side effects, you should tell a study nurse or a doctor as soon as possible. You can reach the clinic staff by phone 24 hours a day. If you have symptoms, we may ask you to come into the clinic for an exam before your next study visit. It is very important that you follow the instructions you get from the clinic staff.

After you get the study injection(s), you will need to come to the clinic once a week for the first 12 weeks. Then you will have clinic visits every other week for another 12 weeks. After 24 weeks, you will have follow-up visits about once a month. After one year of study participation, you will return to the clinic every 6 months for the next 4 years. Total study participation is 5 years.

At each visit, we will check you for any health changes or problems. We will ask you how you are feeling and if you have taken any medications. We will draw your blood at each study visit, and we may also ask for saliva samples (explained below). We will draw about 1 to 13 tubes of

blood from you, depending on the visit. The volume of blood will not exceed the NIH Clinical Center limits.

We will tell you right away if any of your test results show a health problem. We will use some blood samples to study if your body develops immune response (antibody) to the study product. Results of these tests are not for checking your health. We will not give you these results. We may ask you to come into clinic for additional follow-up and blood collection if you have any health changes.

Clinical studies follow a set schedule. This helps us answer the research questions. The visit schedule is a little flexible, but it is important that you work with the staff to follow the schedule as closely as possible. You should try to not miss any visits.

Collection of Saliva for Research

If we find VRC07 in your blood and if you are willing, we will collect saliva samples for research at some clinic visits. Collection of these samples is encouraged but not required. You may choose not to donate these samples but still take part in the study.

We collect these samples to see if VRC07 reaches different parts of your body. These sample collections will not be for checking your health and do not replace routine health care.
If you agree, we may collect saliva samples at 6 visits using small disposable sterile swabs or sponges. The swabs are shaped like a "Q-tip" and placed in your mouth to soak up your saliva.

HIV TESTING AND MANAGEMENT

All participants in this study are HIV infected. As part of your participation in this study, we will frequently test your HIV viral load and $CD4^+$ T cell count for research purposes. We will share these and other test results with you during the study. If you have any questions about your HIV infection, you should discuss them with your primary care doctor. With your permission, we can give copies of your test results to you and your doctor.

This study does not include regular medical care or the management of your HIV infection. You will not be given HIV medications as part of this research study. You should already have a primary health care provider for regular medical care and HIV care. Please tell a study clinician if there is a change in your primary care doctor, or if you are having trouble getting HIV medications. All decisions about starting, stopping, or changing ARV medications will be between you and your primary health care provider.

We expect you to inform us about changes in your HIV medications, but these changes will not affect your continued participation in the study.

MONITORING OF THE STUDY

A group of physicians and scientists at NIH will monitor this study. This group will review the study data and will pay close attention to harmful reactions.

GENETIC TESTING

Some of the research blood drawn as part of this study will be used for genetic tests. Some genetic tests are done in research studies to see if genetic differences in people cause different

types of immune responses. Your blood sample used in these genetic tests will not have your name on it and the results will not be in your medical record. These tests are not used to check your health and we will not tell you the results.

A special genetic test, called HLA typing, may be done through the NIH Clinical Center medical laboratory. If this test is done at the NIH Clinical Center, your HLA type results will be in your medical record. These results are not used to check your health. Any genetic testing, including HLA testing, is for research purposes only. Any genetic information collected or learned about you will be kept confidential. Medical records, including HLA test results are kept securely. We will not give any genetic information that is in your medical record to anyone without your permission.

STORED SAMPLES

We will collect samples (including blood, and possibly saliva) from you during the study. We will keep these samples indefinitely for future research to learn more about monoclonal antibodies, vaccines, the immune system, and/or other medical conditions. Results from research done with your stored samples will not be in your medical record or reported to you.

Labeling of Stored Samples: We will label your stored samples by a numeric code. Only the study team can link this code to you. Any identifying information about you will be kept confidential as much as the law allows. Despite protections, there is a small chance that information identifying you will be given to someone who should not get it.

Risks from Stored Samples: There is a risk of unplanned release of information from your medical records. The chance that this information will be given to an unauthorized person without your permission is very small. Possible problems with the unplanned release of information include discrimination when applying for insurance and employment. Similar problems may occur if you give information yourself or agree to have your medical records released.

Future Studies: In the future, other investigators at NIH or outside of NIH may wish to study your stored samples. When your stored samples are shared, they will be marked with a code. Your samples will not have any identifying information on them. Some information about you, such as your sex, age, health history, or ethnicity may also be shared with other researchers.

Any future research studies using your samples will be conducted in a way that protects the rights and privacy of study participants.

Your stored samples will be used only for research and will not be sold. The research done with your materials may be used to develop new products in the future but you will not receive payment for such products.

Making your Choice: You cannot take part in this study if you do not want us to collect or store your blood samples. If you agree to take part in this study, you must also agree to let us keep any of your samples for future research.

If you decide not to take part in this study, you may still take part in other studies at NIH.

POSSIBLE STUDY RISKS

Possible risks from IM injections: General risks of needle injections include temporary stinging, discomfort, pain, soreness, itchiness, redness, bruising, and or swelling at the needle insertion site. There is a very small chance of infection.

Possible risks from AAV8-VRC07: This is the first time this study product has been given to people. The FDA and other agencies that regulate research reviewed results of AAV8-VRC07 testing in animals. The study product is expected to make VRC07 antibody targeted against HIV-1 using the AAV8 vector. As of May 4, 2020, 8 subjects have received AAV8-VRC07 and no safety concerns were identified. The most frequent symptoms were mild pain/tenderness at the injection site that resolved in about 8-14 days after injection of product. Risks of the antibodies and risks of the vector are described separately below.

Possible risks of VRC07: We will not give VRC07 to people in this study. VRC07 is expected to be made by your own cells after the study product injection. This study is the first time that VRC07 is expected to be made in people.

VRC07 has never been given to people. It is very similar to VRC01 and VRC07-523LS antibodies. More than 3370 adults have received one or more doses of VRC01 given IV (in the vein) or SC (in the fatty tissue). These studies showed that VRC01 is safe for further evaluation in HIV-infected and healthy adults. Twenty five (25) adults have received at least one dose of VRC07-523LS by IV or SC routes.

A small number of subjects in VRC01 and VRC07-523LS studies developed infusion reactions shortly after IV product administration. The symptoms of the infusion reaction were chills, fever, headache, and muscle pain. These are typical of infusion reactions observed with other monoclonal antibodies. All symptoms went away within one day. We believe that infusion reactions should not occur with production of VRC07 antibody by muscle cells. However, VRC07 may have other unknown risks and side effects.

There are several antibody products that FDA approved for use in people. Other research antibody products have been given safely by both the IV and the SC routes. Most side effects tend to occur within the first 24 hours after antibody administration. Side effects of some monoclonal antibodies may include fever, chills, shaking, nausea, vomiting, pain, headache, dizziness, trouble breathing, high or low blood pressure, itchiness, rash, hives, lip or face swelling, diarrhea, racing heart or chest pain. However, we rarely saw these reactions when a monoclonal antibody like VRC07 was given. When reactions were reported, they were usually mild. Antibodies given by the SC route have sometimes caused mild itchiness, redness and/or swelling at the injection site. These symptoms usually cleared within a few minutes to hours after the product was given.

Some antibody products have a risk of serious allergic reactions that can be life-threatening.

- Anaphylaxis is one type of allergic reaction that may happen soon after an antibody product administration. This reaction can include difficulty breathing, low blood pressure, hives or rash, swelling in the mouth and face.
- Serum sickness is a delayed type of allergic reaction that may happen several days to three weeks after an antibody product administration. This reaction can include hives or

rash, fever, swollen lymph nodes, muscle pains, joint pains, chest discomfort and shortness of breath.

The described possible allergic reactions are rare and more often occur with antibodies against human proteins, or with mouse antibodies rather than with human monoclonal antibodies like VRC07. Some antibodies of the type that attack human proteins can increase the risk of serious infections. VRC07 is not expected to increase the risk of serious infections because it attacks a virus and not a human protein.

In addition to the possible risks that are listed above, VRC07 may have other side effects that we do not know about yet.

Taking part in this study may affect your eligibility for future research studies with other HIV-related monoclonal antibodies.

Possible risks of AAV8 vector: AAV vectors have been studied in human clinical trials for testing of experimental products and found to be generally safe.

A theoretical risk of viral vectors in general is the possibility for insertion into the human DNA that could cause cancer. Insertion means that viral DNA becomes a part of human DNA. There was one report that found wild type AAV in human liver tumors. The AAV8 vector in this study product was designed NOT to insert into DNA and has no genes from wild type AAV proteins. There is no evidence that the AAV8 vector would become a part of human DNA. There have been no cancers reported in human studies with AAV vectors. As a precaution, we will test your blood for alpha-fetoprotein, which can indicate a liver tumor.

Another theoretical risk that is even more unlikely, is the insertion of AAV vectors in reproductive cells potentially causing birth defects and cancer in babies. Studies in mice found AAV8-VRC07 in all 15 tested tissues including testes and ovaries. However, the likelihood of AAV8-VRC07 getting into DNA of these cells is very unlikely. There is evidence that these cells may be resistant to AAV. AAV8-VRC07 was designed NOT to insert into DNA.

A potential risk of the study product is the development of immune responses (antibody) to AAV8 vector or to VRC07. This antibody should not affect your health but could decrease effectiveness of future treatments with AAV vectors or effect eligibility for treatment with other experimental antibodies in the future.

There may be other risks of the study product that we don't know about yet.

We will give you any new information about risks or other information that becomes available that may affect your participation in the study.

Possible risks of blood drawing: Blood drawing may cause pain and bruising, and possibly a feeling of lightheadedness or fainting. Rarely, it may cause infection at the blood draw site.

Possible risks of saliva sample collection: Collection of samples by swabs may cause brief discomfort and, rarely, a little bleeding.

Possible risks during pregnancy: We do not know what effects the study product may have on a fetus or nursing infant. Women who can have children must agree to not get pregnant during the first year of study participation. Effective birth control includes not having sex; use of male or female condoms; use of diaphragm or cervical cap with spermicide; use of intrauterine

device; use of birth control pills, patch or injections and other prescription methods; or a male partner who has had a vasectomy. We will discuss effective birth control methods with you.

Women must have a negative pregnancy test before getting the study product. You must notify the clinic staff right away if you have become pregnant during this study or think that you might be pregnant. If you become pregnant, we will not collect any more blood for research. However, you will be asked to continue with study follow-up visits to check on your health. We will ask you to report the outcome of the pregnancy to us, which will be reported to the antiretroviral pregnancy registry (http://www.apregistry.com).

As a precaution, we ask all male participants to use condoms for vaginal sex that could lead to pregnancy during the first year of the study. We also ask all participants to use condoms to prevent spreading of HIV and other sexually transmitted infections.

POSSIBLE BENEFITS

This study will not provide you with any direct health benefit. You and others may benefit in the future from what we learn from the study.

STOPPING STUDY PARTICIPATION

A study sponsor, a regulatory agency or the study investigators may stop the study. If this happens you will be told the reason why.

You may choose to stop participating in the study at any time. If you get a dose of AAV8-VRC07, production of VRC07 may continue in your body for some time. Therefore, we will ask you to keep follow-up visits so we can monitor your health. We may stop collection of samples that are for research purposes only.

ALTERNATIVES

This study is not designed to treat or prevent any disease. You may choose to not participate in this study. You may be eligible for other studies.

CONFLICT OF INTEREST

The NIH research staff is checked yearly for conflicts of interest. You may ask the research team for more information on this process. This study may have investigators who are not NIH employees. Non-NIH investigators are expected to follow the principles of the Protocol Review Guide but are not required to report their personal financial holdings to the NIH.

The NIH, including some members of the VRC scientific staff, developed the study product used in this research study. The results of this study could play a role in whether the FDA will approve the study product for sale at some time in the future. If approved, the future sale of the study product could lead to payments to NIH and some NIH scientists. By U.S. law, government scientists are required to receive such payments for their inventions. You will not receive any money from the development or sale of the study product.

Manufacturing process of the investigational agent used in this trial is currently not available as a stock option by any commercial entity**.**

COMPENSATION, REIMBURSEMENT, AND PAYMENT

Will you receive compensation for participation in the study?

Some NIH Clinical Center studies offer compensation for participation in research. The amount of compensation, if any, is guided by NIH policies and guidelines.

You will get compensation for your time and inconvenience based on VRC policy to help you with transportation costs and other expenses that may occur because of your study participation. It is possible that you may have some expenses not covered by the compensation provided.

The compensation is $200 for scheduled visits with blood drawing and oral swabs. For the enrollment visit that includes study injection(s) and blood draw, compensation depends on the number of injections given: $315 for 1-2 injection(s), $450 for 3-5 injections, $600 for 6-8 injections, and $750 for 9-11 injections. Compensation for timely completion of all 7 days of an online diary will be $25 total. Clinic visits that do not include research or safety blood sample collection will be $100.

Total compensation for completion of the study and all follow-up visits over 5 years is estimated to range from $6,915 to $7,350. Actual compensation is based on the number and type of study visits you complete and the number of injections you get. Your compensation may need to be reported to the internal revenue service (IRS) as taxable income.

If you are unable to finish the study, you will receive compensation for the visits and for the parts you completed. You usually will get the compensation about 2 weeks after each completed visit by direct deposit into a bank account that you specify to the Volunteer Payment Office.

With few exceptions, study compensation is considered taxable income that is reportable to the Internal Revenue Service (IRS). A “Form 1099-Other Income” will be sent to you if your total payments for research participation are $600 or more in a calendar year. If you have unpaid debt to the federal government, please be aware that some or all of your compensation may be automatically reduced to repay that debt on your behalf.

Will you receive reimbursement or direct payment by NIH as part of your participation?

Some NIH Clinical Center studies offer reimbursement or payment for travel, lodging or meals while participating in the research. The amount, if any, is guided by NIH policies and guidelines.

This study does not offer reimbursement for, or payment of, travel, lodging or meals.

Will taking part in this research study cost you anything?

NIH does not bill health insurance companies or participants for any research or related clinical care that you receive at the NIH Clinical Center.

There are no costs to you for participating in this study. You or your health insurance will have to pay for all medical costs for medical care that you get outside this study. It is possible that you may have some expenses that are not covered by the study compensation provided.

CLINICAL TRIAL REGISTRATION AND RESULTS REPORTING

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

CONFIDENTIALITY PROTECTIONS PROVIDED IN THIS STUDY

Will your medical information be kept private?

We will do our best to make sure that the personal information in your medical record will be kept private. However, we cannot guarantee total privacy. Organizations that may look at and/or copy your medical records for research, quality assurance, and data analysis include:

- The NIH and other government agencies, like the Food and Drug Administration (FDA), which are involved in keeping research safe for people.
- National Institutes of Health Intramural Institutional Review Board
- The study Sponsor VRC or their agent(s)
- Qualified representatives from Center for Cellular and Molecular Therapeutics, The Children's Hospital of Philadelphia (CHOP), Philadelphia, PA, the research organization that produced AAV8-VRC07.

The researchers conducting this study and the NIH follow applicable laws and policies to keep your identifying information private to the extent possible. However, there is always a chance that, despite our best efforts, your identity and/or information about your participation in this research may be inadvertently released or improperly accessed by unauthorized persons.

In most cases, the NIH will not release any identifiable information collected about you without your written permission. However, your information may be shared as described in the section of this document on sharing of specimens and data, and as further outlined in the following sections.

Further, the information collected for this study is protected by NIH under a Certificate of Confidentiality and the Privacy Act.

Certificate of Confidentiality

To help us protect your privacy, the NIH Intramural Program has received a Certificate of Confidentiality (Certificate). With this certificate, researchers may not release or use data or information about you except in certain circumstances.

NIH researchers must not share information that may identify you in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings, for example, if requested by a court.

The Certificate does not protect your information when it:

1. is disclosed to people connected with the research, for example, information may be used for auditing or program evaluation internally by the NIH; or

2. is required to be disclosed by Federal, State, or local laws, for example, when information must be disclosed to meet the legal requirements of the federal Food and Drug Administration (FDA);
3. is for other research;
4. is disclosed with your consent.

The Certificate does not prevent you from voluntarily releasing information about yourself or your involvement in this research.

The Certificate will not be used to prevent disclosure to state or local authorities of harm to self or others including, for example, child abuse and neglect, and by signing below you consent to those disclosures. Other permissions for release may be made by signing NIH forms, such as the Notice and Acknowledgement of Information Practices consent.

Privacy Act

The Federal Privacy Act generally protects the confidentiality of your NIH medical records we collect under the authority of the Public Health Service Act. In some cases, the Privacy Act protections differ from the Certificate of Confidentiality. For example, sometimes the Privacy Act allows release of information from your medical record without your permission, for example, if it is requested by Congress. Information may also be released for certain research purposes with due consideration and protection, to those engaged by the agency for research purposes, to certain federal and state agencies, for HIV partner notification, for infectious disease or abuse or neglect reporting, to tumor registries, for quality assessment and medical audits, or when the NIH is involved in a lawsuit. However, NIH will only release information from your medical record if it is permitted by both the Certificate of Confidentiality and the Privacy Act.

RESEARCH-RELATED INJURIES

The NIH Clinical Center will provide short-term medical care for any injury resulting from your participation in research here. In general, no long-term medical care or financial compensation for research-related injuries will be provided by the NIH, the NIH Clinical Center, or the Federal Government. However, you have the right to pursue legal remedy if you believe that your injury justifies such action.

PROBLEMS OR QUESTIONS

If you have any problems or questions about this study, or about your rights as a research participant, or about any research-related injury, contact the Principal Investigator, Joseph Casazza, M.D., Ph.D., [redacted] Other researchers you may call are: Study Coordinator, Laura Novik, RN, [redacted] at [redacted]15 or [redacted] You may also call the NIH Clinical Center Patient Representative at 301-496-2626, or the NIH Office of IRB Operations at 301-402-3713, if you have a research-related complaint or concern.

CONSENT DOCUMENT

Please keep a copy of this document in case you want to read it again.

Adult Research Participant: I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I consent to participate in this study.

Signature of Research Participant	Print Name of Research Participant	Date

Investigator:

Signature of Investigator	Print Name of Investigator	Date

Witness to the oral short-form consent process only:

Witness:

Signature of Witness*	Print Name of Witness	Date

***NIH ADMINISTRATIVE SECTION TO BE COMPLETED REGARDING THE USE OF AN INTERPRETER:**

____ An interpreter, or other individual, who speaks English and the participant's preferred language facilitated the administration of informed consent and served as a witness. The investigator obtaining consent may not also serve as the witness.

____ An interpreter, or other individual, who speaks English and the participant's preferred language facilitated the administration of informed consent but did not serve as a witness. The name or ID code of the person providing interpretive support is: _____.