HIV VACCINE
TRIALS NETWORK

PROTOCOL

HVTN 302

A phase 1, randomized, open-label clinical trial to evaluate the safety and immunogenicity of BG505 MD39.3, BG505 MD39.3 gp151, and BG505 MD39.3 gp151 CD4KO HIV trimer mRNA vaccines in healthy, HIV-uninfected adult participants

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A phase 1, randomized, open-label clinical trial to evaluate the safety and immunogenicity of BG505 MD39.3, BG505 MD39.3 gp151, and BG505 MD39.3 gp151 CD4KO HIV trimer mRNA vaccines in healthy, HIV-uninfected adult participants

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

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Investigator of Record Name (print) Investigator of Record Signature Date

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Acronyms and abbreviations

AE	adverse event
AESI	Adverse events of special interest
AoU	assessment of understanding
ALT	alanine aminotransferase
AUC	area under the curve
BAMA	binding antibody multiplex assay
BCR	B cell receptor
β-HCG	beta human chorionic gonadotropin
BMGF	Bill & Melinda Gates Foundation
bnAb	broadly neutralizing antibody
BP	blood pressure
CAB	Community Advisory Board
CBC	complete blood count
cGMP	current Good Manufacturing Practice
CHAVD	Consortium for HIV/AIDS Vaccine Development
CMV	Cytomegalovirus
CRPMC	NIAID Clinical Research Products Management Center
CRS	clinical research site
CSS	clinical safety specialist
DAIDS	Division of AIDS
EAE	expedited adverse events
EC	ethics committee
eDiary	electronic diary
EIA	enzyme immunoassay
ELISA	enzyme-linked immunosorbent assay
EMPEM	electron microscopy-based polyclonal epitope mapping
EUA	emergency use authorized
EUL	emergency use listing
FDA	(US) Food and Drug Administration
FNA	Fine needle aspiration
GINA	Genetic Information Nondiscrimination Act
GMP	Good Manufacturing Practice
GC	germinal center
GCP	Good Clinical Practice
HLA	human leukocyte antigen
IB	Investigator's Brochure
IBC	Institutional Biosafety Committee

ICS	intracellular cytokine staining
IM	intramuscular
IND	investigational new drug (application)
ISCOMIT	immunostimulatory complex (ISCOM)-like nanoparticle comprised of self-assembled cholesterol, phospholipid, and Quillaja saponin made at Massachusetts Institute of Technology (MIT).
IRB	institutional review board
LABA	long-acting beta agonist
LNP	lipid nanoparticle
MAAE	medically attended adverse events
mRNA	Messenger ribonucleic acid
nAb	neutralizing antibody
NHP	non human primate
NIAID	National Institute of Allergy and Infectious Diseases
NSAID	non-steroidal anti-inflammatory drug
PAB	Pharmaceutical Affairs Branch
PCR	polymerase chain reaction
PBMC	peripheral blood mononuclear cells
PEF	peak expiratory flow
PI	principal investigator
PrEP	pre-exposure prophylaxis
PSRT	Protocol Safety Review Team
RAB	Regulatory Affairs Branch
RE	regulatory entity
RSC	Regulatory Support Center
SAE	serious adverse event
SAP	statistical analysis plan
SDMC	statistics and data management center
SICF	sample informed consent form
SMB	Safety Monitoring Board
SPT	Safety and Pharmacovigilance Team
SSP	Study Specific Procedures
SUSAR	suspected unexpected serious adverse reaction
ULN	upper limit of normal
VISP	vaccine-induced seropositivity
VRC	Vaccine Research Center
WHO	World Health Organization

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1 Executive summary

1.1 Title

A phase 1, randomized, open-label clinical trial to evaluate the safety and immunogenicity of BG505 MD39.3, BG505 MD39.3 gp151, and BG505 MD39.3 gp151 CD4KO HIV trimer mRNA vaccines in healthy, HIV-uninfected adult participants.

1.2 Design

This is an open-label, multicenter, randomized phase 1 study to evaluate the safety and immunogenicity of BG505 MD39.3, BG505 MD39.3 gp151, and BG505 MD39.3 gp151 CD4KO HIV trimer mRNA. These trimers are based on the BG505 MD39 native-like trimer reported in Steichen et al. Immunity 2016 (1). The primary hypothesis is that the BG505 MD39.3 soluble and membrane-bound trimer mRNA vaccines will be safe and well-tolerated among HIV-uninfected individuals and will elicit autologous neutralizing antibodies.

1.3 Study products

- BG505 MD39.3 mRNA (labeled as mRNA-1574v1-GP140) formulated in a lipid nanoparticle (soluble trimer): a prefusion-stabilized, cleavage-independent, glycan-hole-masked soluble trimer. To be administered as 100 mcg or 250 mcg intramuscularly.

- BG505 MD39.3 gp151 mRNA (labeled as mRNA-1574v2-GP151) formulated in a lipid nanoparticle (membrane-bound trimer): a prefusion-stabilized, cleavage-independent, glycan-hole-masked trimer in a membrane-bound format including a transmembrane domain but lacking a C-terminal domain. To be administered as 100 mcg or 250 mcg intramuscularly.

- BG505 MD39.3 gp151 CD4KO mRNA (labeled as mRNA-1574v3-CD4KO-GP151) formulated in a lipid nanoparticle (membrane-bound trimer): a prefusion-stabilized, cleavage-independent, glycan-hole-masked trimer in a membrane-bound format including a transmembrane domain but lacking a C-terminal domain with a point mutation in the CD4bs that reduces CD4 binding. To be administered as 100 mcg or 250 mcg intramuscularly.

- Diluent: Sodium Chloride Injection, USP, 0.9% is commercially sourced from Spectra Medical Devices, Inc. Sodium Chloride Injection, USP, 0.9% is a sterile, nonpyrogenic, isotonic solution of sodium chloride and water for

injection. Each mL contains sodium chloride 9 mg. It contains no bacteriostat, antimicrobial agent or added buffer.

Note: In this protocol we will collectively refer to the study products as BG505 MD39.3 mRNA vaccines or mRNA1574 vaccines for clarity and improved readability.

1.4 Study population

Healthy adults aged 18 to 55 years, inclusive

1.5 Study plan and schema table

Participants will receive BG505 MD39.3 mRNA, BG505 MD39.3 gp151 mRNA or BG505 MD39.3 gp151 CD4KO mRNA, at doses of 100 mcg or 250mcg, administered via intramuscular (IM) injections into the deltoid muscle. Participants will be evaluated for safety and immune responses through blood and lymph node fine-needle aspiration collection at specified timepoints throughout the study. The study schema is below:

Table 1-1 Schema

Study Arm	N	Dose (mcg)	Month 0	Month 2	Month 6
Part A					
Group 1	18	100	BG505 MD39.3 mRNA	BG505 MD39.3 mRNA	BG505 MD39.3 mRNA
Group 2	18	100	BG505 MD39.3 gp151 mRNA	BG505 MD39.3 gp151 mRNA	BG505 MD39.3 gp151 mRNA
Group 3	18	100	BG505 MD39.3 gp151 CD4KO mRNA	BG505 MD39.3 gp151 CD4KO mRNA	BG505 MD39.3 gp151 CD4KO mRNA
Part B					
Group 4	18	250	BG505 MD39.3 mRNA	BG505 MD39.3 mRNA	BG505 MD39.3 mRNA
Group 5	18	250	BG505 MD39.3 gp151 mRNA	BG505 MD39.3 gp151 mRNA	BG505 MD39.3 gp151 mRNA
Group 6	18	250	BG505 MD39.3 gp151 CD4KO mRNA	BG505 MD39.3 gp151 CD4KO mRNA	BG505 MD39.3 gp151 CD4KO mRNA
Total	**108**				

A dose escalation plan will be implemented, whereby sentinel safety groups (n=12, approximately 4 for each of the three low-dose groups) in Part A would

be enrolled and evaluated for safety 2 weeks after the first vaccination. If safety criteria are met, then enrollment of the Part B sentinel safety groups (n=12, approximately 4 per group) and the remainder of the Part A participants would commence. Safety for the sentinel groups in Part B will be assessed after the first vaccination prior to full enrollment of Part B. In addition, standard safety evaluations will occur routinely throughout the trial.

1.6 Duration per participant

Main study: Up to 12 months of scheduled visits.

Extended follow-up: For the small group of participants with unresolved study product-related urticaria-associated symptoms (eg; wheal and flare lesions, pruritis, and/or erythematous lesions), up to an additional 48 months of scheduled contacts.

1.7 Estimated total study duration

Main study: 22 months (includes enrollment, planned safety holds, and follow-up).

Extended follow-up for participants with unresolved study product-related urticaria-associated symptoms (eg; wheal and flare lesions, pruritis, and/or erythematous lesions): estimated total study duration would be up to 70 months (includes main study and extended follow-up).

1.8 Study sites

HVTN Clinical Research Sites (HVTN CRSs) to be specified in the Site Announcement Memo.

2 Introduction

This first-in-human phase 1 clinical trial will evaluate whether messenger ribonucleic acid (mRNA)/lipid nanoparticle (LNP) delivery of soluble or membrane-bound HIV Env trimers is a promising platform for rapid and iterative HIV vaccine development. This platform is expected to be advantageous in terms of the speed and cost of deployment as well as the magnitude and quality of the human immune responses. The comparison of the immune responses elicited by the HIV Env trimers selected for this study will determine whether a modified membrane-bound trimer lacking CD4 affinity elicits improved antibody responses.

Achieving a goal of developing a sequential vaccine regimen that induces sustained protective levels of HIV broadly neutralizing antibodies in humans will require expeditious iterative testing of multiple vaccine regimens in humans. However, the slow timeline and relatively high costs of Good Manufacturing Practice (GMP) protein manufacture present barriers to success. Modified mRNA delivered within LNPs is an emerging vaccine platform that offers the potential for GMP manufacture in a substantially shorter time and with lower costs compared to protein manufacture (2-5).

The primary vaccine strategies investigated by the Scripps Consortium for HIV/AIDS Vaccine Development (CHAVD) include germline targeting and immuno-focusing using a sequence of HIV Env trimer immunogens, serving the general purposes of: (i) priming appropriate precursor B cells; (ii) "shepherding" or guiding affinity maturation toward broadly neutralizing antibody (bnAb) development; and (iii) "polishing" or optimizing the vaccine response in terms of neutralization breadth, potency, magnitude, and durability. This strategy will employ modified trimers to serve at the priming or shepherding stages followed by native-like trimers at the polishing stage. Therefore, identification of a rapid GMP delivery platform for trimers would expedite the process of evaluating human immune responses to HIV vaccines of sufficient strength and quality to enable iterative and measurable progress toward bnAb elicitation.

Fully native trimers are anchored to a lipid membrane on the virus or an infected cell, but most vaccine discovery efforts utilize trimers in a soluble format owing to the difficulties associated with production and purification of membrane proteins. Trimer delivery by mRNA/LNPs allows for deployment of membrane-bound trimer immunogens, which may be advantageous for trimer conformational sampling and glycosylation. This method also allows occluding the base of the trimer that, when exposed on soluble trimers, is immunodominant and elicits non-neutralizing and trimer-degrading antibodies.

The Scripps CHAVD teams have achieved demonstrable successes using soluble trimer immunization to elicit bnAbs or partially mature bnAb in mouse models with restricted repertoires (1, 6, 7); to elicit protective autologous neutralizing

antibodies against BG505 SHIV in rhesus macaques (8, 9); and to improve trimer responses via slow-release (10) and Alum-targeting (11). However, significant challenges are associated with the soluble trimer format. In particular, the "base" of the trimer, the region that is occluded by the membrane on a viral-associated trimer but exposed on soluble gp140, is highly immunogenic or immunodominant in mice, rabbits, and non-human primates (NHPs) (10, 12-14) and Andrew Ward's lab at the Scripps Research Institute has recently shown that base-directed responses are likely associated with trimer degradation in vivo (unpublished data). Furthermore, in some cases, soluble trimers have been shown to have glycosylation profiles that are less native-like than corresponding membrane-bound trimers, potentially leading to differences in the elicitation of neutralizing responses (15).

Experiments with trimer-nanoparticles in which the base of the trimer is fused to self-assembling proteins have found that trimer-nanoparticles can exhibit improved trafficking to follicular dendritic cell networks and increased concentration in germinal centers (16). Compared to soluble trimers, trimer-nanoparticles can elicit stronger neutralizing responses to epitopes well-exposed on the nanoparticle (17). However, there remain significant challenges for this presentation format. In particular, autologous neutralizing and protective responses are not always improved by trimer presentation on a nanoparticle; the nanoparticle core and the trimer bottom both remain immunogenic, in some cases highly immunogenic(18); and trimer-degrading antibodies are still elicited. Elicitation of responses to the nanoparticle core and trimer base is likely due at least in part to particle disassembly in vivo. Thus, while trimer nanoparticles remain of significant interest, the platform requires additional technical improvement.

An important additional uncertainty about trimer immunization in humans is whether trimer binding to host CD4 and the ensuing trimer conformational changes will negatively impact the magnitude and/or specificity of antibody responses. There is a substantial risk that the trimer-CD4 interaction will alter the human antibody response because the germinal center reaction where antibody maturation occurs involves dynamic interactions between CD4+ T cells and B cells in the presence of antigen. The risk of such an undesired response may be heightened for membrane-bound trimers that may interact with CD4+ T cells in a highly multivalent manner via presentation on microvesicles, or exosomes or cell surfaces. Therefore, evaluating a platform which reduces trimer-CD4 interactions is of interest.

A transformative vaccine delivery platform is needed to provide a combination of speed and reduced cost with sufficiently strong and high-quality immunity. In collaboration with Moderna and partnership with IAVI and the Bill & Melinda Gates Foundation (BMGF), rabbit immune responses to BG505-based stabilized trimers delivered as mRNA/LNPs or protein plus an immunostimulatory complex (ISCOM)-like nanoparticle comprised of self-assembled cholesterol,

phospholipid, and Quillaja saponin made at Massachusetts Institute of Technology (MIT) called ISCOMIT (adjuvant) in both soluble trimer and ferritin-nanoparticle formats were evaluated. The ISCOMIT adjuvant is synthesized as described in Tokatlian et al Science 2019 (16). Membrane-bound trimer format via mRNA/LNP delivery was also assessed. As discussed below, the responses have been particularly favorable for the membrane-bound trimer format, in that: (i) autologous neutralizing responses have been at least as good as for purified trimer protein plus adjuvant; (ii) base-directed responses have been substantially reduced, according to enzyme-linked immunosorbent assay (ELISA) and electron microscopy-based polyclonal epitope mapping (EMPEM); and (iii) trimer-degrading responses have been reduced according to EMPEM.

These findings, and the partnership with IAVI and BMGF, along with National Institute of Allergy and Infectious Diseases (NIAID), NIAID/Division of AIDS(DAIDS) and NIAID/Vaccine Research Center (VRC) leadership, have led to the proposed study.

This study involving mRNA/LNP delivery of trimers could greatly accelerate progress toward development of an HIV vaccine, as the rapid timescale and reduced cost of GMP manufacture will enable a larger number of constructs and regimens to be tested in the clinic. Additionally, this approach would likely transform the field and cause most or many future HIV vaccine clinical studies to employ this or similar nucleic acid delivery strategies, at least for the process of iterative regimen optimization. Whether a final licensed vaccine to elicit durable bnAb responses would employ mRNA/LNPs or adjuvanted protein or another technology would remain to be determined.

The proposed trial will also reveal whether or not HIV trimers for human immunization should be engineered to lack CD4 affinity. If knocking out CD4 binding is found to be important for improving human responses, the field will likely adopt this strategy. The VRC is currently carrying out a Phase 1 study (NCT03783130) with an adjuvanted trimer that has 10-fold reduced affinity for CD4 (BG505 DS-SOSIP). However, the VRC molecule still retains high affinity with a KD of 10 nM (19), allowing for potentially avid interactions with cell surface CD4. In contrast, BG505 MD39.3 gp151 CD4KO HIV trimer mRNA has at least 1000-fold reduced avidity for CD4+ T cells (20). Evaluating responses generated by the BG505 MD39.3 gp151 CD4KO trimer and those induced by the DS-SOSIP trimer in a different study should provide guidance to the field on whether and to what degree trimer affinity for human CD4 should be suppressed.

2.1 Rationale for evaluation of BG505 MD39.3, BG505 MD39.3 gp151 and BG505 MD39.3 CD4KO gp151

HVTN 302 will test mRNA/LNP as a platform for delivery of soluble and membrane-bound HIV trimers. The key advantages of mRNA/LNPs are the substantially reduced time and cost for GMP manufacture compared to purified

protein and the feasibility of delivering membrane-bound trimers. Key potential advantages of the following membrane-bound trimers are reduced elicitation of base-directed responses that are non-neutralizing and potentially trimer-degrading; increased elicitation of responses to regions of the trimer other than the base; more native-like glycosylation (15) and more native-like conformational sampling due to the membrane anchor:

1. A prefusion-stabilized, cleavage-independent, glycan-hole-masked soluble trimer (BG505 MD39.3). MD39 is an improved version of BG505 SOSIP with improved antigenicity, trimer yield, and thermal stability that has been engineered via structure-guided mammalian display directed evolution (1). MD39.3 includes the following changes to the original MD39: a linker ("link14") across the gp120-gp41 cleavage site that has also been engineered by directed evolution (21), and mutations that previously described to introduce glycosylation sites in the well-known 241/289 glycan holes of BG505 (20).

2. The matched trimer as in 1. but in a membrane-bound format including a transmembrane domain but lacking a C-terminal domain (BG505 MD39.3 gp151)

3. The same membrane-bound trimer as in 2. but with a point mutation in the CD4bs that was computationally designed and experimentally demonstrated to abrogate CD4 binding (BG505 MD39.3 CD4KO gp151) (20).

This study will determine whether mRNA/LNP delivery of soluble or membrane-bound trimers is a promising platform for expeditious iterative vaccine development in terms of the speed and cost of deployment and the magnitude and quality of the human immune responses. This study will also disclose whether a modified membrane-bound trimer lacking CD4 affinity elicits improved antibody responses.

2.2 Preclinical data

2.2.1 Toxicity studies for BG505 MD39.3 HIV mRNA trimer vaccines

A non-GLP study in Sprague Dawley rats was conducted to characterize the potential toxicity of the BG505 MD39.3, BG505 MD39.3 gp151 and BG505 MD39.3 CD4KO gp151 trimer vaccines at IM dose levels of 30, 60, and 100 mcg administered on Days 1, 22, and 43. Post-dose observations continued for 2 weeks post the third dose. All injections were well tolerated and did not result in any animal deaths. Swelling at the injection site was noted in all animals, whereas transient pale skin resulted in a subset of animals due to lower blood counts. Other clinical pathology findings were consistent with an inflammatory and/or immune process. Overall, the results from the BG505 MD39.3, BG505 MD39.3 gp151 and BG505 MD39.3 CD4KO gp151 trimer vaccines non-GLP

study demonstrate a similar, consistent, and acceptable toxicity profile supportive of this Phase 1 clinical trial.

2.2.2 Immunogenicity studies

2.2.2.1 In Rabbits

BG505 MD39.3 was developed by introducing two glycosylation sites at positions 241 and 289 to BG505 MD39.2, a link14, cleavage-independent native-like trimer. MD39.2 delivered as Moderna mRNA or purified soluble protein in multiple formats was tested in a large rabbit immunization study. Rabbits were immunized by the IM route at weeks 0, 8, and 24, with either 100 mcg mRNA/LNP or 30 mcg protein + 75U ISCOMIT. The different molecular structures of MD39.2 included:

- Soluble trimer: MD39.2 (mRNA and protein) and MD39.2 with wild-type signal peptide leader sequence – MD39.2WTsp (mRNA)
- MD39.2 ferritin nanoparticle (mRNA and protein)
- Two types of membrane-bound trimers tested only as mRNA: membrane-tethered trimer (MD39.2 PDGFR – MD39.2 fused via a long flexible linker to plasma derived growth factor receptor [PDGFR] transmembrane domain) and natively membrane-bound trimer (MD39.2 gp160-dCT).
- BG505 SOSIP trimer (mRNA and protein), MD39 (soluble trimer protein with intact cleavage site), olio6 (a variant of MD39, protein only), and gp120 foldon (protein only) as controls (Figure 2-1).

The hypothesis was that autologous neutralization responses generally provide a readout on the degree to which the trimer constructs maintain a native-like state in vivo. In this study, very little autologous neutralization was detected after two immunizations in any of the groups. However, after three immunizations, the Moderna mRNA-launched MD39.2 gp160-dCT membrane-bound trimer elicited autologous neutralizing antibodies as well or better than any other mRNA group (left side of Figure 2-1).

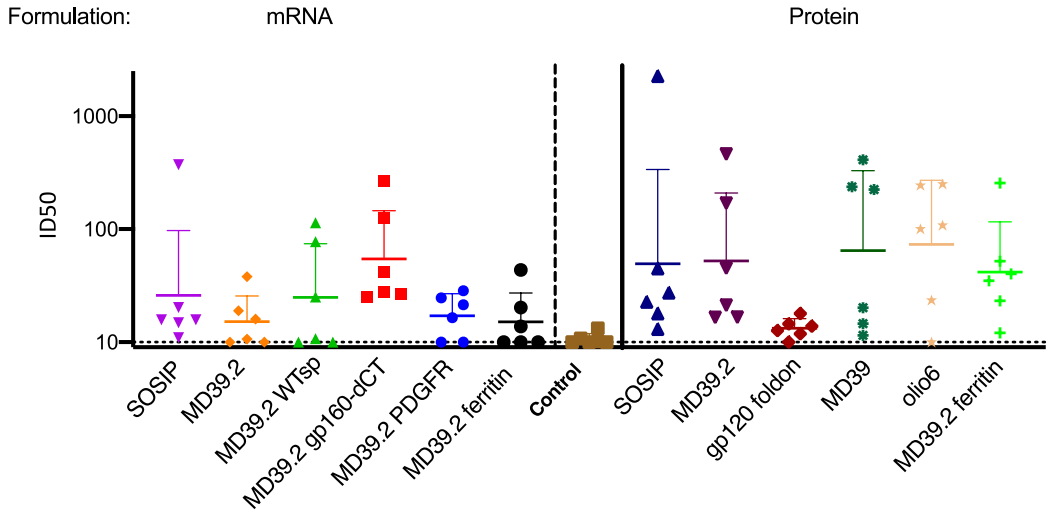


Figure 2-1 BG505-T332N autologous neutralization by purified serum IgG from week 26 (post 3rd vaccination) from rabbit experiment #1

Figure 2-1 also shows that the MD39.2 gp160-dCT mRNA construct performed approximately as well as any of the purified protein groups (right side of Figure 2-1).Using a Kruskal-Wallis statistical test that corrects for multiple comparisons, the MD39.2 gp160-dCT group was not significantly different from any other mRNA or protein group except the MD39.2 ferritin (mRNA) and gp120 foldon (protein). The caveat with the data in Figure 2-1 is that the neutralization titers for both protein and mRNA groups are low compared to historical data for three administrations of BG505 SOSIP protein plus adjuvant in rabbits (22, 23). Assuming that an unknown factor reduced titers in all groups relatively equally, it seems fair to conclude that the membrane-bound trimer performed well against all other mRNA or protein groups.

A smaller follow-up rabbit study was conducted to further evaluate mRNA trimer forms:

- Soluble trimers (MD39.2 and MD39.3)
- Membrane-bound (MD39.2 gp160-dCT)
- Circularly-permuted soluble trimer CPG9 (20) that eliminates the cleavage site and affords a degree of glycan masking on the trimer base.

In both experiments, rabbits were immunized by the IM route at weeks 0, 8, and 24, with 100 mcg mRNA/LNP.

Membrane-bound gp160-dCT MD39.2 and MD39.2 delivered via mRNA elicited autologous neutralizing IgG antibodies at titers similar to or greater than soluble trimer at both week 10 (post two vaccinations) and week 26 (post three

immunizations) (Figure 2-2). Also, the week 26 neutralization titers for MD39.3 gp160-dCT in this experiment were within the range of BG505 autologous neutralization titers elicited by three administrations of stabilized BG505 soluble trimers in rabbits (22, 23) or NHPs (8, 9).

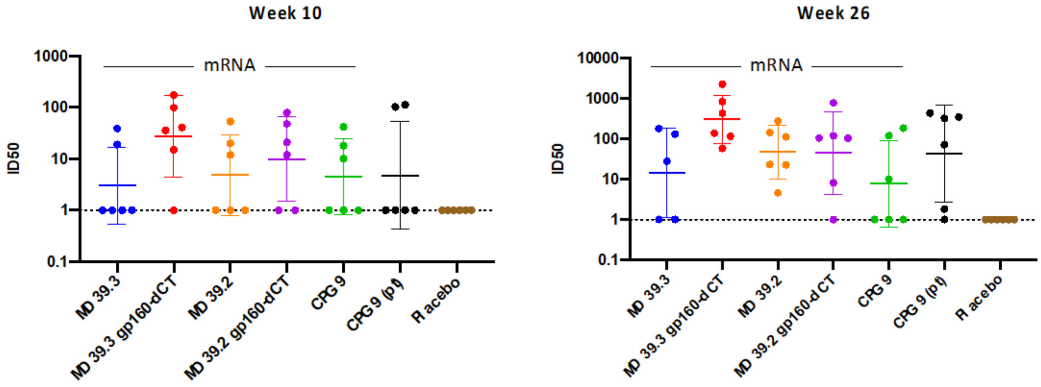


Figure 2-2 BG505-T332N autologous neutralization by purified IgG from week 10 and 26 from rabbit experiment #2. All immunizations were mRNA/LNP except the group labelled CPG9 (pt).

One hypothetical benefit of employing membrane-bound trimer immunogens would be reducing non-neutralizing base-directed responses and a potential increase of responses to other epitopes on the trimer. To investigate this, ELISA analyses were carried out on the week 10 sera from rabbits in both experiments. The responses were characterized by the area under the curve (AUC) of the serum titration. Responses were measured against MD39 captured by PGT128 Fab, a protocol in which MD39 has a native antigenic profile with good binding to bnAbs, including N332 bnAbs and reduced binding to non-nAbs directed to V3 or the CD4bs (not shown). By capturing MD39 with PGT128, the base of the trimer was well-exposed in the ELISA assay. All of the soluble trimer immunogens, whether protein or mRNA, elicited stronger responses to MD39 than either of the membrane-bound gp160-dCT immunogens (Figure 2-3A). To determine the degree of binding to epitopes other than the base, responses to MD39 in the presence of a high concentration (20 mcg/mL) of a base-directed antibody, 19R (also known as 1E6), were measured. In that competition assay, there was no significant difference in the responses to membrane-bound trimers compared to soluble trimers (Figure 2-3B), indicating that membrane-bound trimers elicit comparable responses to the "top" of the trimer as soluble trimers. From these two assays, the fraction of the response to MD39 that was directed to the base (Figure 2-3C) was computed. By this measure, approximately 50% of the response to any of the soluble trimers was directed to the base, whereas the membrane-bound trimers elicited a near-zero anti-base response (blue arrows in Figure 2-3C). In conclusion, mRNA-delivered, membrane-bound trimers can elicit significantly reduced anti-base responses while eliciting similar levels of responses to other trimer epitopes.

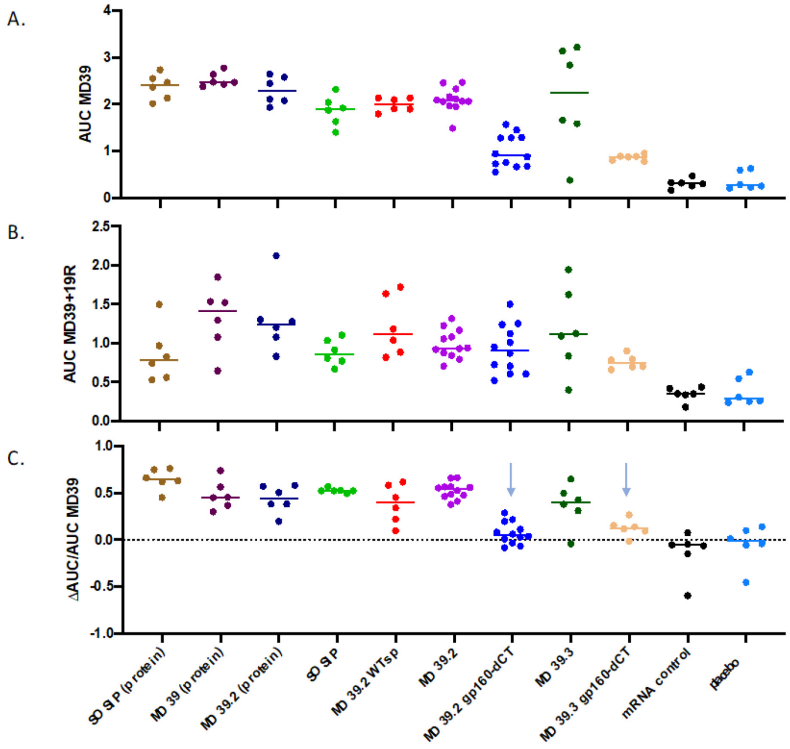


Figure 2-3 ELISA analysis of serum antibody binding responses from week 10 (post two vaccinations) for rabbit experiments 1 and 2 combined. A: Response to MD39; B: Response to MD39 in presence of base-directed antibody 19R, to reveal responses to epitopes other than the base; C: Fraction of response to MD39 that is directed to the base. Immunogens were mRNA/LNP unless indicated otherwise.

Summary of rabbit studies

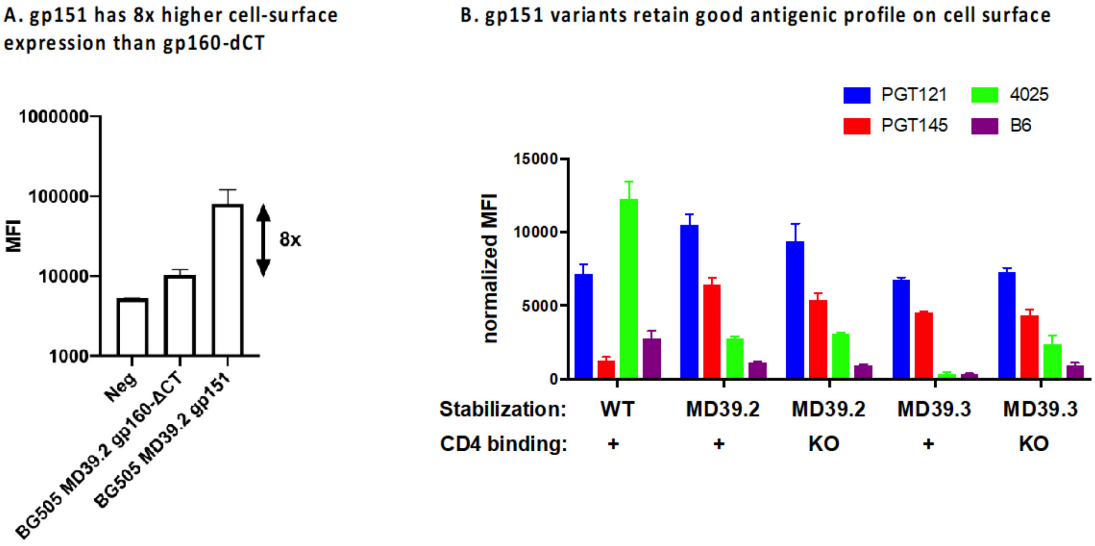
Overall, the mRNA-delivered, natively membrane-bound trimer performed as well as or better than other formats in head-to-head comparisons, based on (a) BG505 autologous neutralization (b) ELISA analyses (c) Electron microscopy (EM) mapping of polyclonal responses (data not shown), and (d) EM analysis of trimer degradation (data not shown). In rabbits, immunization 100 mcg of HIV Trimer mRNA/LNP was well tolerated with no observed morbidity or mortality and only elicited modest autologous neutralization titers.

Improvement of cell surface expression

The rabbit experiments were carried out with a gp160-dCT design that inadvertently included a known endocytosis motif (GYXXØ) at the C-terminus. The designs were modified to eliminate that motif, and the new construct is referred as a gp151 design. The new gp151 design exhibited approximately 8-fold higher cell surface expression compared to the original gp160-dCT design, as measured by in vitro transfection and cytometry (A). The new gp151 MD39.3 mRNA trimers also maintained a favorable antigenic profile (B) (compared to wild type [WT], there is reduced binding to non-neutralizing 4025 and B6 antibodies while retention of binding to bnAbs). For the clinical trial, membrane-

bound trimers are manufactured based on the new gp151 format, as higher cell surface expression combined with favorable antigenicity is expected to lead to stronger favorable immune responses.

Figure 2-4 Cell surface expression and antigenic profile for membrane-bound BG505 trimers as gp160-dCT or gp151



2.2.2.2 In non human primates (NHPs)

Five groups of six rhesus macaques were immunized via IM injection bilaterally in each deltoid at weeks 0, 8 and 24 using BG505 MD39.3 constructs listed below.

1. BG505 MD39.3 soluble trimer delivered by mRNA (100 mcg total dose)

2. BG505 MD39.3 gp151 membrane-bound trimer delivered by mRNA (100 mcg total dose)

3. BG505 MD39.3 CD4KO gp151 membrane-bound trimer delivered by mRNA (100 mcg total dose)

4. BG505 MD39.3 gp151 membrane-bound trimer delivered by mRNA (300 mcg total dose)

5. BG505 MD39.3 soluble trimer delivered with adjuvant (100 mcg protein + 750 mcg SMNP adjuvant total dose)

All five groups elicited similar levels of binding antibodies at assessed at weeks 10 and 26 with the binding titers increasing for all groups following the third immunization at week 24 (Figure 2-5).

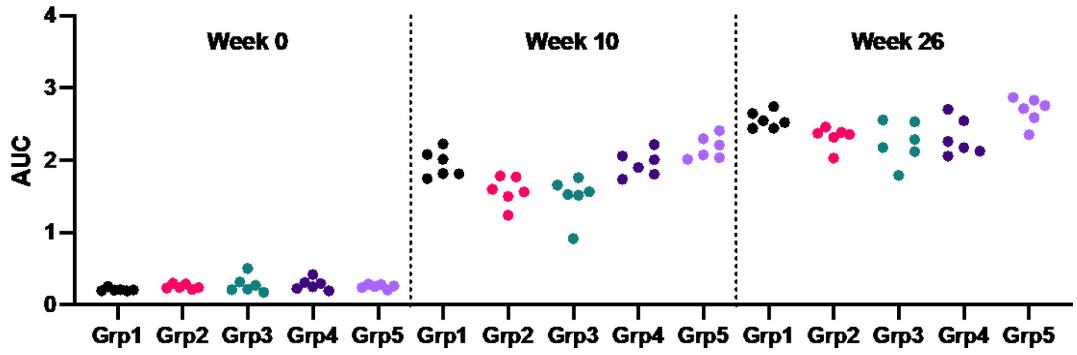


Figure 2-5 ELISA analysis of serum antibody binding responses from week 10 (post two immunizations) and week 26 (post three immunizations) in NHPs

None of the animals receiving BG505 MD39.3 soluble trimer delivered by mRNA developed autologous neutralizing antibodies even after three immunizations (Figure 2-6, Grp1). Half of the animals receiving the 100 mcg dose of BG505 MD39.3 gp151 membrane-bound trimer delivered by mRNA developed detectable autologous neutralizing antibodies at week 10 which increased in potency at week 26 (Figure 2-6, Grp2). Two of six animals receiving the 100 mcg dose of BG505 MD39.3 gp151 CD4KO membrane-bound trimer delivered by mRNA developed autologous neutralizing antibodies at week 26 (Figure 2-6, Grp3). Four out of the six animals in the group receiving the 300 mcg dose of BG505 MD39.3 gp151 membrane-bound trimer delivered by mRNA developed detectable autologous neutralizing antibodies at week 26 (Figure 2-6, Grp4). A similar response was seen in the BG505 MD39.3 soluble trimer delivered with adjuvant group Figure 2-6, Grp5). Overall, BG505 MD39.3 gp151 membrane-bound trimers delivered by mRNA elicited similar antibody responses compare to soluble trimer protein + adjuvant.

To date, most trimer immunogen design and characterization in the HIV vaccine field has been focused on soluble trimers. Although none of the NHP animals receiving BG505 MD39.3 soluble trimer by mRNA (Grp 1) developed autologous neutralizing antibodies in this study, it is known that studies in animal models do not reliably predict human responses. Therefore, to identify the best trimer platform for mRNA delivery in humans, it is essential to test the most common trimer design employed by the HIV vaccine field (soluble gp140) along with the membrane-bound trimers that have performed well in our pre-clinical studies (as shown above) and that are analogous to the membrane-bound trimers in human SARS-CoV-2 mRNA vaccines.

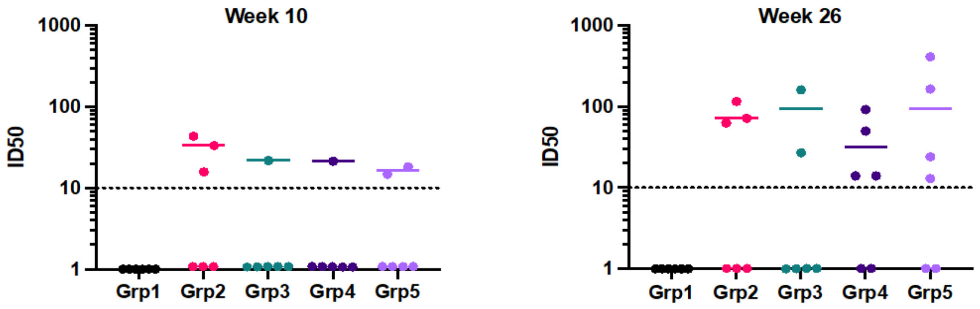


Figure 2-6 BG505-T332N autologous serum neutralization in NHPs from weeks 10 and 26. Bars represent median ID_{50} among animals with detectable neutralization (ie, ID_{50} titer greater than 10).

2.2.2.3 In rats

Rats administered mRNA-1574, mRNA-1574.2 or mRNA-1574.3 demonstrated dose-independent binding Ab titers to autologous trimer antigen on Day 56. 30, 60 and 100 mcg doses of mRNA-1574, mRNA-1574.2 and mRNA-1574.3 elicited serologic responses to autologous trimer antigen in rats by 56 days (2 weeks post 3rd dose) and the magnitude of responses was independent of the dose.

2.3 Rationale for Study Design

The study design, randomized, open-labelled, unblinded and without a placebo control, allows for rapid access of study samples by the laboratory investigators earlier than is typical for blinded, placebo-controlled trials; thereby facilitating expeditious iterative development between the laboratory, pre-clinical and clinical arenas. In particular, exploratory assays may begin on samples before the majority of the participants have reached primary timepoints and prior to completion of the primary immunogenicity assays. This semi-experimental medicine design is being utilized within the HVTN for a suite of trials testing a range of HIV vaccine formulations expected to contribute to the goal of eventually developing HIV vaccines with broad neutralizing capabilities. The BG505 MD39.3 HIV trimer vaccines in HVTN 302 are not on the development pathway toward licensure. The lessons learned from this trial will inform the formulation of future products that will aim toward eliciting increasingly broad neutralization immune responses. Immunogenicity assays do not require comparison between immune responses elicited by vaccine versus by placebo as baseline samples may serve as controls where needed. Additionally, safety assessments do not require placebo recipient comparisons. The unblinded safety reports reviewed weekly by the Protocol Safety Review Team (PSRT) during the vaccination phase will also facilitate safety monitoring between study arms and across dosing groups which would allow potentially for differential action to be

taken by study arm, as appropriate, if safety or tolerability concerns arose for a particular study arm.

2.4 Rationale for Schedule

To match the schedule used in other comparable trials and pre-clinical studies, BG505 MD39.3 HIV Trimer mRNA vaccines will be administered at a dose of 100 mcg or 250 mcg at Month 0, 2 and 6. Rabbit studies described above demonstrated that autologous neutralizing antibody responses were significantly increased following the third compared with the second vaccine administration timepoint. A similar experimental mRNA vaccine being evaluated for Cytomegalovirus (CMV), the Moderna mRNA CMV vaccine (mRNA-1647), is administered at Month 0, 2 and 6. Neutralizing antibody titers increased following the Month 2 vaccination, were further boosted after the Month 6 vaccination and were then sustained for at least 12 months (24-26).

2.5 Rationale for Dose of HIV Trimer mRNA/LNP

We propose to test both the 100 mcg dose and a higher dose of 250 mcg in order to find the maximum tolerable dose that gives strong human antibody responses and that can distinguish responses between the three different immunogens in the trial.

The Moderna Phase 1 trial of the SARS-CoV-2 vaccine candidate mRNA-1273, a membrane-anchored glycoprotein trimer analogous to membrane-anchored trimers proposed here, administered vaccine at 25, 100 and 250 mcg doses at two timepoints. Binding antibody titers were greater with increasing dose levels, although pseudoneutralization antibody titers were generally similar between the 100 and 250 mcg doses. Reactogenicity was generally of greater frequency and intensity at higher doses and following the second administration. Systemic solicited adverse events were more common after the second vaccination and 21% of participants in the 250 mcg dose group reported one or more severe systemic adverse events after the second vaccination, however, no serious adverse events were reported and no stopping rules were met in the trial (4).

Furthermore, the Moderna CMV vaccine (mRNA-1647) has been administered in doses up to 300 mcg, with multiple administrations per volunteer. In the Phase 1 trial, at the 12 month interim analysis, there were no vaccine-related serious adverse events (SAEs) and safety and tolerability at the 300 mcg dose level was similar to that observed at the 180 mcg level (26). Reactogenicity tended to increase in frequency and intensity after the second dose compared to the first, but (for the 90 and 180 mcg doses) decreased or remained similar after the third dose relative to post second dose (25).

Finally, in the rabbit immunization studies with 100 mcg HIV trimer mRNA/LNPs, the autologous neutralization titers have been modest, suggesting that testing a higher dose of the BG505 MD39.3 HIV mRNA trimers is warranted in humans for eliciting neutralizing antibodies. Thus, in order to find the maximum safe dose for mRNA/LNP delivery of HIV Env trimers in humans, testing both 100 mcg and 250 mcg will be important. Tolerability at the 250 mcg dose level for these BG505 MD39.3 HIV mRNA trimer vaccines is not yet predictable given that reactogenicity of mRNA vaccines appears to be antigen-dependent and this trial is a first-in-human assessment of these HIV antigens. The enrollment strategy utilizing safety sentinel cohorts for both of the 100 mcg and 250 mcg dose groups will facilitate careful safety monitoring by group following each administration. A non-GLP toxicity study was performed with three administrations of the mRNA1574 vaccines at 30, 60, and 100 mcg dose in rats. The toxicity report is included in the IB and supports the starting dose level of 100 mcg.

For additional information, see the Investigator's Brochure (IB).

2.6 Rationale for Lymph Node Fine Needle Aspiration

A subset of participants (approximately 1/2 of each group at the 100 mcg dose level) will undergo fine-needle aspiration (FNA) of the axillary draining lymph node after each dose of BG505 MG39.3 mRNA vaccines to gain understanding on how the magnitude and kinetics of germinal center (GC) B and T-cell responses may differ between: a) soluble and membrane trimers and b) CD4KO and WT-CD4bs membrane trimers. The analysis of these FNA/GC responses will be compared to FNA/GC responses in NHPs administered the same immunogens, thus allowing for greater understanding of how and whether NHP responses can be used to predict human responses. The FNA/GC responses here will also provide baseline data on human GC responses to trimer mRNA immunization that can be evaluated relative to future adjuvanted protein trimer immunization studies. This same subset will have samples collected for B-cell sorting and sequencing to obtain information on the B cell receptor (BCR) repertoires of the GC and memory B cells induced by these mRNA immunogens, in particular to look for differences in BCR repertoire responses between: a) soluble and membrane trimers and b) CD4KO and WT-CD4bs membrane trimers.

2.7 Rationale for Leukapheresis

This protocol will employ leukapheresis also in approximately 1/2 of each group at the 100 mcg dose level to ensure adequate specimens for analysis of rare precursors. Leukapheresis will be performed after the second and third doses of BG505 MG39.3 mRNA vaccines have been administered. The rationale for performing leukapheresis two weeks post vaccination is based upon detailed

preclinical investigations into the kinetics of the germinal center response in rhesus macaques (10).

Samples collected through leukapheresis after the second and third vaccine administration will be used for MHC Class II human leukocyte antigen (HLA) typing and CD4+ T-cell epitope mapping to understand the breadth and potency of CD4 epitopes in these trimers. These studies will a) provide insight into how soluble, membrane-bound, and membrane-bound-CD4KO trimers may differ in their induction of CD4+ T-cell responses and b) most importantly, assist design of future sequential immunization regimens that incorporate these trimers to ensure that T-helper epitopes that are cross-reactive over vaccine recipients and common MHC alleles are shared among different immunogens in the regimen.

To assess the expansion of antigen specific precursor B cell populations and CD4+ T-cell repertoire large numbers of peripheral blood mononuclear cells (PBMCs) are required. There are approximately 1 million PBMCs per milliliter of blood and therefore peripheral blood sampling at volumes associated with typical blood donation (~500 mL) would not provide sufficient PBMC numbers. Leukapheresis can provide 6-10 billion PBMCs without depleting red blood cells or other blood components.

2.8 Risks and benefits

2.8.1 Potential risks

While other mRNA vaccines have been evaluated in humans, this is the first study in humans of the MD39.3 mRNA HIV trimer vaccines. The nature of the antigen may affect the safety and tolerability profile of the vaccine. Therefore, most of the risks noted are based on risks of vaccines in general.

On May 25, 2022, study enrollments and vaccinations were paused due to the occurrence of delayed urticarial rash with dermatographism in two participants (out of 106 participants enrolled at that point). The first event was reported by a 42 year-old male participant with a history of seasonal allergies. The onset of the rash was 10 days after receiving dose 1 of BG505 MD39.3gp151 mRNA at 100 mcg (Group 2). The rash was erythematous, pruritic, and diffuse, but without mucosal involvement or respiratory symptoms. Dermatographism was also observed. The event was assessed as related to the investigational product and assigned grade 2 in severity by the investigator. The participant was treated with oral and topical antihistamines. As of June 8, 2022 (approximately 6 weeks after onset), the event was improved but not resolved. The second event was reported by a 39 year-old male participant who developed diffuse pruritis and urticaria approximately 10-17 days after receiving dose 1 of BG505 MD39.3 mRNA at 100 mcg (Group 1). This rash initially was attributed to an environmental exposure and improved with oral antihistamines with residual mild pruritus. On May 19, 2022, six days after the second dose of study vaccine, the participant

developed worsening diffuse pruritis with urticaria and dermatographism. No mucosal involvement or shortness of breath was reported. This participant later developed scrotal edema following recreational activity and presented to an emergency room where he was treated with intravenous and oral steroids and antihistamines. The event was assessed as related to the investigational product and assigned grade 2 in severity by the investigator. As of June 8, 2022, the event was improved but not resolved. Vaccinations were permanently discontinued for both of these participants.

After extensive reviews and further follow up on these 2 events, the vaccination pause for enrolled participants was lifted on June 16, 2022 with participants being informed and re-consented. Subsequently, the protocol and protocol informed consent were amended and the enrollment pause was lifted on July 21, 2022. Enrollment in the trial was completed on August 9, 2022.

Update of urticaria events as of May 23, 2023: The 2 participants with urticaria events described above still have some symptoms, although they have improved over time with less symptom frequency and are using less medication. Since the initial report regarding these 2 participants, 5 additional participants developed mild or moderate hives after receiving their first or second study vaccinations. One participant developed hives about 10 hours after getting the vaccination which went away by the next day without treatment. Four participants developed hives 1-3 weeks after their study vaccination and have required medication to control outbreaks since then. Two participants received additional doses of study vaccine and did not experience a worsening of their initial symptoms. None of the reported reactions included symptoms of anaphylaxis, and all reactions were assessed as mild or moderate in severity.

Overall, urticaria symptoms resolved for 3 of 7 participants and have improved over time with and without medication for the other 4 participants.

Delayed-onset urticarial reactions (occurring >4 hours post-vaccination) have been observed following COVID-19 vaccination, both mRNA and other platforms, and differ distinctly from immediate-onset (<4 hours) Type I hypersensitivity reactions and are not associated with anaphylaxis (27-29). The timing of onset may vary from <1 day to 21 days post-vaccination and the time to resolution may vary from 24 hours to 6 weeks (27-30). Onset is more often reported following the first vaccination and may occur at later doses. It is common for subsequent vaccinations to be received without recurrence of urticarial reactions. Treatment is typically with H1-blocking antihistamines and may also include H2-blocking antihistamines; corticosteroids have also been used. The prevalence of symptomatic dermatographism in the general population is not well known, but is a fraction of those with simple dermatographism (prevalence 1.5-6% of the general population) (31, 32). Urticaria with dermatographism arising anew following receipt of COVID-19 mRNA vaccines has been reported rarely in the literature (32) yet has been observed by practicing

allergy/immunology specialists. Receipt of subsequent COVID-19 mRNA vaccinations was not contra-indicated for these cases, and refractory urticaria with dermatographism (persistent symptoms despite above standard doses of antihistamines) has been treated successfully with omalizumab (32). It is important to note, however, that causality of these events has not been established with respect to vaccines, and a comparative analysis of these events occurring in the background over a similar timeframe has not been done.

Frequent potential side effects resulting from other intramuscular (vaccines) and subcutaneous (local anesthetic prior to FNA) injections include stinging, discomfort, redness and/or swelling of skin, itching, or mild bruising at injection site.

Side effects of varying frequency from other intramuscular vaccines include: headache, fever, chills, rash, aches and pains, nausea, dizziness and fatigue. If these occur, they will be monitored, but with other vaccines, these effects are generally short term, mild to moderate in severity, and usually do not require treatment. Less frequently, severe symptoms that temporarily prevent normal activities or require treatment may occur with some vaccines. Side effects from some vaccines may be more intense or more frequent following a subsequent administration of the vaccine.

Rare risks with any injection procedure include infection at the site of injection. Signs of infection at the injection site include severe pain, erythema, induration, warmth or drainage. There are rare reports of anaphylaxis with many vaccines. There may be side effects from the study products which may be serious or life threatening that we do not know about yet.

If antibodies elicited by the study vaccine result in a positive or indeterminate HIV test (ie, vaccine-induced seropositivity or VISP), this may have a negative impact on employment, health care, insurance, travel, and personal relationships. HIV polymerase chain reaction (PCR) will be used to exclude or confirm HIV infection.

Blood drawing may cause pain, bruising, fainting, and, rarely, infection at the site where the blood is taken. Leukapheresis can also cause pain, bruising, and, rarely infection. Additional risks may depend on local leukapheresis procedures. Fainting can also occur, but occurs less frequently than with blood drawing.

Fine needle aspiration of lymph nodes can uncommonly cause pain, bruising, and/or bleeding at the site where the needle is inserted. Similar to any procedure where a needle is inserted into the skin (such as vaccination, as described above), there is a rare risk of localized infection. The local anesthetic rarely can cause temporary arm numbness or weakness.

Risks during Pregnancy: Because possible effects of the study vaccine on a fetus or nursing infant are unknown, persons assigned female sex at birth who have

reproductive potential will be tested for pregnancy at screening and prior to administration of each dose of study vaccine. Such persons will be asked to notify the site immediately if they suspect or learn they are pregnant during this study. In case of pregnancy, participants will continue to be followed for safety and the participant will not receive any additional vaccinations. The participant will be contacted about the outcome of a pregnancy that begins during the study.

Other Risks: The medical tests performed as part of this research protocol may result in new diagnoses or abnormal values without clinical significance. Depending on the medical findings and consequences of being provided with the results of these tests, the study participant may view this as either a risk or a benefit. Any such information will be shared and discussed with the participant and, if requested by the participant, may be forwarded to the primary health care provider for further workup and management.

Participants in this study risk experiencing discrimination or other personal problems that may result from study participation itself: these are known collectively as negative social impacts. The HVTN clinical research site (CRS) is obliged to provide advocacy for and assistance to participants regarding these negative social impacts associated with the vaccine trial. If HVTN CRS staff have questions regarding ways to assist a participant dealing with a negative social impact, a designated HVTN Core representative can be contacted.

2.8.2 Myocarditis and Pericarditis with mRNA COVID-19 vaccines

In the general population, myocarditis is diagnosed in approximately 10 to 20 individuals per 100,000 per year, occurs more commonly and at younger ages in persons assigned male sex at birth compared with those assigned female, and may be caused by various bacterial, viral and other infectious agents, toxins, drugs, allergens, and autoimmune conditions (33-35). Increased cases of myocarditis and pericarditis have been reported after mRNA COVID-19 vaccination (Pfizer-BioNTech and Moderna), predominantly in adolescents and young adults 16 to 29 years of age assigned male sex at birth; however, cases have also been reported in older persons of both sexes (36). Onset was typically within several days after mRNA COVID-19 vaccination, and cases have occurred more often after the second dose than the first dose.

In most of the reported cases, individuals who presented for medical care have responded well to medications (nonsteroidal anti-inflammatory agents, glucocorticoids, or intravenous immunoglobin) and rest with prompt improvement of symptoms, although some cases have been severe. Follow-up data is limited, and it is not yet known whether the risk of myocarditis or pericarditis remains increased following additional doses of the mRNA COVID-19 vaccine. CDC and its partners are continuing to investigate these reports of myocarditis and pericarditis following mRNA COVID-19 vaccination.

While the pathophysiology of SARS-CoV-2 infection on the cardiovascular system and the association with myocarditis, pericarditis, myocardial infarction, thromboembolic disease has received intense scrutiny (33, 37, 38), the underlying pathophysiology linking COVID-19 mRNA vaccines to cardiac disease is not well understood (27). It is not known whether the risk of myocarditis or pericarditis is increased following other mRNA vaccines. Given this, study participants will be instructed to seek medical attention and to notify study site staff if symptoms of myocarditis and/or pericarditis occur following vaccination, and include chest pain, shortness of breath, palpitations [a fluttering sensation, fast heartbeat, pounding heart, or irregular heartbeat], or syncope.

2.8.3 Benefits

Study participants will not receive direct health benefit from study participation. The public may benefit from knowledge gained in this study that may aid in the future development of an HIV vaccine. The investigational vaccines are not expected to provide protection from HIV infection.

Participants may benefit from more frequent counseling, laboratory tests and physical exams while enrolled in the study. Participants may also experience positive social impacts as a benefit of study participation. When asked, participants from previous HVTN studies say that being in a study made them feel good about helping others, increased their knowledge about HIV, and improved their self-esteem.

2.9 Assessment of immunogenicity endpoints

The primary immunogenicity endpoint assay will measure the occurrence and magnitude of serum antibody neutralization of pseudoviruses (BG505 MD39.3, BG505 MD39.3 gp151, and BG505 MD39.3 gp151 CD4KO) by the TZM-bl assay two weeks after the third vaccination. Occurrence and magnitude of serum IgG binding antibodies to BG505 trimer, and specific epitopes (base of trimer, V3, internal epitope) will be measured by ELISA and/or BAMA two weeks after the third vaccination. Response rate and magnitude of CD4+ T cell responses will be assessed by intracellular cytokine staining assays (ICS) two weeks after the last vaccination.

Lymphocytes from an axillary lymph node obtained by FNA of the node will be examined for a germinal center activity and induction and maturation of germinal center B cells 3-6 weeks after vaccination.

Additional assays may be performed on selected responders and at additional timepoints at the discretion of Protocol Team leadership based on its evaluation of primary results following the third vaccination (see Section 3.1).

3 Objectives and endpoints

3.1 Primary objectives and endpoints

Primary objective 1:

To evaluate the safety and tolerability of BG505 MD39.3 trimer mRNA vaccines in healthy, HIV-uninfected adults

Primary endpoint 1:

Local and systemic reactogenicity signs and symptoms for a minimum of seven days following receipt of any study product. Laboratory measures of safety. All adverse events (AEs) for thirty days after receipt of study vaccination

All serious adverse events (SAEs), medically attended adverse events (MAAEs), adverse events of special interest (AESIs), and AEs leading to early participant withdrawal or permanent discontinuation will be collected throughout the study

Primary objective 2:

To evaluate the ability of BG505 MD39.3 trimer mRNA vaccines to elicit autologous neutralizing antibodies and to compare responses between the membrane-bound vaccine regimen (BG505 MD39.3 gp151 mRNA) and the membrane-bound knockout (BG505 MD39.3 gp151 CD4KO mRNA) and between the membrane-bound vaccine regimen (BG505 MD39.3 gp151 mRNA) and the soluble trimer (BG505 MD39.3 mRNA) vaccine arms, within each dose level

Primary endpoint 2:

Occurrence, magnitude, and response rate of serum antibody neutralization of a vaccine-matched tier 2 HIV-1 strain as measured by the TZM-bl assay two weeks after the third vaccination

3.2 Secondary objectives and endpoints

Secondary objective 1:

To evaluate the ability of BG505 MD39.3 trimer mRNA vaccines to elicit humoral and cellular immune responses and to compare responses between the membrane-bound vaccine regimen (BG505 MD39.3 gp151 mRNA) and the membrane-bound knockout (BG505 MD39.3 gp151 CD4KO mRNA) and between the membrane-bound vaccine regimen (BG505 MD39.3 gp151 mRNA) and the soluble trimer (BG505 MD39.3 mRNA) vaccine arms, within each dose level and to compare responses between dose levels for each vaccine regimen

Secondary endpoints 1:

- Occurrence, magnitude, and response rate of serum antibody neutralization of a vaccine-matched tier 2 HIV-1 strain as measured by the TZM-bl assay two weeks after the second vaccination

- Occurrence, magnitude, and response rate of serum IgG binding antibodies to the BG505 trimer, and specific epitopes (base of trimer, V3, internal epitope) as measured by binding antibody multiplex assay (BAMA) two weeks after the second and third vaccination

- Occurrence, magnitude, and response rate of CD4+ T-cell responses as assessed by intracellular cytokine staining assays (ICS) two weeks after the third vaccination

Secondary objective 2:

To evaluate the durability of humoral immune responses to BG505 MD39.3 trimer mRNA vaccines and to compare durability between arms

Secondary endpoints 2:

- Occurrence, magnitude, and response rate of serum antibody neutralization of a vaccine-matched tier 2 HIV-1 strain, as measured by the TZM-bl assay six months after the third vaccination

- Occurrence, magnitude, and response rate of serum IgG binding antibodies to the BG505 trimer, and specific epitopes (base of trimer, V3, internal epitope), as measured by BAMA six months after the third vaccination

3.3 Exploratory objectives

Exploratory objective 1

To determine whether higher doses of BG505 MD39.3 trimer mRNA vaccines induce stronger and/or more consistent humoral and/or cellular immune responses

Exploratory objective 2

To evaluate the epitope specificity of autologous (BG505/T332N) neutralization

Exploratory objective 3

To evaluate serum antibody specificities and elicitation of trimer degrading antibodies by electron microscopy-based polyclonal epitope mapping (EMPEM)

Exploratory objective 4

To evaluate the magnitude and kinetics of BG505-specific GC B and T-cell responses

Exploratory objective 5

To evaluate BCR repertoires and sequences of the BG505-specific GC and memory B cells

Exploratory objective 6

To evaluate CD4+ T-cell mapping and HLA typing by BG505 MD39.3 trimer mRNA vaccines in a subset of participants

Exploratory objective 7

To evaluate the ability of the vaccine regimen to elicit HIV-1 specific heterologous tier 2 neutralizing antibodies

Exploratory objective 8

To characterize monoclonal antibodies derived from BCR sequences from BG505-specific B cells

Exploratory objective 9

To characterize antibody avidity and Fc-mediated antibody functions (eg, antibody-dependent cellular phagocytosis [ADCP] and antibody-dependent cellular cytotoxicity [ADCC])

Exploratory objective 10

To evaluate binding antibody responses elicited by BG505 MD39.3 trimer mRNA vaccines as measured via ELISA

Additional exploratory objectives

To conduct analyses related to furthering the understanding of HIV, immunology, vaccines, and clinical trial conduct. To further evaluate immunogenicity of each vaccine regimen, additional immunogenicity assays may be performed in a subset of participants, including on samples from other timepoints, based on the HVTN Laboratory Assay Algorithm.

4 Laboratory strategy

The primary goal of HVTN 302 is to determine whether the BG505 MD39.3 HIV trimer mRNA vaccines can drive the induction of tier 2 serum neutralizing antibody responses. To this end, HVTN immunogenicity assays will be used to evaluate induction of autologous and heterologous neutralization as well as vaccine and epitope-specific binding antibodies. These data will also be used to identify and prioritize samples for in-depth immunogenicity analyses, including epitope-mapping of serum neutralizing antibody responses, and isolation and characterization of neutralizing monoclonal antibodies. Germinal center B cells and T cells will be evaluated in fine needle aspirates from lymph nodes to assess the impact of the different immunogen modalities and modifications (such as aberration of CD4 binding) in the trafficking and retention of antigen in the draining lymph nodes and degree of germinal center activity. The detailed laboratory strategy is described in the Central Assay Plan and will be updated as new reagents and techniques are incorporated into assay planning. This document will be available on the protocol webpage. Descriptions of the standard HVTN laboratory assays can be found online at https://www.hvtn.org/content/dam/hvtn/scientific-programs/hvtn-laboratory-assay-descriptions.pdf.

5 Study design

This is a multicenter, randomized, open-label, Phase 1 study to evaluate the safety and immunogenicity of BG505 MD39.3, BG505 MD39.3 gp151, and BG505 MD39.3 gp151 CD4KO HIV trimer mRNA in healthy adults. The primary hypothesis is BG505 MD39.3 soluble and membrane-bound trimer mRNA vaccines will be safe and well-tolerated among HIV-uninfected individuals and will elicit autologous neutralizing antibodies.

5.1 Study population

All inclusion and exclusion criteria must be met for eligibility. Screening procedures to determine eligibility must be performed within 56 days prior to enrollment.

Investigators should always use good clinical judgment in considering a volunteer's overall fitness for trial participation. Some volunteers may not be appropriate for enrollment even if they meet all inclusion/exclusion criteria. Medical, psychiatric, occupational, or other conditions may make evaluation of safety and/or immunogenicity difficult, and some volunteers may be poor candidates for retention.

5.1.1 Inclusion criteria

1. Able and willing to complete the informed consent process, including an Assessment of Understanding: volunteer demonstrates understanding of this study; completes a questionnaire prior to first vaccination with verbal demonstration of understanding of all questionnaire items answered incorrectly.

2. 18-55 years old, inclusive, on day of enrollment.

3. Agrees to comply with planned study procedures and be available for clinic follow-up through the last clinic visit.

4. Agrees not to enroll in another study of an investigational agent during participation in the trial. If a potential participant is already enrolled in another clinical trial, approvals from the other trial sponsor and the HVTN 302 PSRT are required prior to enrollment into HVTN 302.

5. In good general health according to the clinical judgement of the site investigator.

6. Physical examination and laboratory results without clinically significant findings that would interfere with assessment of safety or reactogenicity in the clinical judgement of the site investigator.

7. Assessed as low risk for HIV acquisition per low risk guidelines (see Appendix H), agrees to discuss HIV infection risks, agrees to risk reduction counseling, and agrees to avoid behavior associated with high risk of HIV exposure through the final study visit. Low risk may include persons stably taking pre-exposure prophylaxis (PrEP) as prescribed for 6 months or longer.

8. Hemoglobin

    - ≥ 11.0 g/dL for volunteers who were assigned female sex at birth
    - ≥ 13.0 g/dL for volunteers who were assigned male sex at birth and transgender males who have been on hormone therapy for more than 6 consecutive months
    - ≥ 12.0 g/dL for transgender females who have been on hormone therapy for more than 6 consecutive months
    - For transgender participants who have been on hormone therapy for less than 6 consecutive months, determine hemoglobin eligibility based on the sex assigned at birth

9. White blood cell (WBC) count > 3,500/mm^3

10. Platelets ≥125,000 /mm^3

11. Alanine aminotransferase (ALT) < 2.5 x upper limit of normal (ULN) based on the institutional normal range

12. Serum creatinine ≤1.1 x ULN based on the institutional normal range

13. Negative results for HIV infection by an (US) Food and Drug Administration (FDA)-approved enzyme immunoassay (EIA) or chemiluminescent microparticle immunoassay (CMIA).

14. Negative for anti-Hepatitis C antibodies (anti-HCV) or negative HCV nucleic acid test (NAT) if anti-HCV antibodies are detected.

15. Negative for Hepatitis B surface antigen.

16. For a volunteer capable of becoming pregnant:

    - Volunteers who were assigned female sex at birth and are of reproductive potential must agree to use effective means of birth control from at least 21 days prior to enrollment through 3 months after their third vaccination timepoint (see Appendix I)

- Has negative β-HCG (beta human chorionic gonadotropin) pregnancy test (urine or serum) at screening and prior to study product administration on day of enrollment.

5.1.2 Exclusion criteria

1. Volunteer who is breast-feeding or pregnant.

2. Hypertension that is not well controlled. If a person has been found to have elevated blood pressure or hypertension during screening or previously, exclude for blood pressure that is not well controlled. Well-controlled blood pressure is defined in this protocol as consistently < 140 mm Hg systolic and < 90 mm Hg diastolic, with or without medication, with only isolated, brief instances of higher readings, which must be ≤ 150 mm Hg systolic and ≤ 100 mm Hg diastolic. For these volunteers, blood pressure must be < 140 mm Hg systolic and < 90 mm Hg diastolic at enrollment. If a person has NOT been found to have elevated blood pressure or hypertension during screening or previously, exclude for systolic blood pressure ≥ 150 mm Hg at enrollment or diastolic blood pressure ≥ 100 mm Hg at enrollment.

3. Diabetes mellitus type 1 or type 2. (Not exclusionary: type 2 cases controlled with diet alone or a history of isolated gestational diabetes).

4. Previous or current recipient of an investigational HIV vaccine (previous placebo recipients are not excluded).

5. Acutely ill or febrile (temperature ≥ 38.0°C/100.4°F) on the day of the first vaccination. Participants meeting this criterion may be rescheduled within the enrollment window period. Afebrile participants with minor illnesses can be enrolled at the discretion of the Investigator.

6. Immunosuppressive medications received within 168 days before first vaccination (Not exclusionary: [1] corticosteroid nasal spray; [2] inhaled corticosteroids; [3] topical corticosteroids for mild, uncomplicated dermatologic condition; or [4] a single course of oral/parenteral prednisone or equivalent at doses ≤ 60 mg/day and length of therapy < 11 days with completion at least 30 days prior to enrollment).

7. Blood products or immunoglobulin within 16 weeks prior to enrollment; receipt of immunoglobulin within 16 weeks prior to enrollment requires PSRT approval.

8. Receipt of any of the following:

    - Within 4 weeks prior to enrollment:
        - Any licensed live, attenuated vaccine

- Any mRNA-based SARS-CoV-2 vaccine with FDA licensure, FDA emergency use authorization (EUA), or World Health Organization (WHO) emergency use listing (EUL)

- Within 2 weeks prior to enrollment:
  - Any licensed killed/subunit/inactivated vaccine
  - Any adenoviral-vectored or protein SARS-CoV-2 vaccine with FDA licensure, FDA EUA, or WHO EUL

Receipt of any SARS-CoV-2 vaccination series should be completed 4 weeks prior to enrollment when possible; however, exceptions may be made by approval of the HVTN 302 PSRT.

9. Initiation of antigen-based immunotherapy for allergies within the previous year (stable immunotherapy is not exclusionary); inclusion of participants who initiated immunotherapy within the previous year requires PSRT approval.

10. Receipt of investigational research agents with a half-life of 7 or fewer days within 4 weeks prior to enrollment. If a potential participant has received investigational agents with a half-life greater than 7 days (or unknown half-life) within the past year, PSRT approval is required for enrollment.

11. History of serious reaction (eg, hypersensitivity, anaphylaxis) to any vaccine or any component of the study vaccine.

12. History of myocarditis and/or pericarditis.

13. Hereditary angioedema, acquired angioedema, or idiopathic forms of angioedema.

14. Idiopathic urticaria within the past year.

15. Bleeding disorder diagnosed by a doctor (eg, factor deficiency, coagulopathy, or platelet disorder requiring special precautions).

16. Seizure disorder; febrile seizures as a child or seizures secondary to alcohol withdrawal more than 5 years ago are not exclusionary.

17. Asplenia or functional asplenia.

18. Active duty and reserve US military personnel.

19. Any other chronic or clinically significant condition that in the clinical judgement of the investigator would jeopardize the safety or rights of the study participant, including, but not limited to: clinically significant forms of drug or alcohol abuse, serious psychiatric disorders, or cancer that, in the clinical

judgement of the site investigator, has a potential for recurrence (excluding basal cell carcinoma).

20. Asthma is excluded if the participant has ANY of the following:

    - Required either oral or parenteral corticosteroids for an exacerbation two or more times within the past year; OR

    - Needed emergency care, urgent care, hospitalization, or intubation for an acute asthma exacerbation within the past year (eg, would NOT exclude individuals with asthma who meet all other criteria but sought urgent/emergent care solely for asthma medication refills or co-existing conditions unrelated to asthma); OR

    - Uses a short-acting rescue inhaler more than 2 days/week for acute asthma symptoms (ie, not for preventive treatment prior to athletic activity); OR

    - Uses medium-to-high-dose inhaled corticosteroids (greater than 250 mcg fluticasone or therapeutic equivalent per day), whether in single-therapy or dual-therapy inhalers (ie, with a long-acting beta agonist [LABA]); OR

    - Uses more than one medication for maintenance therapy daily. Inclusion of anyone on a stable dose of more than one medication for maintenance therapy daily for greater than two years requires PSRT approval.

21. A participant with a history of an immune-mediated disease, either active or remote. Not exclusionary: 1) remote history of Bell's palsy (>2 years ago) not associated with other neurologic symptoms, 2) mild psoriasis that does not require ongoing systemic treatment.

22. Immunodeficiency

5.1.3 Additional eligibility criteria for subset undergoing FNA and/or leukapheresis procedures

1. Meeting local site requirements related to these procedures (eg, any specific hemoglobin level requirements by pheresis center)

2. No concern by site investigator for difficulty with venous access based upon clinical history and physical examination. For example, history of IV drug abuse or substantial difficulty with previous blood draws.

3. Willing to undergo lymph node FNA three times and/or leukapheresis twice per schedule of procedures.

4. Participant may not be taking warfarin, oral antithrombin equivalents (including, but not limited to, apixiban, rivaroxiban, dabigatran), enoxaparin injections, or

other medications that would increase the risk of bleeding as assessed by the site investigator.

5. No history of allergy to local anesthetic (eg, Novocaine, Lidocaine).

6. No evidence of localized condition that would pose a contraindication to performance of axillary FNA of the draining lymph nodes ipsilateral to vaccination site as assessed by the site investigator. Note that FNA will only be performed following injections in the deltoid; in the event that the thigh is the injection site location, FNA will not be performed.

5.2 Participant departure from vaccination schedule or withdrawal

5.2.1 Delaying vaccinations for a participant

Under certain circumstances, a participant's scheduled vaccination will be delayed. The factors to be considered in such a decision include but are not limited to the following:

- Intercurrent illness or pre-vaccination abnormal vital signs or clinical symptoms that may mask assessment of vaccine reaction

- Treatment with systemic glucocorticoids (eg, prednisone or other glucocorticoid), immune targeting monoclonal antibodies or other immunomodulators (other than nonsteroidal anti-inflammatory drugs [NSAIDs]), with the exception that study injection may continue per principal investigator (PI) discretion if the next study injection occurs at least 2 weeks following completion of glucocorticoid treatment;

- Receipt of any live attenuated vaccines within 4 weeks prior to study vaccine administration

- Receipt of any inactivated vaccines within 2 weeks prior to study vaccine administration with exception as below:

- Timing of study vaccination with respect to SARS-CoV-2 vaccination:

    - After administration of SARS-CoV-2 vaccination, a minimum of 4 weeks is required before administration of study vaccine

    - Before administration of SARS-CoV-2 vaccine, a minimum of 4 weeks is recommended after administration of study vaccine

- Timing of study vaccination with respect to monkeypox vaccination:

    - JYNNEOS vaccine:

- After administration of JYNNEOS vaccine, a minimum of 4 weeks is required before administration of study vaccine
- Before administration of JYNNEOS vaccine, a minimum of 2 weeks is recommended after administration of study vaccine

- ACAM2000 vaccine:
  - After administration of ACAM2000 vaccine, a minimum of 4 weeks is required before administration of study vaccine if vaccination scab is no longer present
  - If vaccination scab is still present at 4 weeks after receiving ACAM2000, delay study vaccination until the scab is no longer present
  - Before administration of ACAM2000 vaccine, a minimum of 4 weeks is recommended after administration of study vaccine

Vaccinations should not be administered outside the visit window period specified in Appendix D and Appendix E without PSRT approval.

Where not otherwise specified above, participants should be counseled to also avoid the above products in the interval between a study vaccination and completion of the 2-week postvaccination follow-up visit, whenever possible. However, the health and welfare of participants will be prioritized, so there is no need to delay any of the licensed or authorized vaccines or systemic glucocorticoids after the study vaccine, if necessary.

5.2.2 Discontinuation of study vaccine administration

Under certain circumstances, an individual participant's vaccinations will be permanently discontinued. Specific events that will result in stopping a participant's vaccination schedule include:

- SAE that is subsequently considered to be related to vaccination
- Pregnancy (regardless of outcome)
- HIV infection
- Grade 3 AE assessed as related to study vaccine, with the following exceptions:
  - Grade 3 subjective reactogenicity and injection site reactions: injection site pain/tenderness and erythema/induration (grade 3 by size only), fatigue, generalized myalgia, generalized arthralgia, chills, headache, nausea (unless IV rehydration required)

- AEs reviewed by the PSRT and approved for vaccination continuation

- Grade 4 AE assessed as related to study vaccine

- Clinically significant type 1 hypersensitivity associated with study vaccine

For ease of reference and review, the clinically significant type 1 hypersensitivity definition, as per the Second National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network symposium criteria for anaphylaxis (39), is provided below:

Anaphylaxis is highly likely when any <u>one</u> of the following criteria are fulfilled:

1 Acute onset of an illness (minutes to several hours) with involvement of the skin, mucosal tissue, or both (eg, generalized hives, pruritus or flushing, swollen lips-tongue-uvula)

AND AT LEAST ONE OF THE FOLLOWING:

a. Respiratory compromise (eg, dyspnea, wheeze-bronchospasm, stridor, reduced peak expiratory flow(PEF), hypoxemia)

b. Reduced blood pressure (BP) or associated symptoms of end-organ dysfunction (eg, hypotonia [collapse], syncope, incontinence)

2 Two or more of the following that occur rapidly after exposure to a likely allergen for that patient [participant] (minutes to several hours):

a. Involvement of the skin-mucosal tissue (eg, generalized hives, itch-flush, swollen lips-tongue-uvula)

b. Respiratory compromise (eg, dyspnea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia)

c. Reduced BP or associated symptoms (eg, hypotonia [collapse], syncope, incontinence)

d. Persistent gastrointestinal symptoms (eg, crampy abdominal pain, vomiting)

3 Reduced BP after exposure to known allergen for that patient [participant] (minutes to several hours). Adults: systolic BP of less than 90 mm Hg or greater than 30% decrease from that person’s baseline

- PI assessment that it is not in the best interest of the participant to continue receiving study vaccine.

- Co-enrollment in a study with an investigational research agent (rare exceptions allowing for the continuation of vaccinations may be granted with the unanimous consent of the HVTN 302 PSRT)

Participants discontinuing study vaccine for reasons other than HIV infection should be encouraged to participate in follow-up visits and procedures per the protocol. At the discretion of the CRS clinician and the PSRT (for composition of PSRT see Section 9.4), some clinic procedures and sample collections may be modified or discontinued.

If a participant becomes HIV-infected during the course of the study, no additional study vaccine will be administered. Participants will be encouraged to continue until the final scheduled study visit. At post-infection follow-up visits, only samples for protocol-specified clinical labs (with the exception of HIV diagnostic testing) will be collected. In addition, some clinic procedures may be modified or discontinued.

5.2.3 Participant departure from vaccine schedule

If a participant misses a scheduled vaccination, they are eligible for future vaccinations.

5.2.4 Discontinuation of study participation

A participant may be discontinued from protocol participation for the following reasons:

- Participant voluntarily withdraws;

- CRS determines the participant is lost to follow-up;

- The investigational new drug (IND) application Sponsor or regulatory authorities stop the study; or,

- PI assessment that it is not in the best interest of the participant to continue participation in the study or that the participant's compliance with the study is not sufficient.

If a participant terminates participation in the study early for any reason, the site principal investigator should consider if the following assessments are appropriate: end-of-study HIV test, complete blood count (CBC) with differential, serum chemistry (alanine aminotransferase (ALT) and creatinine), physical examination, and if indicated, a pregnancy test (see Appendix A and Appendix B). For participants with HIV infection, please see Section 8.8. If the

site principal investigator has questions regarding a termination visit, they should consult with the PSRT.

6 Statistical considerations

6.1 Sample size justification and accrual

Recruitment will target enrolling 108 healthy, HIV-uninfected adult participants.

108 healthy, HIV-uninfected adult participants will be enrolled into three 100 mcg vaccine dose groups in Part A and into three 250 mcg vaccine dose groups in Part B (n=18 per group). The study will begin by enrolling a Part A sentinel safety cohort with 1:1:1 randomization into Groups 1-3 (of n=12) to be evaluated for safety two weeks after the first vaccination. Participants that are randomized but not enrolled will be replaced to ensure that at least 12 participants are enrolled before initiation of the planned enrollment pause.

The PSRT will convene after the 12th participant in the Part A sentinel safety cohort has received their first vaccination and completed the 2 week post vaccination safety visit to determine if further participants should be enrolled. If safety criteria are met, enrollment will proceed with Part B sentinel group of n=12 with 1:1:1 randomization and the remainder of Part A (n=42) with 1:1:1 randomization. Once the safety criteria for the Part B sentinel safety cohort have been met, the remaining Part B participants (n=42) will be enrolled with 1:1:1 randomization. Figure 6-1 illustrates this plan.

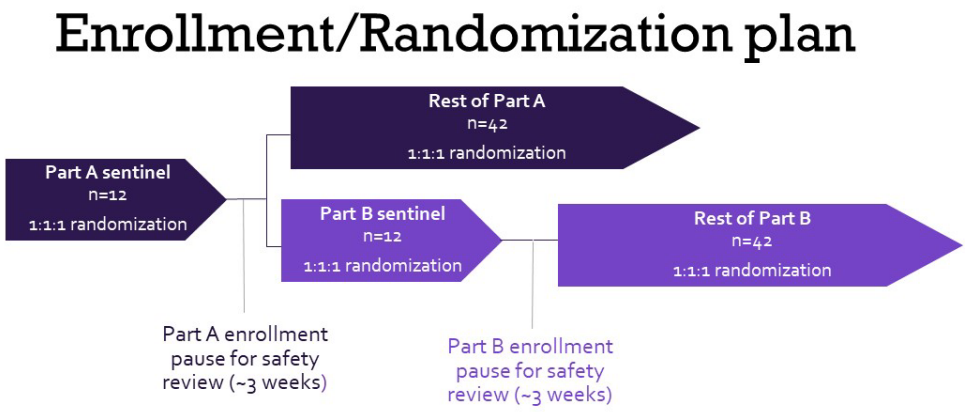


Figure 6-1 Schema of enrollment strategy

Since enrollment is concurrent with receiving the first study vaccination, all participants will provide some safety data. However, for immunogenicity analyses, it is possible that data may be missing for various reasons, such as participants terminating from the study early, problems in shipping specimens, low cell viability of processed peripheral blood mononuclear cells (PBMCs), or high assay background. Immunogenicity data from 17 phase 1 and 2 phase 2a HVTN vaccine trials, which began enrolling after June 2005 (data as of

September 2014), indicate that 15% is a reasonable estimate for the rate of missing data at Month 6.5. For this reason, the sample size calculations in below account for 15% enrolled participants having missing data for the primary immunogenicity endpoints.

6.1.1 Power calculations for immunogenicity

The immunogenicity sample size calculations allow for a 15% rate of missing immunogenicity data at Month 6.5. Of the n=18 enrolled per arm, we assume n=15 will be available for analysis.

A primary objective is to evaluate the ability of the BG505 MD39.3 trimer mRNA vaccines to elicit autologous neutralizing antibodies. The primary endpoint will be the occurrence and magnitude of serum antibody neutralization of pseudoviruses (BG505 T332N) as measured by the TZM-bl assay two weeks after the third vaccination which will be compared between the membrane-bound vaccine regimen (BG505 MD39.3 gp151 mRNA) and the membrane-bound knockout (BG505 MD39.3 gp151 CD4KO mRNA) and between the membrane-bound vaccine regimen (BG505 MD39.3 gp151 mRNA) and the soluble trimer (BG505 MD39.3 mRNA) vaccine arms, within each dose level. Secondary analyses will test for differences between dose levels. If no significant difference is seen between the 100mcg and 250mcg dose (eg, between Groups 1 and 4, between Groups 2 and 5 or between Groups 3 and 6), secondary analyses may aggregate over dose groups for comparisons between the vaccine regimens. For example, if no significant difference is seen between doses for the soluble trimer regimen in Groups 1 and 4 or between doses for the membrane-bound vaccine regimen in Groups 2 and 5, we may aggregate over the dose to compare responses of Groups 1 and 4 (n=30) to Groups 2 and 5 (n=30).

The precision with which the true immune response rate can be estimated from the observed data depends on the true underlying response rate and the sample size. Two-sided 95% confidence intervals for the response rate based on observing a particular rate of responses in the vaccinees is shown in Table 6-1. Calculations are done using the score test method (29).

Table 6-1 Two-sided 95% confidence intervals for the true response rate based on observing a particular rate of responses in the vaccinees (n = 15, 30)

No. of responses	Observed response rate (%)	95% Confidence interval
4/15	26.7	(9.0, 51.9)
7/15	46.7	(24.8, 69.9)
11/15	73.3	(48.0, 89.1)
12/15	80.0	(54.8, 93.0)
8/30	26.7	(14.2, 44.4)
15/30	50.0	(33.2, 66.8)
22/30	73.3	(55.6, 85.8)
24/30	80.0	(62.7, 90.5)

As shown in Table 6-2, there is limited power for a formal comparison of immunogenicity response rates between vaccine arms of size n = 15. For either 80% or 90% power, the sizes of differences that the trial is powered to detect are large. These calculations use a Fisher's exact 2-sided test with a Type I error rate of 0.05. The trial is, however, adequately powered to detect group differences based on data from a study in rabbits led by Dr. William Schief. There is 90% power to detect an 11-fold difference in ID50 pseudovirus neutralization and a 1.9-fold difference in serum binding responses between two dose groups or between two dose levels of n=15 evaluable samples each. For the comparisons of BG505 MD39.2 gp151 mRNA vs. BG505 MD39.3 mRNA (or vs. BG505 MD39.3 gp151 CD4KO mRNA) that aggregate arms over the low and high dose levels, the trial has 90% power to detect a 5.2-fold difference in ID50 pseudovirus neutralization and a 1.6-fold difference in serum binding responses between two groups, aggregated over dose level (n=30 evaluable samples in each of the aggregated groups). These calculations use a two-sided t-test with a Type I error rate of 0.05 and estimated standard deviation of 0.85 for log10 ID50 values and of 0.23 for ELISA AUC MD39 at day 182 (shown in Figure 6-2). The standard deviation estimates were based on the mean standard deviation across four groups of rabbits, with n=6 per group, given either soluble or membrane-bound MD39.3 or MD39.2 vaccine at 2 weeks after 3rd vaccination.

Table 6-2 Power for comparison of response rates between 2 arms (n1 = n2 = 15)

True response rate Arm 1 (%)	Minimum true response rate in Arm 2 in order to detect a difference	
	80% power	90% power
10	64	71
20	76	82
30	85	90
40	92	95
50	97	99

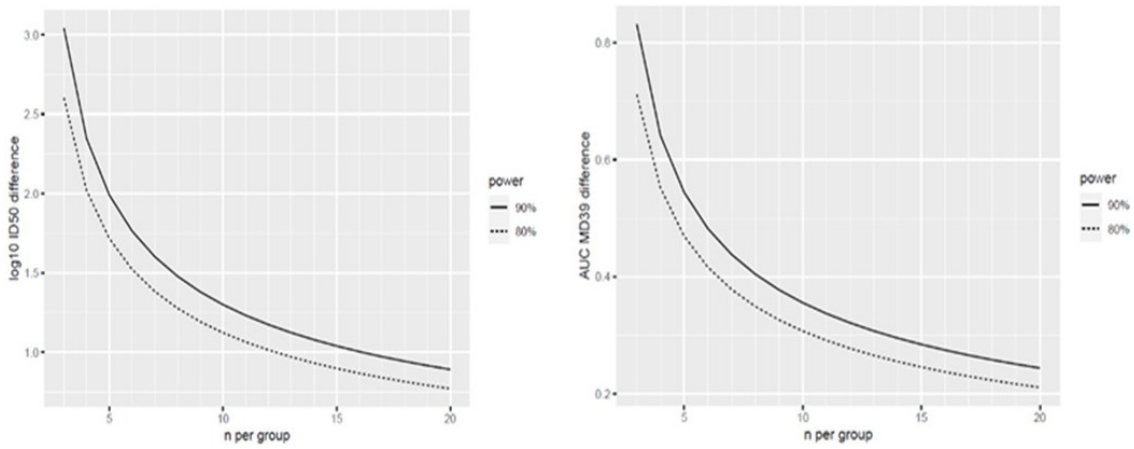


Figure 6-2 Effect size necessary to detect a difference between groups for n=3 to 20 per group. Estimated standard deviation of log10 ID50 values from day 182 is 0.85 (left). Estimated standard deviation of ELISA AUC MD39 is 0.23 (right). The two figures are identical except for the y-axis scale which depends on the outcome-specific estimated standard deviation

In addition, "generic" power calculations inform the power of the study to compare immune response magnitudes between arms. These presume a continuous immune response, transformed to a 1-standard deviation scale, and a mean shift in scaled immune response between arms. Table 6-3 shows the power for comparing immune responses between two arms at Month 6.5 (n = 15 per arm, n = 30 per group). Note that the study is powered to detect moderate 1.1-standard deviation differences in immune responses between arms for n=15 per arm and to detect 0.8- standard deviation differences in immune responses between groups for n=30 per group.

6.1.2 Power calculations for safety

The goal of the safety evaluation for this study is to identify safety concerns associated with vaccine administration. The ability of the study to detect SAEs can be expressed by the true event rate above which at least one SAE would likely be observed and the true event rate below which no events would likely be observed. Specifically, with n=4 in the sentinel safety group for a given arm, there is at least 90% chance of observing at least one safety event among those 4 if the true rate of such an event is 45% or more; and there is at least a 90% chance of observing no events among the 4 if the true rate is 2.5% or less. Safety calculations are evaluated using historical controls. As a reference, in HVTN vaccine trials conducted in the US from April 2008 through March 2018, about 1% of participants who received placebos experienced an SAE.

Table 6-3 Power for comparing the magnitude of a generic immune response between arms. Immune responses are compared using 0.025-level one-sided Wilcoxon rank sum tests. Continuous immune responses are assumed to follow a normal distribution, transformed to a 1-standard deviation scale, with a mean of zero in one arm and the same standard deviation in the two arms. To allow for missing immunogenicity data, analyses include 15 participants in each arm and 30 participants if aggregating over dose level in Part A and Part B. Power is based on 5000 simulations.

Mean difference between arms (in standard-deviation units)	Power, n=15 per arm	Power, n=30 per group (aggregating over dose)
0.8 SD	51%	84%
0.9 SD	63%	91%
1.0 SD	71%	96%
1.1 SD	80%	98%
1.2 SD	86%	100%
1.3 SD	91%	100%

Binomial probabilities of observing 0, 1 or more events among the n=4 sentinel participants receiving each study vaccine are presented in Table 6-4 for a range of possible true adverse event rates. Probabilities are also provided for the total safety sentinel group for each dose group (n=12) as well as for each vaccine arm (n=18). These calculations provide a more complete picture of the sensitivity of this study design to identify potential safety problems with the vaccine.

Table 6-4 Probability of observing 0 events, 1 or more events among arms of n=4, 12, 18 for different true event rates

True event rate (%)	arm size	0 events	1+ events
10	4	0.66	0.34
20	4	0.41	0.59
30	4	0.24	0.76
10	12	0.28	0.72
20	12	0.07	0.93
30	12	0.01	0.99
10	18	0.15	0.85
20	18	0.02	0.98
30	18	0	1

An alternative way of describing the statistical properties of the study design is in terms of the 95% confidence interval for the true rate of an adverse event based on the observed data. Table 6-5 shows the 2-sided 95% confidence intervals for the probability of an event based on a particular observed rate for each arm of the sentinel groups (n=4), for the sentinel group for each dose group (n=12), and for each vaccine arm (n=18). Calculations are done using the score test method for CI described in Agresti and Coull formula 2 (40). If none of the 4 participants receiving the study vaccine in one of the sentinel safety cohort arms experience a safety event, the 95% 2-sided upper confidence bound for the true rate of such events in the total vaccinated population is 49.0%. If none of the 54 participants receiving the vaccine regimen in Part A or B experience a safety event, the 95% 2-sided upper confidence bound for the true rate of such events in the total vaccinated population at that dose level is 6.7%.

Table 6-5 Two-sided 95% confidence intervals for the probability of observing a safety event based on observing a particular rate of safety endpoints in an arm of 4, 12, 18, 54 study participants

Observed event rate	95% Confidence interval (%)
0/4	[0 ; 49.0]
1/4	[4.6 ; 69.9]
2/4	[15.0 ; 85.0]
0/12	[0: 24.3]
1/12	[1.5: 35.4]
2/12	[4.7: 44.8]
0/18	[0: 17.6]
1/18	[1.0: 25.8]
2/18	[3.1: 32.8]
0/54	[0: 6.7]
1/54	[0.3: 9.8]
2/54	[1.0: 12.5]

6.2 Randomization

Participants will first be randomized in a 1:1:1 ratio into the Part A sentinel safety cohort. If safety criteria are met, enrollment will proceed with Part B sentinel group of n=12 with 1:1:1 randomization and the remainder of Part A (n=42) with 1:1:1 randomization, with some sites enrolling into the Part B sentinel cohort while other sites are enrolling the remainder of Part A. Once the safety criteria for the Part B sentinel safety cohort have been met, the remaining Part B participants (n=42) will be enrolled with 1:1:1 randomization.

Randomization to Groups 1-3 will be stratified by willingness to consent to FNA and/or leukapheresis. A maximum of 27 participants in total that do NOT consent to either FNA and leukapheresis collection will be enrolled.

A participant's randomization assignment will be computer generated and provided to the HVTN CRS pharmacist through a web-based randomization system. At each institution, the pharmacist with primary responsibility for dispensing study products is charged with maintaining security of the treatment assignments (except in emergency situations as specified in the HVTN Manual of Operations [MOP]).

6.3 Statistical analyses

This section describes the final study analysis, unblinded as to treatment arm assignment. All data from enrolled participants will be analyzed regardless of how many vaccinations they received. In the rare instance that a participant receives the wrong treatment at a specific vaccination time, the Statistical Analysis Plan (SAP) will address how to analyze the participant's safety and immunogenicity data. Analyses are modified intent-to-treat in that individuals who are randomized but not enrolled do not contribute data and hence are excluded. Because of blinding and the brief length of time between randomization and enrollment—typically no more than 4 working days—very few such individuals are expected.

Analyses for primary endpoints will be performed using SAS and R. All other descriptive and inferential statistical analyses will be performed using SAS, StatXact, or R statistical software.

For the primary and secondary immunogenicity analysis comparisons, the following groups may be pooled: groups 1 and 4, groups 2 and 5, groups 3 and 6. Unpooled group comparisons will also be considered.

No formal multiple comparison adjustments will be employed for multiple safety endpoints, multiple primary immunogenicity endpoints, or secondary endpoints. However, multiplicity adjustments will be made for certain immunogenicity assays, as discussed below, when the assay endpoint is viewed as a collection of hypotheses (eg, testing multiple peptide pools to determine a positive response).

6.3.1 Analysis variables

The analysis variables consist of baseline participant characteristics, safety, and immunogenicity for primary- and secondary-objective analyses.

6.3.2 Baseline comparability

Treatment arms will be compared for baseline participant characteristics using descriptive statistics.

6.3.3 Safety/tolerability Analyses

Since enrollment is concurrent with receiving the first vaccination, all participants will have received at least 1 vaccination and therefore will provide some safety data.

6.3.3.1 Reactogenicity

The number and percentage of participants experiencing each type of reactogenicity sign or symptom will be tabulated by severity and treatment arm and the percentages displayed graphically by arm. For a given sign or symptom, each participant's reactogenicity will be counted once under the maximum severity for all injection visits. In addition, to the individual types of events, the maximum severity of local pain or tenderness, induration or erythema, and of systemic symptoms will be calculated. Kruskal-Wallis tests will be used to test for differences in severity between arms.

6.3.3.2 Adverse events (AEs) and serious adverse events (SAEs)

Adverse events (AEs) will be summarized using Medical Dictionary for Regulatory Activities (MedDRA) System Organ Class and preferred terms. Tables will show by treatment arm the number and percentage of participants experiencing an AE within a System Organ Class or within preferred term category by severity or by relationship to study product. For the calculations in these tables, a participant with multiple AEs within a category will be counted once under the maximum severity or the strongest recorded causal relationship to study product. Formal statistical testing comparing arms is not planned since interpretation of the magnitude of differences must rely heavily upon clinical judgment.

A listing of SAEs reported to the DAIDS Regulatory Support Center (RSC) Safety Office will provide details of the events including severity, relationship to study product, time between onset and last vaccination, and number of vaccinations received.

6.3.3.3 Local laboratory values

Box plots of local laboratory values will be generated for baseline values and for values measured during the course of the study by treatment arm and visit. Each box plot will show the first quartile, the median, and the third quartile. Outliers (values outside the box plot) will also be plotted. If appropriate, horizontal lines representing boundaries for abnormal values will be plotted.

For each local laboratory measure, summary statistics will be presented by treatment arm and timepoint, as well as changes from baseline for postenrollment values. In addition, the number (percentage) of participants with local laboratory values recorded as meeting Grade 1 AE criteria or above as specified in the

Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1 – July 2017, will be tabulated by treatment arm for each postvaccination timepoint. Reportable clinical laboratory abnormalities without an associated clinical diagnosis will also be included in the tabulation of AEs described above.

6.3.3.4 Reasons for vaccination discontinuation and early study termination

The number and percentage of participants who discontinue vaccination and who terminate the study early will be tabulated by reason and treatment arm.

6.3.4 Immunogenicity analyses

6.3.4.1 General approach

For the statistical analysis of immunogenicity endpoints, data from enrolled participants will be used according to the initial randomization assignment regardless of how many injections they received. Additional analyses may be performed, limited to participants who received all scheduled injections per protocol. Assay results that are unreliable are excluded; results from specimens collected outside of the visit window, or from HIV-infected participants post-infection, may be excluded. Since the exact date of HIV infection is unknown, any assay data from blood draws 4 weeks prior to an infected participant's last seronegative sample and thereafter may be excluded. If an HIV-infected participant does not have a seronegative sample postenrollment, then all data from that participant may be excluded from the analysis.

Discrete categorical assay endpoints (eg, response rates) will be analyzed by tabulating the frequency of positive response for each assay by antigen and treatment arm at each timepoint for which an assessment is performed. Crude response rates will be presented with their corresponding 95% confidence interval estimates calculated using the score test method (40). Barnard or Fisher's exact tests, as specified in the SAP, will be used to compare the response rates of any 2 vaccine arms, with a significant difference declared if the 2-sided p-value is $\leq$ 0.05. In general Barnard's is preferred since under most circumstances it is more powerful than Fisher's exact test (41).

For quantitative assay data (eg, percentage of positive cells from ICS assay), graphical and tabular summaries of the distributions by antigen, treatment arm, and timepoint will be made. For all primary and secondary immunogenicity endpoints, box plots and plots of estimated reverse cumulative distribution curves will be used for graphical display of all of the study arms. Typically, the results will be shown for each vaccine arm.

The difference between arms at a specific timepoint will be tested with a nonparametric Wilcoxon rank sum test if the data are not normally distributed and with a 2-sample t-test if the data appear to be normally distributed. The primary

analysis consists of 4 individual tests comparing the membrane-bound vaccine regimen (BG505 MD39.3 gp151 mRNA) to the membrane-bound knockout (BG505 MD39.3 gp151 CD4KO mRNA) and to the soluble trimer (BG505 MD39.3 mRNA) vaccine arms within each dose level, unless prespecified. That is, we compare Groups 2 vs 1, Groups 2 vs 3, Groups 5 vs 4, and Groups 5 vs 6. Secondary analyses will compare responses between dose levels for each regimen. If no differences are seen between dose levels for a given vaccine regimen, subsequent comparisons of that vaccine regimen may be based on the aggregated arms (eg, pooling over dose). If rank-based tests are used, then the tests will be inverted to construct Hodges-Lehmann point estimates and 2-sided (1-0.05/15) × 100% CIs about the differences in location centers of the 3-15 pair-wise comparisons of vaccine arms. If actual-value tests are used then the Dunnett's procedure will be used to construct simultaneous confidence intervals about the pairs of mean differences for the many-to-one comparisons (42) when multiple vaccine arms are each compared with one common control arm. When all pair-wise comparisons between the multiple vaccine arms are of interest, the Tukey procedure (43) will be used. If only specific comparisons between pairs of the multiple vaccine arms are of interest, the Holm-Bonferroni procedure will be used. An appropriate data transformation (eg, $\log_{10}$ transformation) may be applied to better satisfy assumptions of symmetry and homoscedasticity (constant variance). Significance of the differences between pairs will be evaluated using two procedures, first based on whether the simultaneous 95% CIs exclude zero and secondly based on whether the nominal (unadjusted) 95% CIs exclude zero.

Some immunologic assays have underlying continuous or count-type readout that are dichotomized into responder/nonresponder categories (eg, BAMA, TZM-BL). If treatment arm differences for these assays are best summarized by a mixture model, then either Lachenbruch's test statistic (44) or an alternative two-part test (45) (as defined in the SAP) will be used to evaluate the composite null hypothesis of equal response rates in the 2 arms and equal response distributions.

Based upon previous HVTN trials, missing 15% of immunogenicity results for a specific assay is common due to study participants terminating from the study early, problems in shipping specimens, or low cell viability of processed peripheral blood mononuclear cells (PBMCs). To achieve unbiased statistical estimation and inferences with standard methods applied in a complete-case manner (only including participants with observed data in the analysis), missing data need to be missing completely at random (MCAR). Following the most commonly used definition, MCAR assumes that the probability of an observation being missing does not depend on any participant characteristics (observed or unobserved data [ie, the observed data are just a random sample of all the potential data]). When missing data are minimal (specifically if no more than 20% of participants are missing any values), then standard complete-case methods will be used, because violations of the MCAR assumption will have little impact on the estimates and hypothesis tests.

If a substantial amount of immunogenicity data are missing for an endpoint (at least 1 value missing from more than 20% of participants), then using the methods that require the MCAR assumption may give misleading results. In this situation, analyses of the immunogenicity endpoints at a specific timepoint will be performed with methods such as targeted minimum loss-based estimation (TMLE), parametric generalized linear models fit by maximum likelihood, augmented inverse probability weighting (AIPW) methods or multiple imputation methods. TMLE and AIPW are typically preferred because they are doubly robust, providing consistent estimation and appropriate inferences if either the outcome regression or the probability of observing the response value are modeled correctly, under the assumption that the missing data are missing at random (MAR). MAR assumes that the probability of an observation being missing only depends upon the observed responses and upon observed covariates, but not upon any unobserved data. TMLE methods will typically be implemented with the drtmle R package (46) available at CRAN, and will entail specification of a library of learners for the outcome regression and for the probability of observing the response value. When data are not missing at random, sensitivity analyses may be performed as an exploratory analysis. For assessing repeated immunogenicity measurement, linear mixed effects models will be used. If the immunological outcomes are left- and/or right- censored, then the linear mixed effects models of Hughes (47) will be used, because they accommodate the censoring. In addition, secondary analyses of repeated immunogenicity measurements may be done using weighted GEE (48) methods, which are valid under MAR. All of the models described above in this paragraph will include as covariates all available baseline predictors of the missing outcomes.

Some “resource-intensive” immunogenicity endpoints are only measured in subset of participants, eg, fine needle lymph node aspirates and leukapheresis will be performed only on participants consenting to these optional procedures, and immunogenicity endpoints based on these samples will only exist for those participants. For such endpoints, exploratory analyses will be conducted to assess the correlation of participant characteristics measured in (nearly) all participants with the resource-intensive endpoints. For example, if the same assay is performed on blood and fine needle lymph node aspirate samples, then a scatterplot and Spearman rank correlation coefficient (r) will be used to assess the correlation of responses. If at least moderate correlations exist (eg, $r \geq 0.3$), then TMLE is the preferred method estimate the mean of the resource-intensive endpoint for each group and to compare means between groups. Another potential method to apply is the semiparametric locally efficient analysis method of Rotnitzky and Robins (49) as described in Gilbert, Sato et al. for application to vaccine studies (50).

6.3.4.2 Multivariate display of immunogenicity endpoints

Data visualization techniques may be used to explore the relationship among immunogenicity readouts. The set of readouts may be based on one of the primary endpoints (eg, TZM-BL), on the set of primary endpoints, or on immunogenicity

endpoints that also include secondary or exploratory endpoints. To understand the relationship between pairs of readouts, scatter plots may be used when the number of readouts is small or for a larger number of readouts, a heatmap showing the degree of correlation between any two pairs. Principal component analysis (PCA) and associated 'biplots' of the scores and loadings are particularly useful to understand associations between readouts, especially when readouts are correlated (51). PCA is a method to reduce the dimensionality of the number of readouts to a smaller set of values (principal components) that are normalized linear combinations of the readouts in such a way that the first principal component accounts for the most variability in the data and subsequent components, while maximizing variability, are uncorrelated with each other. A 'biplot' displays the first and second principal component scores and principal component loadings. The x-axis is the value from the first principal component and the y-axis is the second principal component, where each axis label includes the percentage of variation in the total set of readouts captured by the principal component. The top axis is the first principal component loadings and the right axis is the second principal component loadings. An arrow is drawn for each immunogenicity readout (eg, Env-specific CD4+ T cell polyfunctionality score, Env-specific CD8+ T cell total magnitude) from the origin to the point defined by its first two principal component loadings. The length of the arrow represents the amount of total variation of the set of readouts captured by the given readout. The direction of an arrow conveys the extent to which the variation of a readout is in the direction of the first or second principal component. The angle between two arrows conveys information about the correlation of the two readouts, with a zero-degree angle denoting perfect correlation and a 90 degree angle denoting no correlation. Each arrow on the biplot is labeled by the immunogenicity readout it represents. A biplot is annotated with key meta-information such as the treatment arm (most common application) or a demographic category. Depending on the application, K-means clustering and hierarchical clustering may also be applied for multivariate graphical display of immunogenicity readouts.

The occurrence and magnitude of serum antibody neutralization of pseudoviruses (BG505 MD39.3, BG505 MD39.3 gp151, and BG505 MD39.3 gp151 CD4KO) measured by the TZM-bl assay, the occurrence and magnitude of serum IgG binding antibodies to BG505 trimer, and specific epitopes (base of trimer, V3, internal epitope) measured by ELISA and/or BAMA, and the response rate and magnitude of CD4+ T-cell responses measured by intracellular cytokine staining assays (ICS) will be summarized using descriptive statistics. Data will be tabulated and graphically displayed by visit number and by number of doses received.

Discrete categorical assay endpoints (eg, response rates) will be analyzed by tabulating the frequency of positive response. Crude response rates will be presented with their corresponding 95% confidence interval estimated based on the score test method (40). Barnard's exact tests may be employed to compare the response rates of immune responses between groups.

Quantitative assay endpoints will be summarized by time point. Nonparametric paired comparisons will be performed using Wilcoxon signed-rank tests, and the Wilcoxon rank sum test will be used to compare independent samples. More details of the analyses of each assay will be provided in the statistical analyses plan (SAP).

6.3.4.3 Analysis of CD4+ and CD8+ T-cell responses as measured by the ICS assay

The analysis of CD4+ and CD8+ T-cell response rates as measured by the ICS assay will be evaluated and compared as described under the general approach. For each T-cell subset, the positivity call for each peptide pool will include a multiple comparison adjustment for the number of peptide pools used in the assay. In general, the Mixture Models for Single-cell Assays (MIMOSA) statistical framework (52) and/or the Fisher's exact test-based positivity criteria will be used. Details of the positivity criteria will be discussed in the SAP. The magnitude of marginal response will be analyzed as described for quantitative data in the general approach section. For each T-cell subset, graphs will be used to display the background-subtracted magnitudes for each participant by protein, treatment arm and timepoint. When 3 or more cytokines are being measured by the ICS assay, the polyfunctionality of ICS responses may also be analyzed as an exploratory endpoint. Besides descriptive plots of the magnitude of polyfunctional responses, the COMPASS (Combinatorial Polyfunctionality analysis of Antigen-Specific T-cell Subsets) statistical framework (53) may also be used to perform joint modelling of multiple T-cell subsets of different cytokine combinations. For example, the functionality score (FS) and the polyfunctionality score (PFS) may be used to summarize the multi-parameter ICS responses.

7 Study vaccine preparation, storage, and administration

7.1 Vaccine Regimen

The schedule of vaccination is shown in Section 1 and additional information is given below.

Group 1

100 mcg of BG505 MD39.3 mRNA (labeled as mRNA-1574v1-GP140) to be administered as 0.5 mL doses intramuscularly (IM) at months 0, 2, and 6

Group 2

100 mcg of BG505 MD39.3 gp151 mRNA (labeled as mRNA-1574v2-GP151) to be administered as 0.5 mL doses intramuscularly (IM) at months 0, 2, and 6

Group 3

100 mcg of BG505 MD39.3 gp151 CD4KO mRNA (labeled as mRNA-1574v3-CD4KO-GP151) to be administered as 0.5 mL doses intramuscularly (IM) at months 0, 2, and 6

Group 4

250 mcg of BG505 MD39.3 mRNA (labeled as mRNA-1574v1-GP140) to be administered as 0.5 mL doses intramuscularly (IM) at months 0, 2, and 6

Group 5

250 mcg of BG505 MD39.3 gp151 mRNA (labeled as mRNA-1574v2-GP151) to be administered as 0.5 mL doses intramuscularly (IM) at months 0, 2, and 6

Group 6

250 mcg of BG505 MD39.3 gp151 CD4KO mRNA (labeled as mRNA-1574v3-CD4KO-GP151) to be administered as 0.5 mL doses intramuscularly (IM) at months 0, 2, and 6

7.2 Study Product Formulation and Storage

7.2.1 BG505 MD39.3 mRNA (labeled as mRNA-1574v1-GP140 Injection) is formulated in a lipid nanoparticle (soluble trimer).

The visual appearance of the formulated product is a white to off-white dispersion which may contain visible, white or translucent product-related particulates. The drug product is supplied as a frozen liquid in a 2 mL borosilicate glass vial. Each vial contains 0.6 mL of mRNA-1574v1-GP140 at a concentration of 0.5 mg/mL and is stored at -60°C to -90°C.

7.2.2 BG505 MD39.3 gp151 mRNA (labeled as mRNA-1574v2-GP151 Injection) formulated in a lipid nanoparticle (membrane-bound trimer).

The visual appearance of the formulated product is a white to off-white dispersion which may contain visible, white or translucent product-related particulates. The drug product is supplied as a frozen liquid in a 2 mL borosilicate glass vial. Each vial contains 0.6 mL of mRNA-1574v2-GP151 at a concentration of 0.5 mg/mL and is stored at -60°C to -90°C.

7.2.3 BG505 MD39.3 gp151 CD4KO mRNA (labeled as mRNA-1574v3-CD4KO-GP151 Injection) formulated in a lipid nanoparticle (membrane-bound trimer)

The visual appearance of the formulated product is a white to off-white dispersion which may contain visible, white or translucent product-related particulates. The drug product is supplied as a frozen liquid in a 2 mL borosilicate glass vial. Each vial contains 0.6 mL of mRNA-1574v3-CD4KO-GP151 at a concentration of 0.5 mg/mL and is stored at -60°C to -90°C.

7.2.4 Diluent (Sodium Chloride Injection, USP, 0.9%)

Sodium Chloride Injection, USP, 0.9% is supplied only in single dose ampules (5 mL and 10 mL Glass Ampules). The diluent is stored at controlled room temperature 15-30°C, avoid freezing.

7.3 Product Preparation

There are 6 mRNA 1574 vaccine Dose Preparation and Administration Worksheets in total, two for each of the three vaccines for the 100 mcg and 250 mcg doses. These worksheets, as well as detailed instructions with respect to their use, can be found in the HVTN 302 Study Specific Procedures (SSP).

Below are general guidelines for the product preparation.

- Two vials of vaccine are required to prepare each dose since the maximum extractable volume is 0.3-0.4mL.

- Pharmacists must follow appropriate aseptic technique and sterile preparation procedures/guidance as outlined in USP <797> [medium risk], utilizing a pharmacy biosafety cabinet/isolator or better. Local regulations and site institutional policies and procedures for use of personal protective equipment, such as gloves, gowns, masks, and safety glasses, must be followed. Pharmacists should follow the requirements of their country, their institution, and their pharmacy regulatory authority regarding these procedures.

- Any unused portion of study product will not be used for another participant. (Exception note: If two participants are scheduled for the 100 mcg dose of the same vaccine on the same day, both doses may be prepared from the mixing vial) Empty vials, unused portion of entered vials, or unused prepared study product should be discarded in a biohazard container and disposed of in accordance with institutional or pharmacy policy.

7.3.1 Preparation of the 100 mcg doses of mRNA-1574 vaccines

- Remove two vials of the appropriate mRNA-1574 vaccine from the -60°C to -90°C freezer and allow to thaw at room temperature for a maximum of 30 minutes.

- Once thawed the product must be diluted within 30 mins using 0.9% sodium chloride for injection, USP.

- Clean the neck of the ampule of 0.9% sodium chloride for injection, USP with an alcohol swab. Grasp the head of the ampule with the alcohol swab and snap the neck open. Dispose of the ampule head and swab in an approved sharps collector.

- Using a filter needle (eg, Becton Dickinson Catalog no. 305211) and 3 mL syringe withdraw 0.9 mL of 0.9% sodium chloride for injection, USP from the ampule. Passively recap the filter needle and remove the filter needle and dispose the filter needle in an approved sharps collector.

- Attach a 21G x1 ½ inch or 22G x1 ½ inch needle to the syringe and inject the 0.9 mL of 0.9% sodium chloride for injection, USP into a 2 mL sterile empty mixing vial.

- Using a 21G x1 ½ inch or 22G x1 ½ inch needle and 1 mL syringe withdraw as much of the contents as possible of one of the mRNA-1574 vaccine vials and inject into the second mRNA-1574 vaccine vial.

- Gently invert vial number 2 of the mRNA-1574 vaccine 20 times until the components are mixed. Do not mix vigorously, sonicate, shake or vortex.

- Using a new 1 mL syringe and new 21G x1 ½ inch or 22G x1 ½ inch needle withdraw 0.6 mL of mRNA -1574 vaccine and inject into the mixing vial containing the 0.9% sodium chloride for injection, USP. Gently invert the mixing vial 20 times. The mixing vial now contains 1.5 mL of a 200 mcg/mL vaccine.

- Using a new 1 mL syringe and a new 21G x1 ½ inch or 22G x1 ½ inch needle withdraw slightly more than 0.5 mL into the dosing syringe. Invert the syringe and vial and remove any air bubble from the dosing syringe. Adjust the volume of solution to 0.5 mL for a dose of 100 mcg and withdraw the needle from the vial.

- Draw back the plunger until air is just visible in the tip of the syringe and remove the needle from the dosing syringe.

- Add either a luer lock cap or an administration needle (22G to 25 G 1 to1 ½ inch) for IM injection as agreed upon by pharmacy and clinic. Label the syringe and assign expiration time of 8 hours after syringe has been filled. It is recommended that the dose is administered immediately after syringe preparation. However, a maximum of 8 hours is allowed for holding dosing solution in dosing syringes prior to completion of administration

- If two participants are scheduled for the 100 mcg dose of the same vaccine on the same day, both doses may be prepared from the mixing vial.

7.3.2 Preparation of the 250 mcg doses of mRNA-1574 vaccine

- Remove two vials of the appropriate mRNA-1574 vaccine from the -60°C to -90°C freezer and allow to thaw at room temperature for a maximum of 30 minutes.

- Using a 21G x1 ½ inch or 22G x1 ½ inch needle and 1 mL syringe withdraw as much of the contents as possible of one of the mRNA-1574 vaccine vials and inject into the second mRNA-1574 vaccine vial. Gently invert vial number 2 of the mRNA-1574 vaccine 20 times until the components are mixed. Do not mix vigorously, sonicate, shake or vortex.

- Using a new 1 mL syringe and a new 21G x1 ½ inch or 22G x1 ½ inch needle withdraw slightly more than 0.5 mL into the dosing syringe. Invert the syringe and vial and remove any air bubble from the dosing syringe. Adjust the volume of solution to 0.5 mL for a dose of 250 mcg and withdraw the needle from the vial.

- Draw back the plunger until air is just visible in the tip of the syringe and remove the needle from the dosing syringe.

- Add either a luer lock cap or an administration needle (22G to 25 G 1 to1 ½ inch) for IM injection as agreed upon between pharmacy and clinic.

- Label the syringe and assign expiration time of 8 hours after syringe has been filled. It is recommended that the dose is administered immediately after syringe preparation. However, a maximum of 8 hours is allowed for holding dosing solution in dosing syringes prior to completion of administration.

7.4 Study product labeling

Label the study product as follows:

- Participant identifier(s)

- Study Product

- Dose 100 mcg OR Dose 250 mcg (depending on group randomization)

- Final volume (0.5 mL)

- Route (IM)

- Beyond use date and time

- Any additional information required by jurisdiction

7.5 Study Vaccine Administration

Study product will be administered as one 0.5 mL injection intramuscularly into the deltoid muscle by needle and syringe.

If the injection cannot be administered in either deltoid muscle, the injection should be administered in separate alternate body site (eg, lateral thigh). The appropriate study staff should document this clearly. Under this circumstance, this is NOT a protocol violation.

When preparing a dose in a syringe and administering the dose, consideration should be given to the volume of solution in the needle before and after the dose is administered. Particularly if the needle used to withdraw the product is replaced prior to vaccine administration, consideration should be given to conserving the full dose of product. The pharmacy and clinic staff members are encouraged to work together to administer the dose specified in the protocol.

7.6 Acquisition of study products

BG505 MD39.3 mRNA (labeled as mRNA-1574v1-GP140), BG505 MD39.3 gp151 mRNA (labeled as mRNA-1574v2-GP151) and BG505 MD39.3 gp151 CD4KO mRNA (labeled as mRNA-1574v3-CD4KO-GP151) were current Good Manufacturing Practice cGMP manufactured by – Moderna, Cambridge MA and will be provided by IAVI (New York, NY, USA). IAVI will also provide 0.9% sodium chloride for injection, USP. These products will be available through the CRPMC.

The following items must be procured locally and will not be available through the NIAID Clinical Research Products Management Center (CRPMC): 2 mL sterile empty mixing vials, 1 mL syringes, 3 mL syringes, 21G x1 ½ inch needles or 22G x1 ½ inch dose preparation needles, filter needles, 21G x1 ½ inch to 25G x1 ½ inch administration needles, sterile luer lock syringe caps.

Once an HVTN CRS is protocol registered, the pharmacist can obtain study products from the NIAID CRPMC by following the ordering procedures given in Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks (https://www.niaid.nih.gov/research/daids-clinical-research-pharmacy-and-study-products-management).

7.7 Study Vaccine Accountability

The HVTN CRS pharmacist is required to maintain complete records of all study products. The pharmacist of record is responsible for maintaining randomization codes and randomization confirmation notices for each participant in a secure manner.

7.8 Final Disposition of study product

For US clinical research sites, all unused study products must be returned to the CRPMC after the study is completed or terminated unless otherwise instructed by the study sponsor. For non-US clinical research sites, all unused study products must be destroyed after the study is completed or terminated unless otherwise instructed by the study sponsor. The procedures are included in the Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks.

8 Clinical procedures

8.1 Screening

Screening for eligibility will be performed after informed consent has been obtained and properly documented before enrollment. Screening evaluations and sample collection include medical history review, physical exam, and any clinical laboratory tests as detailed in the Schedule of procedures (Appendix A and Appendix B) needed to confirm eligibility. Persons assigned female sex at birth who are of reproductive potential will be given a pregnancy test. Additional assessments of health may be conducted at screening based on clinical judgment.

An Assessment of Understanding (AoU) will be completed prior to enrollment. Records will be kept documenting the reason that screened participants did not enroll.

8.2 Definition of Study Day and Study Visit

Study Day 1 is defined as the day of the first vaccination. A study visit may be conducted remotely, such as via phone, text message, email, or other electronic means, in lieu of, or in combination with, in-person visits. As long as they are completed within the visit window (see Appendix D and Appendix E), procedures for a study visit can be completed over multiple days.

8.3 Reactogenicity Assessments

Pre-Vaccine Administration: Medical history and evaluations including vital signs and planned injection site evaluation are performed prior to each vaccine administration.

Post-Vaccine Administration Follow-Up in Clinic: Following each vaccine administration, participants will be observed for a minimum of 30 minutes (between 30-60 minutes will be acceptable) post-injection, vital signs (at a minimum temperature and heart rate are assessed; other vital signs [blood pressure and respiratory rate] are assessed as clinically indicated) will be assessed, the injection site will be inspected for evidence of local reaction, and any evidence of systemic symptoms will be assessed.

Post-Clinic Follow-Up: Participants are asked to record symptoms on a daily basis using an electronic participant diary. Signs and symptoms considered to represent reactogenicity from the vaccine include systemic events of increased body temperature, fatigue, generalized myalgia, generalized arthralgia, headache, chills, nausea, and local events at the injection site including pain/tenderness, induration and erythema. Participant diaries will be reviewed by a clinician and

reconciled for accuracy and completeness. No attribution assessment will be performed for systemic reactogenicity events reported in the participant diary without additional evaluation of the participant by clinician.

Participants will be given a thermometer for oral temperature measurement, a ruler, and provided access to the electronic study diary (eDiary). The information collected by eDiary might include personal health information, contact information, and information about app usage. The eDiary does not collect personal information about participant activities over time or from other websites or online services. It also does not allow third parties to collect that information. Participants will be encouraged to use the preferred electronic diary but will have the option to use a paper diary. The paper diary, if used, will be transcribed into the study database and stored in the participant file for monitoring purposes. The participant will use the diary to record daily their highest temperature as well as local and systemic signs and symptoms for 7 full days following each study vaccination. Participants will be provided training on diary completion, proper thermometer usage, and the use of the measuring device to measure any injection site induration and/or erythema.

Remote or in-person contact between the participant and the site staff should take place at least once on the third- or fourth-day following vaccination. The reactogenicity assessment period is seven days following vaccine administration. Clinicians will follow and collect resolution information for any reactogenicity signs and symptoms that have not resolved within 7 days.

Any postvaccination reaction grade 2 or higher will be assessed by a clinician within 48 hours after onset, unless the reaction is improving and/or has completely resolved. Additionally, other clinical concerns may prompt a study visit based on the judgment of a study clinician.

8.4 Cardiac symptom management

Participants will be instructed to observe for any cardiac symptoms (chest pain, shortness of breath, feeling of an abnormal heartbeat [fluttering sensation, fast heartbeat, heart pounding, or irregularity], or syncope that occur within days to weeks following each vaccination, and to seek medical attention and notify study site staff if any of these symptoms occur. Site staff will request medical records for any cardiac workup that has occurred. Any events of myocarditis and/or pericarditis occurring during this trial will be reported as AESIs (see Section 9.2); and are subject to study pause rules (see Section 9.7). Serum samples stored at baseline or post-vaccination study visits could be retrospectively assayed for additional safety labs if indicated on a case-by-case basis.

8.5 Additional procedures for Part A participants

Approximately half of the participants in Part A (groups 1-3) will undergo lymph node FNA and half will have leukapheresis performed. These procedures require that participants meet additional eligibility criteria (see Sections 5.1.3, 8.5.1 and 8.5.2). The participants willing to undergo these procedures may choose to have only lymph node FNA, only leukapheresis, or both procedures. Other participants in Part A may choose to have neither procedure performed.

8.5.1 Lymph node FNA

Tissue sampling of an axillary lymph node ipsilateral to the deltoid injection site will be carried out percutaneously by FNA. Lymph node FNA will not be performed if the injection is given in the thigh. This procedure will be performed in accordance with the standard practices of the participating provider and/or facility. The procedure involves tissue retrieval with a needle via a small skin incision under sonographic guidance. Approximately 2-4 passes will be made to retrieve cytologic material.

Post-procedural safety assessments will be performed in accordance with the standard practices of the participating provider and/or facility. The participant will be advised to contact the study site if they experience severe pain, fever (post-procedural body temperature ≥ 38.0°C or 100.4°F) or other evidence of infection (inflammation and/or pus) at the aspiration site, or arm numbness or weakness.

Eligibility for lymph node FNA:

- Participant may not be taking warfarin, oral antithrombin equivalents (including, but not limited to, apixiban, rivaroxiban, dabigatran), enoxaparin injections, or other medications that would increase the risk of bleeding as assessed by the clinician performing the procedure.
- The vaccine injection was given in the deltoid and there is no evidence of localized infection directly superior to the axillary aspiration site
- No other contraindication to procedure as assessed by the clinician performing the procedure
- Participants who can become pregnant must have a negative pregnancy test within 48 hours prior to the procedure.

8.5.2 Leukapheresis

Collection of PBMC via leukapheresis will be performed in accordance with the standard practices of the participating apheresis center. Ideally, in this procedure, approximately 1.5 liters of blood will be processed over about 1 hour using

peripheral veins for venous access. The blood will be anti-coagulated in accordance with standard practice of the apheresis center.

Post-procedural safety assessments will be performed in accordance with the standard practices of the participating apheresis center. Additionally, the participant will be advised to contact the study site if they experience any adverse events following the procedure.

Eligibility for leukapheresis:

- Prior to leukapheresis, participant must meet all apheresis center requirements for this procedure.
- Participants who can become pregnant must have a negative pregnancy test within 48 hours prior to the procedure.

8.6 Extended follow-up for participants with unresolved study product-related urticaria-associated symptoms

Participants with unresolved study product-related urticaria-associated symptoms (eg; wheal and flare lesions, pruritis, and/or erythematous lesions) at Visit 15 (month 12) will be asked to have follow-up remote contacts (eg, via phone) with CRS staff until all associated signs and symptoms resolve, or for up to an additional 48 months (4 years) after the month 12 visit, whichever time is shorter (see HVTN 302 SSP for detail). In addition, participants will come into the clinic for serum collections for banking at the following times (See Appendix C):

- Visit 102
- At clinic visit(s) scheduled in response to a urticaria-associated symptom. This can be multiple visits for a single flare or during recurrent visits for multiple flares.
- Participant's exit visit

A sample addendum informed consent form is provided in Appendix G. Participants who consent for this extended follow-up period will follow the schedule of visits and evaluations shown in Appendix C.

CRS staff will record the following:

- Updates on dermatological AEs deemed related to study product;
- New or updates to current concomitant medications;
- A brief urticaria-related symptom questionnaire;

- New SAEs, regardless of attribution to study product;
- New dermatological AEs, regardless of attribution to study product; and
- Targeted physical exam findings (if a physical exam is conducted).

Resolution of symptoms that would allow for termination of extended follow-up are defined as the following:

- For participants not taking antihistamines prior to enrollment (aka baseline): absence of study product-related urticaria-associated symptoms (eg; wheal and flare lesions, pruritis, and/or erythematous lesions) while off antihistamines for at least 8 weeks. The HVTN 302 PSRT will be consulted prior to terminating a participant from study follow-up.
- For participants taking antihistamines for pre-existing allergic conditions prior to enrollment (aka baseline): back-to-baseline dosage of antihistamines, with lack of wheal and flare lesions and/or pruritic, erythematous lesions for at least 8 weeks. The HVTN 302 PSRT will be consulted prior to terminating a participant from study follow-up.

8.7 Visit procedures, schedule, windows and missed visits

The schedule of visits and evaluations performed at each visit are shown in Appendix A (for participants undergoing LN FNA and/or leukapheresis) and Appendix B (for participants undergoing neither LN FNA nor leukapheresis) and Appendix C (for participants in extended follow-up). Visit windows are shown in Appendix D and Appendix E. The procedures for documenting missed visits and out-of-window visits are described in the HVTN 302 SSP. If the missed visit is one that required safety assessments or local safety labs, HVTN CRS staff should attempt to bring the participant in for an interim visit as soon as possible.

8.8 Monitoring for HIV infection

Study participants will be tested for HIV infection periodically throughout the study as indicated in the schedules included in Appendix A and Appendix B. The Laboratory Program (or approved diagnostic laboratory) will follow the HVTN HIV testing algorithm (see HVTN Laboratory Center MOP) which is able to distinguish vaccine-induced antibody responses from actual HIV infections. Participants will be promptly informed and counseled if they become HIV-infected during the study and referred for treatment (see Section 5.2.2).

Study participants will receive regularly scheduled counseling regarding avoidance of HIV infection in accordance with the most recent Centers for Disease Control and Prevention HIV counseling guidelines.

Although the study vaccine will not cause HIV infection, it may induce antibodies detectable by standard HIV infection screening techniques. This is referred to as vaccine induced seropositivity (VISP). The following steps will be taken to protect participants from adverse consequences associated with VISP:

- Participants will be counseled to avoid HIV antibody testing outside of the HVTN CRS during study participation.
- Participants can receive HIV diagnostic testing from the CRS following their last scheduled visit until they are told they do not have VISP.
- Participants with VISP will be offered poststudy HIV diagnostic testing (per the HVTN poststudy HIV testing algorithm) periodically free of charge as medically/socially indicated (approximately every 6 months) unless or until HIV antibody testing is no longer standard in clinical settings.
- Unless they request that their names be removed, the names of all participants in HVTN studies are entered into a secure VISP registry in order to verify that an individual received an HIV vaccine (and therefore has the potential for VISP) and to qualify former participants for post-study HIV testing to distinguish between VISP and HIV infection. Information in the VISP registry is not used for research.

8.9 Early termination visit

If a participant terminates participation in the study early for any reason, the site principal investigator should consider if the following assessments are appropriate: end-of-study HIV test, CBC with differential, serum chemistry (ALT and creatinine), physical examination, social impact assessment, and if indicated, a pregnancy test (see Appendix A and Appendix B). If the site principal investigator has questions regarding a termination visit, they should consult with the PSRT.

For participants with HIV infection, please see Section 5.2.2. If the site principal investigator has questions regarding a termination visit, they should consult with the PSRT.

Early termination to extended follow-up consider all study procedures and sample collection in Appendix C.

9 Safety and adverse events

9.1 Adverse events

Unsolicited AEs will be collected over a period of 30 days after each vaccination. All collected AEs are captured in the clinical database on the appropriate CRF. Clinic staff should evaluate every AE to determine if (1) the AE meets the requirements for expedited reporting (see Section 9.3.1), (2) if the AE meets the criteria for a safety pause/prompt AE review (see Section 9.7), (3) if the AE meets the criteria for an MAAE, and (4) if the AE is an adverse event of special interest (AESI). Reportable AESIs will be myocarditis and pericarditis.

In addition, a limited set of AEs will be collected and reported through the end of the main study.

- SAEs/expedited adverse events (EAEs)
- MAAEs (defined as any adverse events leading to an unscheduled visit to a healthcare professional)
- AEs leading to early participant withdrawal or early discontinuation of study vaccine(s) administration.
- Adverse Events of Special Interest (AESIs):
    - myocarditis
    - pericarditis
- Participants in the Extended Follow-up (see Section 8.6) will have this subset of AEs collected and reported through the end of their study participation:
    - New dermatologic AEs (indicate if an MAAE)
    - SAEs

Adverse events (AEs) will be graded according to the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1 – July 2017 (https://rsc.niaid.nih.gov/clinical-research-sites/daids-adverse-event-grading-tables), with the following exceptions:

- Unintentional Weight Loss is required to be reported as an AE only if it is considered to be potentially deleterious to the participant's health (see HVTN 302 SSP);

- Creatinine is required to be reported as an AE only if it is gradable per the increase from local lab ULN parameter. Do not grade elevated creatinine based on the change from the baseline parameter.

- Do not grade creatinine clearance or eGFR based on the change from the baseline parameter. Do not grade on the basis of eGFR if there is clinical concern for kidney injury.

- Injection Site Erythema or Redness and Injection Site Induration or Swelling will not consider surface area and interference with usual social and functional activities such that:

    - Grade 1 is: 2.5 to < 5 cm in diameter;

    - Grade 2 is: ≥ 5 to < 10 cm in diameter;

    - Grade 3 is: ≥ 10 cm in diameter OR ulceration OR secondary infection OR phlebitis OR sterile abscess OR drainage;

    - Grade 4 is: Potentially life-threatening consequences (eg, abscess, exfoliative dermatitis, necrosis involving dermis or deeper tissue);

9.2 AESIs

Any possible, probable, or confirmed/definitive case of myocarditis and/or pericarditis, should be reported as an AESI whether diagnosed from a local clinical evaluation or if meeting the CDC or Brighton Collaboration case definitions. The event should also be reported as an SAE if it meets seriousness criteria (Section 9.3). The CDC and Brighton Collaboration case definitions are provided in Appendix J, as guidance.

9.3 Serious adverse events

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,

- 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. Similarly, a hospital admission for an elective procedure is not considered an SAE.

9.3.1 Expedited reporting of adverse events to DAIDS

Requirements, definitions and methods for expedited reporting of AEs are outlined in Version 2.0 (January 2010) of the *Manual for Expedited Reporting of Adverse Events* to DAIDS (DAIDS EAE Manual), which is available on the RSC website at https://rsc.niaid.nih.gov/clinical-research-sites/manual-expedited-reporting-adverse-events-daids.

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

For questions about DAERS, please contact CRMSsupport@niaid.nih.gov or from within the DAERS application itself.

For questions about EAE reporting, please contact the DAIDS RSC Safety Office at (DAIDSRSCSafetyOffice@tech-res.com).

The SAE Reporting Category will be used throughout the study. After completion of the study the suspected unexpected serious adverse reaction (SUSAR) reporting category will be used if clinical staff becomes aware of an event on a passive basis.

The study products for which expedited reporting are required are: mRNA-1574v1-gp140, mRNA-1574v2-gp151, mRNA-1574v3-CD4KO. There is no placebo product administered in this protocol. The NIAID/DAIDS will report all

unexpected SAEs related to the study products observed in this clinical trial to the FDA in accordance with 21 CFR 312.32 (IND Safety Reports).

In addition to the expedited Reporting Category identified above, other AEs that must be reported in an expedited manner are: myocarditis and/or pericarditis (AESIs).

9.4 Safety monitoring

9.4.1 Protocol Safety Review Team (PSRT)

The PSRT comprises the Study Chairs, participating CRS PI(s), HVTN Protocol Team Leader, DAIDS Medical Officer, HVTN Clinical Safety Specialist (CSS), and other Study Clinicians. The Protocol Team clinic coordinator, clinical data manager, vaccine developer representative, clinical trial manager, and others may also be included in HVTN 302 PSRT meetings. The PSRT will review study safety information on a weekly basis through two weeks after the last participant receives the final study injection. Because participants can be enrolled in the study with a Grade 1 ALT value (up to <2.5 x ULN), the PSRT will monitor all gradable ALT abnormalities reported as adverse events. Less frequent safety reviews will be conducted at the discretion of the PSRT.

9.4.2 HVTN Safety Monitoring Board (SMB)

The safety monitoring board (SMB) is a multidisciplinary group consisting of biostatisticians, clinicians, and experts in HIV vaccine research that, collectively, has experience in the conduct and monitoring of vaccine trials. Members of the SMB are not directly affiliated with the protocols under review.

The SMB reviews safety data (including cumulative reactogenicity events, AEs, laboratory safety data, and individual SAE reports) approximately every 4 months. The SMB conducts additional special reviews at the request of the HVTN 302 PSRT.

Study sites will receive SMB summary minutes and are responsible for forwarding them to their institutional review board (IRB)/ethics committee (EC) and any applicable Regulatory Entity (RE).

9.5 Total blood volume

Required blood volumes per visit are shown in Appendix A, Appendix B, and Appendix C. Not shown is any additional blood volume that would be required if a safety lab needs to be repeated, or if a serum pregnancy test needs to be performed. The additional blood volume would likely be minimal. The total blood volume drawn for each participant will not exceed 500 mL in any 56-day (8-

week) period as per American Red Cross guidelines for blood donation (https://www.redcrossblood.org/donate-blood/how-to-donate/eligibility-requirements.html).

The preferred laboratory specimen tube types for research samples are shown in Appendix A, Appendix B, and Appendix C. Alternate tube types may be used under certain circumstances (eg, ACD tube shortages) upon approval of the HVTN Laboratory Center. Refer to the HVTN 302 Specimen Collection SSP for more information.

9.6 Sentinel Safety Reviews

Enrollment will start with Groups 1-3 simultaneously until a total of 12 participants, approximately 4 participants in each group (Part A sentinel safety cohort) have been enrolled. Enrollment will then pause. The PSRT will review cumulative safety information recorded through the safety visit scheduled two weeks post first vaccination and will determine whether it is safe to proceed with enrolling the participants in Groups 4-6 and the rest of Groups 1-3.

Enrollment will then start again for the first 12 participants in Groups 4-6 (Part B sentinel safety cohort) and the rest of Part A (Groups 1-3) in parallel. Once the Part B sentinel safety cohort (Groups 4-6) has been fully enrolled, enrollment in Groups 4-6 will then pause while the remainder of Part A (Groups 1-3) continues enrollment. The PSRT will review cumulative safety information recorded for Groups 4-6 (Part B sentinel safety cohort) through the safety visit scheduled two weeks post first vaccination and will determine whether it is safe to proceed with enrolling the remainder of participants in Groups 4-6 (see also Section 6.1). As PSRT safety reviews are performed weekly during the vaccination phase (Section 9.4.1), this review will also automatically include cumulative safety data from Part A participants available at this time.

This enrollment and safety review strategy allows for a single enrollment pause whilst maintaining separate randomization schemas for Part A and Part B participants. Additionally, this strategy allows for intervention of enrollment and vaccination plans for the higher dose group if safety concerns are identified in either Part A participants or the sentinel safety cohort of Part B.

9.7 Safety pause and prompt PSRT AE review

The PSRT (see Section 9.4) will closely monitor participant safety. The trial overall, or by a particular dose level or group can be paused at any time for any reason by the PSRT. When a safety pause is implemented, enrollment and all vaccinations within the dose level(s), group(s) or overall trial that was placed on pause will be held until further notice. The AEs that will lead to a safety pause or prompt HVTN 302 PSRT AE review are summarized in Table 9-1. Vaccinations

may be suspended for safety concerns other than those described in the table, or before pause rules are met, if, in the judgment of the HVTN 302 PSRT, participant safety may be threatened. The enrollment strategy described above may allow for a safety pause for Part B participants alone if appropriate. Criteria for an individual participant's departure from the schedule of vaccinations are listed in Section 5.2.3.

Table 9-1 Pause Rules Table

Event and relationship to study vaccine	Severity Grade	HVTN Site Actions	HVTN Core action
SAE, **related**	5 or 4	**Phone** 24/7 Safety Phone immediately **Email** vtn.clin.safety.spec@hvtn.org **Submit** CRFs immediately	Immediate pause
AESI[a], **related or unrelated**	Any grade	**Phone** 24/7 Safety Phone immediately **Email** vtn.clin.safety.spec@hvtn.org **Submit** CRFs immediately	Immediate pause
SAE, **related**	3, 2, or 1	**Email** clinical safety specialist **Submit** CRFs immediately	Prompt PSRT AE review to consider a pause
AE, **related (see Grade 3 exceptions)**	4 or 3	**Email** clinical safety specialist **Submit** CRFs immediately	Prompt PSRT AE review to consider a pause

[a] Adverse Events considered AESIs for the protocol are myocarditis and pericarditis.

Exceptions to the related Grade 3 AEs (for Grade 3 subjective reactogenicity events and local reactions):

- injection site pain/tenderness and erythema/induration (grade 3 by size only)
- fatigue
- generalized myalgia
- generalized arthralgia
- chills
- headache

- nausea (unless IV rehydration required)

Unrelated Participant Death: Sites will call the CSS office phone upon learning of any unrelated participant deaths. The site will also email the CSS and immediately submit CRFs. The PSRT will then be immediately notified.

If you need to contact the CSS, refer to phone numbers and email addresses found on the Protocol home page on the HVTN Members' site (https://members.hvtn.org/protocols/hvtn302).

9.7.1 Plan for review of pause rules

For all safety pauses, HVTN Core notifies the HVTN 302 PSRT, HVTN Regulatory Affairs, DAIDS Pharmaceutical Affairs Branch (PAB), DAIDS Regulatory Affairs Branch (RAB), DAIDS Safety and Pharmacovigilance Team (SPT), and participating HVTN CRSs. When an immediate safety pause is triggered, HVTN Core notifies the SMB.

Once a trial is paused, the HVTN 302 PSRT reviews safety data and decides whether the pause can be lifted or permanent discontinuation of vaccination is appropriate, consulting the SMB if necessary. HVTN Core notifies the participating HVTN CRSs, HVTN Regulatory Affairs, DAIDS PAB, DAIDS RAB, and DAIDS SPT of the decision regarding resumption or discontinuation of study vaccinations. Based on the HVTN 302 PSRT assessment, DAIDS RAB notifies the FDA as needed.

If an immediate HVTN 302 PSRT notification or prompt HVTN 302 PSRT AE review is triggered, HVTN Core notifies the HVTN 302 PSRT as soon as possible during working hours (local time)—or, if the information was received during off hours, by the morning of the next workday. If a prompt HVTN 302 PSRT AE review cannot be completed within 72 hours of notification (excluding weekends and US federal holidays), an automatic safety pause occurs.

The HVTN requires that each CRS submit to its IRB/EC and any applicable RE protocol-related safety information (such as IND safety reports, notification of vaccine holds due to the pause rules, unanticipated problems involving risks to participants or others, and notification of other unplanned safety pauses). CRSs must also follow all applicable RE reporting requirements.

9.8 Study termination

This study may be terminated early by the determination of the HVTN 302 PSRT, the NIH, the United States Department of Health and Human Services Office for Human Research Protections (OHRP), the FDA, or study product developers. In addition, the conduct of this study at an individual HVTN CRS may be terminated by the determination of the IRB/EC and any applicable RE.

9.9 Pregnancy

If a participant becomes pregnant during the course of the study, no more injections of study product will be given, but remaining visits and study procedures should be completed unless medically contraindicated or applicable regulations require termination from the study. During follow-up of persons who are confirmed pregnant, pregnancy testing is not required, unless clinically indicated. If the participant terminates from the study prior to the pregnancy outcome, the site should make every effort to keep in touch with the participant in order to ascertain the pregnancy outcome. Pregnancies and pregnancy outcomes will be reported as described in the HVTN 302 SSP section on Pregnancy Management and Reporting. If the participant is no longer pregnant, refer to Section 5.2.2.

10 Protocol conduct and informed consent

10.1 Protocol conduct

This research study will be conducted in compliance with the protocol, Good Clinical Practice (GCP) (ICH E6 (R2)), HVTN and DAIDS policies and procedures as specified in the *HVTN Manual of Operations* and DAIDS Clinical Research Policies and Standard Procedures Documents, and all applicable regulatory requirements. These policies and procedures include protocol monitoring (on-site and remote) and compliance. DAIDS and HVTN policies and procedures are available for review by any IRB/EC/RE upon request. Any policies or procedures that vary from DAIDS and HVTN standards or require additional instructions (eg, instructions for randomization specific to this study) will be described in the HVTN 302 SSP. Regarding protocol registration, sites should follow procedures outlined in the current version of the DAIDS Protocol Registration Manual.

HVTN scientists and operational staff are committed to substantive community input into the planning, conduct, and follow-up of its research, ensuring that locally appropriate cultural and linguistic needs of study populations are met. Community Advisory Boards (CAB) are required by DAIDS and supported at all HVTN research sites to ensure community input in accordance with Good Participatory Practices (GPP) and all local and national guidelines.

10.2 Compliance with NIH guidelines for research involving products containing recombinant or synthetic nucleic acid molecules

Because this study is evaluating products containing recombinant or synthetic nucleic acid molecules, it must comply with regulations set forth in the NIH's *Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* (April 2019). Information about the study must be submitted to the Institutional Biosafety Committee (IBC) for each CRS. Investigators at each CRS are responsible for obtaining IBC approval per NIH guideline *section IV-B7-a-(1)*. IBC review and approval must be documented by the investigator and submitted as part of DAIDS's initial protocol registration for this trial before participants are enrolled at the CRS. If this protocol is amended, investigators should follow the requirements of their respective IBC.

10.3 Informed consent

The sample informed consent form (SICF) in Appendix F describes the investigational vaccine and all aspects involved in study participation. Documentation of appropriate informed consent must be in place prior to conducting study procedures with participants. Periodic assessment of

participants' continued understanding of key study concepts and informed consent must also be documented. Study sites are strongly encouraged to have their local CABs review their site-specific consent forms. This review should include, but should not be limited to, issues of cultural competence, local language considerations, and the level of understandability.

If any new information is learned that might affect the participants' decisions to stay in the trial, this information will be shared with them. If necessary, participants will be asked to sign revised informed consent forms.

An HVTN CRS may employ recruitment efforts prior to the participant consenting. For example, some HVTN CRSs use a telephone script to prescreen people before they come to the clinic for a full screening visit. Participants must sign a screening or protocol-specific consent before any procedures are performed to determine eligibility. HVTN CRSs must submit recruitment and prescreening materials to their IRB/EC and any applicable RE for human subjects protection review and approval.

10.3.1 Screening Consent Form

Without a general screening consent, screening for a specific study cannot take place until the site receives protocol registration from the DAIDS RSC Protocol Registration Office.

Some HVTN CRSs have approval from their IRB/EC and any applicable RE to use a general screening consent form that allows screening for an unspecified HIV vaccine trial. In this way, HVTN CRS staff can continually screen potential participants and, when needed, proceed quickly to obtain protocol-specific enrollment consent. Sites conducting general screening or prescreening approved by their IRB/EC and any applicable RE may use the results from this screening to determine eligibility for this protocol, provided the tests are conducted within the time periods specified in the eligibility criteria.

11 Exploratory studies

Samples may be used for other testing and research related to furthering the understanding of HIV immunology or vaccines. In addition, cryopreserved samples may be used to perform additional assays to support standardization and validation of existing or newly developed methods.

Only genetic testing that is in accord with the language in the sample informed consent form (Appendix F and Appendix G) may be performed on samples.

11.1 Specimen storage and other use of specimens

The HVTN stores specimens from all study participants indefinitely, unless a participant requests that specimens be destroyed or if destruction or a time limit for storage is required by IRB/EC, or RE.

Other use of specimens is defined as studies not covered by the protocol or the informed consent form for the main study (see Appendix F).

This research may relate to HIV, vaccines, the immune system, and other diseases. This could include genetic testing and, potentially, genome-wide studies. This research is done only to the extent authorized in each study site's informed consent form, or as otherwise authorized under applicable law. Other research on specimens ("other use") will occur only after review and approval by the HVTN, the IRB/EC of the researcher requesting the specimens, and the IRBs/ECs/REs of the CRSs if required.

As part of consenting for the study, participants document their initial decision to allow or not allow their specimens to be used in other research, and they may change their decision at any time. The participant's initial decision about other use of their specimens, and any later change to that decision, is recorded by their CRS in a Web-based tool that documents their current decisions for other use of their specimens. The HVTN will only allow other research to be done on specimens from participants who allow such use.

CRSs must notify HVTN Regulatory Affairs if institutional or local governmental requirements pose a conflict with or impose restrictions on specimen storage or other use of specimens.

12 Literature cited

1. Steichen JM, Kulp DW, Tokatlian T, Escolano A, Dosenovic P, Stanfield RL, et al. HIV Vaccine Design to Target Germline Precursors of Glycan-Dependent Broadly Neutralizing Antibodies. Immunity. 2016;45(3):483-96.

2. Corbett KS, Edwards DK, Leist SR, Abiona OM, Boyoglu-Barnum S, Gillespie RA, et al. SARS-CoV-2 mRNA vaccine design enabled by prototype pathogen preparedness. Nature. 2020.

3. Corbett KS, Flynn B, Foulds KE, Francica JR, Boyoglu-Barnum S, Werner AP, et al. Evaluation of the mRNA-1273 Vaccine against SARS-CoV-2 in Nonhuman Primates. N Engl J Med. 2020.

4. Jackson LA, Anderson EJ, Rouphael NG, Roberts PC, Makhene M, Coler RN, et al. An mRNA Vaccine against SARS-CoV-2 - Preliminary Report. N Engl J Med. 2020.

5. Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA vaccines - a new era in vaccinology. Nat Rev Drug Discov. 2018;17(4):261-79.

6. Escolano A, Steichen JM, Dosenovic P, Kulp DW, Golijanin J, Sok D, et al. Sequential Immunization Elicits Broadly Neutralizing Anti-HIV-1 Antibodies in Ig Knockin Mice. Cell. 2016;166(6):1445-58 e12.

7. Briney B, Sok D, Jardine JG, Kulp DW, Skog P, Menis S, et al. Tailored Immunogens Direct Affinity Maturation toward HIV Neutralizing Antibodies. Cell. 2016;166(6):1459-70 e11.

8. Pauthner M, Havenar-Daughton C, Sok D, Nkolola JP, Bastidas R, Boopathy AV, et al. Elicitation of Robust Tier 2 Neutralizing Antibody Responses in Nonhuman Primates by HIV Envelope Trimer Immunization Using Optimized Approaches. Immunity. 2017;46(6):1073-88 e6.

9. Pauthner MG, Nkolola JP, Havenar-Daughton C, Murrell B, Reiss SM, Bastidas R, et al. Vaccine-Induced Protection from Homologous Tier 2 SHIV Challenge in Nonhuman Primates Depends on Serum-Neutralizing Antibody Titers. Immunity. 2019;50(1):241-52 e6.

10. Cirelli KM, Carnathan DG, Nogal B, Martin JT, Rodriguez OL, Upadhyay AA, et al. Slow Delivery Immunization Enhances HIV Neutralizing Antibody and Germinal Center Responses via Modulation of Immunodominance. Cell. 2019;177(5):1153-71 e28.

11. Moyer TJ, Kato Y, Abraham W, Chang JYH, Kulp DW, Watson N, et al. Engineered immunogen binding to alum adjuvant enhances humoral immunity. Nat Med. 2020;26(3):430-40.

12. Hu JK, Crampton JC, Cupo A, Ketas T, van Gils MJ, Sliepen K, et al. Murine Antibody Responses to Cleaved Soluble HIV-1 Envelope Trimers Are Highly Restricted in Specificity. J Virol. 2015;89(20):10383-98.

13. Bianchi M, Turner HL, Nogal B, Cottrell CA, Oyen D, Pauthner M, et al. Electron-Microscopy-Based Epitope Mapping Defines Specificities of Polyclonal Antibodies Elicited during HIV-1 BG505 Envelope Trimer Immunization. Immunity. 2018;49(2):288-300 e8.

14. Nogal B, Bianchi M, Cottrell CA, Kirchdoerfer RN, Sewall LM, Turner HL, et al. Mapping Polyclonal Antibody Responses in Non-human Primates Vaccinated with HIV Env Trimer Subunit Vaccines. Cell Rep. 2020;30(11):3755-65 e7.

15. Cao L, Pauthner M, Andrabi R, Rantalainen K, Berndsen Z, Diedrich JK, et al. Differential processing of HIV envelope glycans on the virus and soluble recombinant trimer. Nat Commun. 2018;9(1):3693.

16. Tokatlian T, Read BJ, Jones CA, Kulp DW, Menis S, Chang JYH, et al. Innate immune recognition of glycans targets HIV nanoparticle immunogens to germinal centers. Science. 2019;363(6427):649-54.

17. Brouwer PJM, Antanasijevic A, Berndsen Z, Yasmeen A, Fiala B, Bijl TPL, et al. Enhancing and shaping the immunogenicity of native-like HIV-1 envelope trimers with a two-component protein nanoparticle. Nat Commun. 2019;10(1):4272.

18. Antanasijevic A, Sewall LM, Cottrell CA, Carnathan DG, Jimenez LE, Ngo JT, et al. Polyclonal antibody responses to HIV Env immunogens resolved using cryoEM. bioRxiv. 2021.

19. Kwon YD, Pancera M, Acharya P, Georgiev IS, Crooks ET, Gorman J, et al. Crystal structure, conformational fixation and entry-related interactions of mature ligand-free HIV-1 Env. Nat Struct Mol Biol. 2015;22(7):522-31.

20. Kulp DW, Steichen JM, Pauthner M, Hu X, Schiffner T, Liguori A, et al. Structure-based design of native-like HIV-1 envelope trimers to silence non-neutralizing epitopes and eliminate CD4 binding. Nat Commun. 2017;8(1):1655.

21. Steichen JM, Lin YC, Havenar-Daughton C, Pecetta S, Ozorowski G, Willis JR, et al. A generalized HIV vaccine design strategy for priming of broadly neutralizing antibody responses. Science. 2019;366(6470).

22. de Taeye SW, Ozorowski G, Torrents de la Pena A, Guttman M, Julien JP, van den Kerkhof TL, et al. Immunogenicity of Stabilized HIV-1 Envelope

Trimers with Reduced Exposure of Non-neutralizing Epitopes. Cell. 2015;163(7):1702-15.

23. Sanders RW, van Gils MJ, Derking R, Sok D, Ketas TJ, Burger JA, et al. HIV-1 VACCINES. HIV-1 neutralizing antibodies induced by native-like envelope trimers. Science. 2015;349(6244):aac4223.

24. Moderna Inc. Press release. Moderna Announces Positive Interim Results from Phase 1 Cytomegalovirus (CMV) Vaccine (mRNA-1647) Study and Progress Toward Phase 2 and Pivotal Trials 2019 [Available from: https://investors.modernatx.com/news-releases/news-release-details/moderna-announces-positive-interim-results-phase-1.

25. Moderna Inc. CMV vaccine (mRNA-1647) 7-Month Interim Analysis Call Presentation 2020 [Available from: https://investors.modernatx.com/static-files/be885344-ba1c-466a-ad01-7053cd8f2d3c.

26. Moderna Inc. Annual R&D Day Presentation 2020 [Available from: https://investors.modernatx.com/static-files/e5fd7ac7-53c9-4804-b89d-4e51d5121960.

27. Kroumpouzos G, Paroikaki ME, Yumeen S, Bhargava S, Mylonakis E. Cutaneous Complications of mRNA and AZD1222 COVID-19 Vaccines: A Worldwide Review. Microorganisms. 2022;10(3).

28. Pitlick MM, Joshi AY, Gonzalez-Estrada A, Chiarella SE. Delayed systemic urticarial reactions following mRNA COVID-19 vaccination. Allergy Asthma Proc. 2022;43(1):40-3.

29. McMahon DE, Amerson E, Rosenbach M, Lipoff JB, Moustafa D, Tyagi A, et al. Cutaneous reactions reported after Moderna and Pfizer COVID-19 vaccination: A registry-based study of 414 cases. J Am Acad Dermatol. 2021;85(1):46-55.

30. Shah R, Tsao L, Zheng J, Tang M, Otani I. Urticarial and Eczematous Eruptions Following mRNA COVID-19 Vaccination in Patients With and Without Chronic Urticaria and Atopic Dermatitis. Journal of Allergy and Clinical Immunology. 2022;149(2):AB175.

31. Wong RC, Fairley JA, Ellis CN. Dermographism: a review. J Am Acad Dermatol. 1984;11(4 Pt 1):643-52.

32. Wolfson AR, Freeman EE, Blumenthal KG. Urticaria 12 Days After COVID-19 mRNA Booster Vaccination. JAMA. 2022;327(17):1702-3.

33. Bozkurt B, Kamat I, Hotez PJ. Myocarditis With COVID-19 mRNA Vaccines. Circulation. 2021;144(6):471-84.

34. Law YM, Lal AK, Chen S, Cihakova D, Cooper LT, Jr., Deshpande S, et al. Diagnosis and Management of Myocarditis in Children: A Scientific Statement From the American Heart Association. Circulation. 2021;144(6):e123-e35.

35. NHLBI. Heart Inflammation [Available from: https://www.nhlbi.nih.gov/health-topics/heart-inflammation.

36. Centers for Disease Control and Prevention. Clinical Considerations: Myocarditis and Pericarditis after Receipt of mRNA COVID-19 Vaccines Among Adolescents and Young Adults [Available from: https://www.cdc.gov/vaccines/covid-19/clinical-considerations/myocarditis.html.

37. Siddiqi HK, Libby P, Ridker PM. COVID-19 - A vascular disease. Trends Cardiovasc Med. 2021;31(1):1-5.

38. Nishiga M, Wang DW, Han Y, Lewis DB, Wu JC. COVID-19 and cardiovascular disease: from basic mechanisms to clinical perspectives. Nat Rev Cardiol. 2020;17(9):543-58.

39. Sampson HA, Munoz-Furlong A, Campbell RL, Adkinson NF, Jr., Bock SA, Branum A, et al. Second symposium on the definition and management of anaphylaxis: summary report--second National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network symposium. Ann Emerg Med. 2006;47(4):373-80.

40. Agresti A, Coull BA. Approximate is better than "exact" for interval estimation of binomial proportions. Am Stat. 1998;52(2):119-26.

41. Lydersen S, Fagerland MW, Laake P. Recommended tests for association in 2 x 2 tables. Stat Med. 2009;28(7):1159-75.

42. Dunnett C. A multiple comparisons procedure for comparing several treatments with a control. Journal of the American Statistical Association. 1995;50:1096-121.

43. Brookes ST, Whitely E, Egger M, Smith GD, Mulheran PA, Peters TJ. Subgroup analyses in randomized trials: risks of subgroup-specific analyses; power and sample size for the interaction test. J Clin Epidemiol. 2004;57(3):229-36.

44. Lachenbruch PA. Comparisons of two-part models with competitors. Stat Med. 2001;20(8):1215-34.

45. Lynch RM, Boritz E, Coates EE, DeZure A, Madden P, Costner P, et al. Virologic effects of broadly neutralizing antibody VRC01 administration during chronic HIV-1 infection. Sci Transl Med. 2015;7(319):319ra206.

46. Benkeser D, Carone M, Laan MJV, Gilbert PB. Doubly robust nonparametric inference on the average treatment effect. Biometrika. 2017;104(4):863-80.

47. Hughes JP. Mixed effects models with censored data with application to HIV RNA levels. Biometrics. 1999;55(2):625-9.

48. Rotnitzky A, Robins J. Analysis of semi-parametric regression models with non-ignorable non-response. Stat Med. 1997;16(1-3):81-102.

49. Rotnitzky A, Robins JM. Semiparametric regression estimation in the presence of dependent censoring. Biometrika. 1995;82(4):805-20.

50. Gilbert PB, Sato A, Sun X, Mehrotra DV. Efficient and robust method for comparing the immunogenicity of candidate vaccines in randomized clinical trials. Vaccine. 2009;27(3):396-401.

51. James G, Witten D, Hastie T, Tibshirani R. An introduction to statistical learning with applications in R. New York: R. Springer; 2013.

52. Finak G, McDavid A, Chattopadhyay P, Dominguez M, De RS, Roederer M, et al. Mixture models for single-cell assays with applications to vaccine studies. Biostatistics. 2014;15(1):87-101.

53. Lin L, Finak G, Ushey K, Seshadri C, Hawn TR, Frahm N, et al. COMPASS identifies T-cell subsets correlated with clinical outcomes. Nat Biotechnol. 2015;33(6):610-6.

54. Gargano JW, Wallace M, Hadler SC, Langley G, Su JR, Oster ME, et al. Use of mRNA COVID-19 Vaccine After Reports of Myocarditis Among Vaccine Recipients: Update from the Advisory Committee on Immunization Practices - United States, June 2021. MMWR Morb Mortal Wkly Rep. 2021;70(27):977-82.

Appendix A Schedule of procedures for participants undergoing LN FNA and/or leukapheresis

Visit Number		01	02	03	04	05[16]	06	07	08	09[16]	10	11	12	13[16]	14	15
Study Month			**0**	**0.25**	**0.5**	**1.5**	**2**	**2.25**	**2.5**	**3**	**6**	**6.25**	**6.5**	**7**	**8**	**12**
Study Week			**0**	**1**	**2**	**6**	**8**	**9**	**10**	**12**	**24**	**25**	**26**	**28**	**32**	**52**
Study Day		-56 to 1	1	8	15	43	57	64	71	85	169	176	183	197	225	365
Procedure		Screen[1]	**Vac 1**				**Vac 2**				**Vac 3**					
Study procedures																
Assessment of Understanding		√														
Informed consent		√														
Medical history[2]		√														
Physical exam[3]		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Contraception status assessment[4]		√	√				√				√				√	
Risk reduction counseling[5]		√	√		√		√		√		√		√		√	√
Concomitant medications[6]		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Adverse Events (AEs)			√	√	√	√	√	√	√	√	√	√	√	√	√	√
MAAEs/AESIs/SAEs			√	√	√	√	√	√	√	√	√	√	√	√	√	√
Vaccination[7]			√				√				√					
Reactogenicity assessment[8]			√				√				√					
Social impact assessment			√	√	√	√	√	√	√	√	√	√	√	√	√	√
Social impact questionnaire							√				√					√
Clinical labs	Tube	Vol (mL)														
Pregnancy test (urine or serum) [9, 10]		√	√			√[15]	√		√[17]	√[15]	√		√[17]	√[15]	√	
HBsAg/anti-HCV[10]	SST	5														
HIV screening test [10,11]	SST	5														
CBC/Differential[10]	EDTA	5		5				5				5			5	
ALT/Creatinine[10]	SST	5		5				5				5				
Storage for additional safety labs[12]	SST		y	5	y			5	y			5	y			
HIV diagnostic test	EDTA								10		10				10	20
Research samples[13]																
PBMC for assays and storage	ACD		42.5		42.5		85		144.5[14]		85		204[14]			255
Serum for assays and storage	SST		25.5		25.5	25.5	25.5		25.5	25.5	25.5		25.5	25.5	25.5	25.5
Lymph Node Fine Needle Aspirate[15]						√[15]				√[15]				√[15]		
Leukapheresis[17]									√[17]				√[17]			
Daily volume maximum (mL)[18]		20	68	15	68	25.5	110.5	15	180	25.5	120.5	15	229.5	25.5	40.5	300.5
56-day total volume maximum (mL)[18]		20	88	103	171	196.5	307	234	399	356.5	120.5	135.5	365	390.5	431	300.5

Green shading = Vaccination Visit; Blue shading = LN FNA visits, which are only applicable to participants undergoing LN FNA

[1] **Screening** evaluations at Visit 01 are performed no more than 56 days before Day 1.

[2] **Medical history**: A complete medical history is performed during screening. At enrollment and at subsequent visits, an interim medical history may be performed.

[3] A complete **physical exam** is performed at screening and last clinic visit, to include height, weight, vital signs, and clinical assessments of head, ears, eyes, nose, and throat; neck, lymph nodes; heart; chest; abdomen; extremities; neurological function; and skin. At other visits, a **targeted physical exam** will be performed as needed, based on participant report or indications of illness.

[4] **Contraception status assessment** is required only for participants who were assigned female sex at birth and are capable of becoming pregnant.

[5] **Risk reduction counseling** per CRS Standard Operating Procedures.

[6] **Concomitant medications**, including prescription and non-prescription drugs, vitamins, topical products, alternative/complementary medicines, recreational drugs, vaccinations, and allergy shots are recorded during screening, at enrolment, and at each subsequent clinic visit.

[7] **Vaccination (in clinic assessments)**: At least 30 minutes after each vaccination and prior to clinic discharge, participants will have vital signs taken, the injection site will be assessed, and systemic symptoms will be assessed.

[8] **Reactogenicity**: Clinic staff will follow new or unresolved reactogenicity symptoms present at day 7 until resolution.

[9] **Pregnancy test**: For participants assigned female sex at birth. Pregnancy test may be performed on urine or blood specimens. Persons who are NOT capable of becoming pregnant due to total hysterectomy or bilateral oophorectomy (verified by medical records), or menopause (no menses for ≥ 1 year) are not required to undergo pregnancy testing. Persons who are confirmed pregnant, pregnancy testing is not required, unless clinically indicated.

[10] Local labs may assign appropriate alternative tube types for locally performed tests.

[11] **HIV screening test**: see Section 5.1.1.

[12] For additional assays to be performed if clinically indicated (See Section 8.4)

[13] **Research samples**: Blood draw volumes for each tube type shown.

[14] **PBMC for assays and storage:** To be collected only from participants who will not undergo leukapheresis.

[15] **Lymph node fine needle aspirate:** Will only be collected from participants who agreed to provide this sample type and who have met lymph node FNA eligibility criteria (see Section 8.5.1). A pregnancy test must be performed and confirmed negative within 48 hours prior to lymph node FNA (see also: footnote 9). For participants who decide not to continue providing Lymph Node FNA samples, procedures shown in Appendix B should be followed.

[16] Visits 5, 9 and 13 are **required only for participants undergoing lymph node FNA.**

[17] **Leukapheresis**: Will only be collected from participants who agreed to provide this sample type and who have met leukapheresis eligibility criteria (see Section 8.5.2). A pregnancy test must be performed and confirmed negative within 48 hours prior to leukapheresis (see also: footnote 9). For participants who decide not to continue providing leukapheresis samples, procedures shown in in Appendix B should be followed.

[18] Daily and 56-day total blood draw volumes will be lower for participants who are providing leukapheresis samples.

y=SST blood collected for assays and serum storage will also cover specimen needs for storage for additional safety labs; no separate blood draw is needed.

Appendix B Schedule of procedures for participants undergoing neither LN FNA nor leukapheresis

Visit Number		01	02	03	04	05	06	07	08	09	10	11	12	13	14	15
Study Month			**0**	**0.25**	**0.5**	**1.5**	**2**	**2.25**	**2.5**	**3**	**6**	**6.25**	**6.5**	**7**	**8**	**12**
Study Week			**0**	**1**	**2**	**6**	**8**	**9**	**10**	**12**	**24**	**25**	**26**	**28**	**32**	**52**
Study Day		-56 to 1	1	8	15	43	57	64	71	85	169	176	183	197	225	365
Procedure		Screen[1]	**Vac 1**				**Vac 2**				**Vac 3**					
Study procedures																
Assessment of Understanding		√														
Informed consent		√														
Medical history[2]		√														
Physical exam[3]		√	√	√	√		√	√	√		√	√	√		√	√
Contraception status assessment[4]		√	√				√				√				√	
Risk reduction counseling[5]		√	√		√		√		√		√		√		√	√
Concomitant medications[6]		√	√	√	√		√	√	√		√	√	√		√	√
Adverse Events (AEs)			√	√	√		√	√	√		√	√	√		√	√
MAAEs/AESIs/SAEs			√	√	√		√	√	√		√	√	√		√	√
Vaccination[7]			√				√				√					
Reactogenicity assessment[8]			√				√				√					
Social impact assessment			√	√	√		√	√	√		√	√	√		√	√
Social impact questionnaire							√				√					√
Clinical labs	Tube	Vol (mL)														
Pregnancy test (urine or serum) [9, 10]		√	√				√				√				√	
HBsAg/anti-HCV[10]	SST	5														
HIV screening test [10,11]	SST	5														
CBC/Differential[10]	EDTA	5		5				5				5			5	
ALT/Creatinine[10]	SST	5		5				5				5				
Storage for additional safety labs[12]	SST		y	5	y			5	y			5	y			
HIV diagnostic test	EDTA								10		10				10	20
Research samples[13]																
PBMC for assays and storage	ACD		42.5		42.5		85		144.5		85		204			255
Serum for assays and storage	SST		25.5		25.5		25.5		25.5		25.5		25.5		25.5	25.5
Daily volume total (mL)		20	68	15	68		110.5	15	180		120.5	15	229.5		40.5	300.5
56-day total volume (mL)		20	88	103	171		281.5	208.5	373.5		120.5	135.5	365		405.5	300.5

Green shading = Vaccination Visit; Gray shading = FNA visits, which are not applicable to this subgroup

[1] **Screening** evaluations at Visit 01 are performed no more than 56 days before Day 1.
[2] **Medical history**: A complete medical history is performed during screening. At enrollment and at subsequent visits, an interim medical history may be performed.
[3] A complete **physical exam** is performed at screening and last clinic visit, to include height, weight, vital signs, and clinical assessments of head, ears, eyes, nose, and throat; neck, lymph nodes; heart; chest; abdomen; extremities; neurological function; and skin. At other visits, a **targeted physical exam** will be performed as needed, based on participant report or indications of illness.
[4] **Contraception status assessment** is required only for participants who were assigned female sex at birth and are capable of becoming pregnant.
[5] **Risk reduction counseling** per CRS Standard Operating Procedures.
[6] **Concomitant medications**, including prescription and non-prescription drugs, vitamins, topical products, alternative/complementary medicines, recreational drugs, vaccinations, and allergy shots are recorded during screening, at enrolment, and at each subsequent clinic visit.
[7] **Vaccination (in clinic assessments)**: At least 30 minutes after each vaccination and prior to clinic discharge, participants will have vital signs taken, the injection site will be assessed, and systemic symptoms will be assessed.
[8] **Reactogenicity**: Clinic staff will follow new or unresolved reactogenicity symptoms present at day 7 until resolution.
[9] **Pregnancy test**: For participants assigned female sex at birth. Pregnancy test may be performed on urine or blood specimens. Persons who are NOT capable of becoming pregnant due to total hysterectomy or bilateral oophorectomy (verified by medical records), or menopause (no menses for ≥ 1 year) are not required to undergo pregnancy testing. Persons who are confirmed pregnant, pregnancy testing is not required, unless clinically indicated.
[10] Local labs may assign appropriate alternative tube types for locally performed tests.
[11] **HIV screening test**: see Section 5.1.1.
[12] For additional assays to be performed if clinically indicated (See Section 8.4)
[13] **Research samples**: Blood draw volumes for each tube type shown.
y=SST blood collected for assays and serum storage will also cover specimen needs for storage for additional safety labs; no separate blood draw is needed.

Appendix C Schedule of procedures for extended follow-up for participants with unresolved study product-related urticaria-associated symptoms

Visit Number		101	102	103	104	105	106	107	108	Urticaria-Associated Symptom Visit[4]
Study Month		**15**	**18**	**21**	**24**	**30**	**36**	**48**	**60**	
Study Week		**65**	**78**	**91**	**104**	**130**	**156**	**208**	**260**	
Study Day		456	547	638	729	911	1093	1457	1821	
Study procedures										
Informed consent		√								
Urticaria-related symptom questionnaire		√	√	√	√	√	√	√	√	√
Medical history[1]		√	√	√	√	√	√	√	√	√
Concomitant medications[2]		√	√	√	√	√	√	√	√	√
Dermatological Adverse Events (AEs)		√	√	√	√	√	√	√	√	√
SAEs		√	√	√	√	√	√	√	√	√
Targeted Physical exam (if clinically indicated)[3]		√	√	√	√	√	√	√	√	√
Research samples										
Serum for storage and assays[4]	SST	0	17	As clinically indicated and at the participant's exit visit[4]					17	17
Daily volume total (mL)		0	17	0	0	0	0	0	17	17
56-day total volume (mL)		0	17	0	0	0	0	0	17	

[1] **Medical history**: Based on the responses on urticaria-related symptom questionnaire, an interim medical history may be performed and documented in the participant's chart.

[2] **Concomitant medications**, including review of prescription and non-prescription drugs, vitamins, topical products, alternative/complementary medicines, recreational drugs, vaccinations, and allergy shots are updated and any new information is recorded at each contact.

[3] Site may ask the participant to come in for a targeted (abbreviated) **physical exam** if during the remote visit, the participant reports anything that may require further evaluation. See Section 8.6.

[4]See Section 8.6. Participants in extended follow will have clinic visits to collect 17 mls serum for banking at Visit 102, their exit visit, and at any clinic visit(s) scheduled to further evaluate urticaria-associated symptoms.

Appendix D Visit windows for main study

Visit Number	Visit Type	Lower Allowable Window (-)	Lower Target Window (-)	Target Day[1]	Upper Target Window (+)	Upper Allowable Window (+)
01.0	Screening	-56	-		-	Up to Day 1
02.0	**Enrollment/Vaccination 1**	**-**	**-**	**1**	**-**	**-**
03.0	1 week post-vaccination 1		-3	8	+2	
04.0	2 weeks post-vaccination 1		-4	15	+4	+7
05.0**	6 weeks post-vaccination 1 FNA	-7	-4	43	+4	+7
06.0	**Vaccination 2**	**-7**	**-4**	**57**	**+4**	**+7**
07.0	1 week post-vaccination 2		-3	**64**	+2	
08	2 weeks post-vaccination 2	-	-4	71	+4	+7
	Leukapheresis		-4	71	+1	+4
09.0**	4 weeks post-vaccination 2 FNA	-7	-4	85	+4	+7
10.0	**Vaccination 3**	**-21**	**-14**	**169**	**+14**	**+21**
11.0	1 week post-vaccination 3		-3	176	+2	
12.0	2 weeks post-vaccination 3 *Primary Immunogenicity*		-4	183	+4	+7
	Leukapheresis		-4	183	+1	+4
13.0**	4 weeks post-vaccination 3 FNA	-7	-4	197	+4	+7
14.0	8 weeks post-vaccination 3	-14	-7	225	+7	+14
15.0	Final Visit	-28	-14	365	+14	+28

Green shading = Vaccination Visit; Blue shading = FNA visit

* All target dates are relative to Day 1, with the exception of the post-vaccination visits (visits 3.0-5.0, 7.0-9.0, and 11.0-14.0) which are relative to the vaccination immediately preceding the visit.

** Visits 5.0, 9.0, and 13.0 are only required for participants who are providing lymph node FNA collections.

Appendix E Visit windows for follow-up of participants with unresolved study product-related urticaria-associated symptoms

Visit Number	Visit Type	Lower Allowable Window (-)	Lower Target Window (-)	Target Day*	Upper Target Window (+)	Upper Allowable Window (+)
101	Initial contact	-42	-28	456	+28	+42
102	1.5-year contact	-42	-28	547	+28	+42
103	1.75-year contact	-42	-28	638	+28	+42
104	2-year contact	-42	-28	729	+28	+42
105	2.5-year contact	-84	-56	911	+56	+84
106	3-year contact	-84	-56	1093	+56	+84
107	4-year contact	-84	-56	1457	+56	+84
108	Final 5-year contact	-84	-56	1821	+56	+84

* All Target Days are relative to Day 1 of the main study (enrollment date); informed consent for follow-up can be collected any time prior and up to Visit 101

Appendix F Sample informed consent form

Sponsor / Study Title:	**National Institutes of Health / "A phase 1, randomized, open-label clinical trial to evaluate the safety and immunogenicity of BG505 MD39.3, BG505 MD39.3 gp151, and BG505 MD39.3 gp151 CD4KO HIV trimer mRNA vaccines in healthy, HIV-uninfected adult participants"**
Protocol Number:	**HVTN 302**
Principal Investigator: (Study Doctor)	**«PiFullName»**
Telephone:	**«IcfPhoneNumber»**
Address:	**«PiLocations»**

Thank you for your interest in our research study. Please read this consent form or ask someone to read it to you. If you decide to join the study, we will ask you to sign or make your mark on this form. We will offer you a copy to keep. We will ask you questions to see if we have explained everything clearly. You can also ask us questions about the study.

Research is not the same as treatment or medical care. The purpose of a research study is to answer scientific questions.

Key information

- This is the first study in which the study vaccines will be given to people.
- Being in this research study is voluntary. Whether you join or not is your choice.
- You are being asked to take part in this study because you are age 18-55, HIV negative and healthy.
- The purpose of this study is to see how a person's immune system responds to the 3 experimental vaccines (referred to as study vaccines throughout this form).
- The study will also see if the study vaccines are safe to give to people and do not make people too uncomfortable.
- You will be in this study for up to 12 months of clinic visits.
- Procedures will include blood draws and injections of study vaccines.
    - You may be asked if we can collect white blood cells from your blood and cells from your lymph nodes. White blood cells will be collected by a procedure called leukapheresis. Lymph node cells will be collected by a doctor using a very thin needle guided by

ultrasound. We will tell you more about these procedures later in this consent form.

- There are risks from participating:
  - Because these study vaccines have not been given to people before, we do not know what all of the risks may be. Common side effects from all vaccines include headache, fever, chills, rash, aches and pains, nausea, dizziness, and fatigue (feeling tired).
  - Taking blood and giving injections can cause bruising, pain, fainting, soreness, redness, swelling, itching, a sore and bleeding.
  - Collection of white blood cells (leukapheresis) and collecting cells from your lymph node can cause pain, bruising, and rarely an infection.
  - We will tell you more information about risks later in this consent form.
- We do not expect the study vaccines to benefit you in any way.

About the study

The HIV Vaccine Trials Network (HVTN) and the study site are doing this study to test 3 human immunodeficiency virus (HIV) vaccines. HIV is the virus that causes acquired immunodeficiency syndrome (AIDS).

Up to 108 people will take part in this study. The researcher in charge of this study at this clinic is called the study doctor. The US National Institutes of Health (NIH) is paying for the study.

1. **We are doing this study to answer several critical questions.**
   - Are the study vaccines safe to give to people?
   - Are people able to take the study vaccines without becoming too uncomfortable?
   - How do people's immune systems respond to the study vaccines? (Your immune system protects you from infections and disease.)
2. **The study vaccines cannot give you HIV.**

   The study vaccines are not made from HIV. It is impossible for the study vaccines to give you HIV. Also, they cannot cause you to give HIV to someone else.

   We do not know if the study vaccines will decrease, increase, or not change your risk of getting HIV if you are exposed to the virus.

3. **These study vaccines are experimental.**

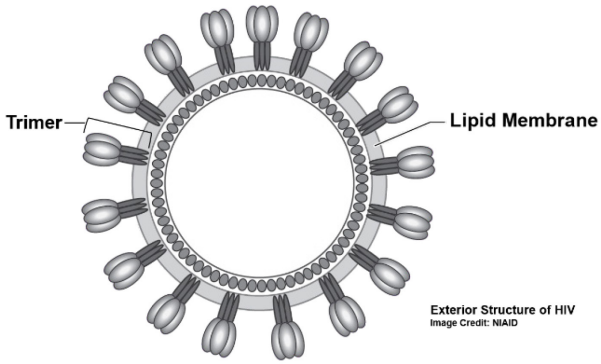
We are testing three experimental vaccines: BG505 MD39.3 mRNA, BG505 MD39.3 gp151 mRNA, and BG505 MD39.3 gp151 CD4KO mRNA. Experimental means they have not been approved by the U.S. Food and Drug Administration (FDA).

Together, they are called the either BG505 MD39.3 mRNA or mRNA1574 vaccines. From here on, we will call the vaccines the study vaccines. If you join the study, you will only be given 1 of the 3 study vaccines. These study vaccines were developed by researchers at Scripps Research, and are provided for the study by IAVI.

Each of these 3 study vaccines is made using messenger ribonucleic acid (mRNA) technology developed by Moderna, Inc. mRNA is a piece of genetic code carried into your body by the vaccine as a message with instructions in the same way that the mRNA vaccines against COVID instruct the body's cells to make the SARS-CoV-2 spike protein. Instead of showing your immune system actual pieces of HIV, the study vaccines carry instructions that show human muscle cells how to make small pieces that look like parts of HIV.

When people get a vaccine injection in the muscle of their arm, the cells in that muscle will get the instructions and start to make the different types of HIV pieces described below, and to display these pieces on the muscle cell's surface. The immune system will be able to see these HIV pieces and learn how to recognize them. Researchers hope that the immune system will respond by making antibodies and T-cells that could fight HIV if a person is ever exposed to the real virus in the future.

The mRNA instructions do their work in the part of the muscle cell called the cytoplasm. The mRNA does not get into the nucleus of the cells, which is where human DNA is located. The mRNA cannot interact with your DNA. The mRNA instructions only remain in the body for a couple of days before they break down naturally.



As shown in the picture, HIV has protein spikes on its surface. These spikes are made from 3 pieces, known as a trimer. The trimer has an outer end that it uses to bind onto human cells, and a "stalk" that connects to the inner part of the virus. This "stalk" extends through the outer membrane layer of the virus. Each of the 3 study vaccines is made slightly differently to see how the immune system will respond to these different structures.

- BG505 MD39.3 mRNA has the code for just the outermost part of the trimer.

- BG505 MD39.3 gp151 mRNA has the codes for the outermost part of the trimer, part of the "stalk," and a bit of the membrane layer.

- BG505 MD39.3 gp151 CD4KO mRNA also has the codes for the outermost part of the trimer, part of the "stalk," and a bit of the membrane layer, but a piece of the trimer called the CD4 binding site has been removed.

The study vaccines have not been given to people before. Studies in rats and rabbits showed that animals that got the vaccines had symptoms similar to the general risks of other vaccines described below. Even if something looks like it is safe or works in animals, it may not be true for people.

General risks of vaccines:

All vaccines can cause headache, fever, chills, rash, aches and pains, nausea, dizziness, and feeling tired. Vaccines can also cause redness, swelling or itching where you got the injection. Most side effects do not interfere with daily activities or make a person visit the doctor. With mRNA vaccines for other diseases, people may experience more of these side effects after getting the second dose of the vaccine.

Rarely, a vaccine can cause an allergic reaction, including a rash, hives, or trouble breathing. Allergic reactions can be life-threatening. Tell us if you ever had a bad reaction to an injection or vaccine.

Risks of the study vaccines:

- Hives: We previously told you about 2 participants that had developed itchy hives within 1-3 weeks after their first vaccinations. As of November 2022, these 2 participants still have some symptoms, although they have improved over time. They are using less medication and have less frequent symptoms.

  Since then, we have seen 5 more participants develop hives after getting the first or second injection of a study vaccine (out of 108 participants enrolled total). These reactions have been mild or moderate. Of these 5 new people, 4 began to experience hives 1-3 weeks after their study vaccination and have required medication to control outbreaks since then. The fifth person developed hives about 10 hours after getting the vaccination which went away by the next day without medication.

  All 7 participants have been able to continue with their daily activities even though most of them needed to use antihistamine medications like Zyrtec. As of November 8, 2022, all but one of the participants' symptoms have lasted at least 5 weeks, and the reactions have resolved or are resolving with use of medications.

  Enrollment in the trial is now complete, but participants continue to receive study vaccinations. Of the 7 participants who reported these reactions, two have

received additional doses of study vaccine and have not had a worsening of their initial symptoms.

Many people get hives in reaction to foods, creams or lotions or medicines such as ibuprofen. We don't have enough information to know why some study participants got hives after getting a study vaccine, but we will let you know if we learn anything new that might change your mind about participating in this study.

- **Myocarditis and pericarditis:** Myocarditis (inflammation of the heart muscle) and pericarditis (inflammation of the lining around the heart) have occurred very rarely in some people who got mRNA vaccines against COVID-19. As discussed above, the vaccines in this study also use mRNA technology. Reports have been more common in young adults who were assigned male sex at birth than in older people. In most of these people, symptoms began within a few days after the second dose of the vaccine. Almost all people have responded well to medications and rest, and their symptoms improved quickly. The chance of having this inflammation occur is very low. It is not known whether the risk of myocarditis or pericarditis is increased following mRNA vaccines for other diseases.

  You should seek medical attention right away and inform your site staff if you have any of the following symptoms after getting the study vaccines:
  - Chest pain
  - Shortness of breath
  - Fainting
  - Having a fast-beating, fluttering, or pounding heart.

There may be other side effects from these study vaccines that we don't know about. We will tell you if we learn about new side effects that could affect your willingness to stay in the study.

Joining the study

4. **It is up to you whether or not to join the study.**

   Take your time. Talk to people you trust. If you decide not to join this study or if you leave after you have joined, that will not affect your other care at this clinic and the benefits or rights you would normally have.

   You cannot be in this study while you are in another study where you get a study product. If you do not join this study, you may be able to join another study.

   During the study, you should not donate blood or tissue.

   Site: Remove item 5 if you use a separate screening consent that covers these procedures.

5. **If you want to join the study, we will screen you to see if you are eligible.**

   Screening involves a physical exam, HIV test, and health history. A physical exam may include, but is not limited to:

   - Checking your weight, temperature, and blood pressure
   - Looking in your mouth and throat
   - Listening to your heart and lungs
   - Feeling your abdomen (stomach and liver)
   - Asking about any vaccines you have gotten recently.

   We will also do blood tests. These tell us about the health of your kidneys and liver and whether or not you have Hepatitis B or C. The study doctor may be required by law to report the result of these tests to the local health authority.

   We will ask you about medicines you are taking, including HIV pre-exposure prophylaxis (PrEP). We will ask you about behaviors that might put you at risk for getting HIV. If you were assigned female sex at birth, we will test you for pregnancy.

   We will review the test results with you. They may show you are not eligible for the study, even if you want to participate.

   Site: adapt the following section per the care available at your site

6. **If we find that you have a health problem during screening or during the study, we will tell you about the care that we can give here at no cost.**

   For the care that we cannot give, we will help you get care elsewhere. For health problems unrelated to the study, we will not pay for care.

7. **If you were assigned female sex at birth and could become pregnant, you must use birth control to join this study.**

   Site: If you want to include Appendix I, Approved birth control methods (for sample informed consent form), in this consent form, paste it below and delete paragraph below.

   You should not become pregnant during the study because we do not know how the study vaccine could affect a developing baby. For this reason, you must agree to use effective birth control from 21 days before your first injection until 3 months after the time of your third study injection (9 months total). We will talk to you about effective birth control methods. They are listed on a handout that we will give to you.

Being in the study

If you join the study, here is what will happen:

8. **You will come to the clinic for scheduled visits about 12-15 times over 12 months.**

   Site: Insert number of visits and range of visit lengths. (There is site-specific variation in screening protocols and in the number of possible follow-up visits between protocol-mandated visits.)

   Visits can last from [#] to [#] hours.

   You may have to come for more visits if you have a lab or health issue.

   We may contact you after the study ends (for example, to tell you about the study results). We may also contact you about other studies you may want to join.

9. **We will give you compensation for each study visit you complete.**

   «Compensation»

   You will be paid up to a total of $xx.xx if you complete this study. You will be paid for the visits you complete according to the following schedule:

   c. $xx.xx for Visits xxx.

   d. $xx.xx for Visits xxx.

   e. $xx.xx for Visits xxx.

   This amount is to cover the costs of [*Site: Insert text*]. You will receive _____ ***["following each completed visit", "monthly", "quarterly", "at the end of your participation in the research study", "following each completed visit or at the end of your participation in the research study, whichever you prefer"]***.

   There is also additional compensation for participants in Groups 1-3 who have the optional study procedures. We will tell you more about these optional study procedures in Section 12 below.

   You will receive $xx.xx each time you have leukapheresis. This amount is to cover the costs of [*Site: Insert text*]. You will be paid _____ ***["following each completed visit", "monthly", "quarterly", "at the end of your participation in the research study", "following each completed visit or at the end of your participation in the research study, whichever you prefer"]***.

   You will receive $xx.xx each time you have a lymph node cell collection. This amount is to cover the costs of [*Site: Insert text*]. You will be paid _____ ***["following each completed visit", "monthly", "quarterly", "at the end of your participation in***

the research study", "following each completed visit or at the end of your participation in the research study, whichever you prefer"].

Payments you receive for being in the study may be taxable. We may need to ask you for your Social Security number for tax reasons.

Costs

You do not have to pay anything to be in this study. The study vaccine and all study procedures will be provided at no cost to you.

10. **We will give you the study vaccines on a schedule.**

You will be in one of the 6 study groups shown in the table below. You will get 3 injections of one of the study vaccines into the muscle of your upper arm during the study according to the schedule shown below. You have an equal chance of getting any of the 3 study vaccines. Whether you are enrolled in a lower dose group or higher dose group will depend on when you join the study.

Because this is the first time the study vaccines are being given to people, the study has planned pauses when safety information will be reviewed to decide if it is safe to continue the study. The first pause will be after the first 4 participants in Groups 1-3 have their first injection. If the safety review of these participants shows it is safe to continue, then enrollment will resume for the rest of the participants in the lower dose groups. At the same time, enrollment will start for the first 4 participants in Groups 4-6. There will be another pause for a review of the safety information after they receive their first injection. If the review shows it is safe to continue, then enrollment will resume for the rest of the participants in the higher dose groups.

Group	Number of participants	Injection schedule		
		At enrollment	At 2 months	At 6 months
1	18	lower dose of BG505 MD39.3 mRNA	lower dose of BG505 MD39.3 mRNA	lower dose of BG505 MD39.3 mRNA
2	18	lower dose of BG505 MD39.3 gp151 mRNA	lower dose of BG505 MD39.3 gp151 mRNA	lower dose of BG505 MD39.3 gp151 mRNA
3	18	lower dose of BG505 MD39.3 gp151 CD4KO mRNA	lower dose of BG505 MD39.3 gp151 CD4KO mRNA	lower dose of BG505 MD39.3 gp151 CD4KO mRNA
4	18	higher dose of BG505 MD39.3 mRNA	higher dose of BG505 MD39.3 mRNA	higher dose of BG505 MD39.3 mRNA
5	18	higher dose of BG505 MD39.3 gp151 mRNA	higher dose of BG505 MD39.3 gp151 mRNA	higher dose of BG505 MD39.3 gp151 mRNA
6	18	higher dose of BG505 MD39.3 gp151 CD4KO mRNA	higher dose of BG505 MD39.3 gp151 CD4KO mRNA	higher dose of BG505 MD39.3 gp151 CD4KO mRNA

You will have to wait in the clinic for about 30-60 minutes after getting injections to see if there are any problems. Then for that night and for 7 more days, you will use a secure online symptom log or a paper log to keep track of how you are feeling. If you are unable or unwilling to use the online symptom log, please talk with us about the option to use a paper log. Within 4 days after each injection visit, we will contact you to ask how you are doing. Contact the clinic staff if you have any issues or concerns after getting an injection.

As part of this research, you may need to use a phone or web app or an electronic study diary (eDiary). While using these, information about you may be collected and shared with the researchers or people outside of the study who have been approved by the researchers. This data might include personal health information, your contact information, and information about your use of the app such as the amount of time you spend on each screen. The eDiary does not collect personal information about your activities over time or from other websites or online services. It also does not allow third parties to collect that information. A complete description of the data collection and sharing can be found in the Privacy Policy associated with the app. If you would like to read these documents, the study doctor can tell you how to access this information.

The Privacy Policy may include statements that limit your rights if you are harmed by using the app in this study. You do not release the study doctor, sponsor, this institution, or the research staff for responsibilities from mistakes. You also do not waive any of your rights as a research participant.

11. **In addition to giving you the study vaccines, we will do the procedures shown in the following table.**

		Time after first injection visit													
Procedure	**Screening visit(s)**	**First injection visit**	**1 week**	**2 weeks**	**1½ months**	**2 months**	**2 ¼ months**	**2½ months**	**3 months**	**6 months**	**6 ¼ months**	**6½ months**	**7 months**	**8 months**	**12 months**
Injection		√				√				√					
Medical history	√														
Complete physical	√														√
Brief physical		√	√	√	√	√	√	√	√	√	√	√	√	√	
Blood drawn	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Lymph node cell collection*					√				√				√		
Leukapheresis*								√				√			
Pregnancy test**	√	√			√	√		√	√	√		√	√	√	
HIV testing	√							√		√				√	√
Risk reduction counseling	√	√		√		√		√		√		√		√	√
Interview/questionnaire	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

Notes:
Grey shading: These additional visits are only for participants who provide lymph node cell samples.
* Leukapheresis and lymph node cell collection will be done if you agree to these optional procedures and are eligible (Groups 1-3 only).
** For persons who were assigned female sex at birth and who are capable of becoming pregnant. A negative pregnancy test is required within 48 hours before lymph node cell collection and leukapheresis. Persons who have had a hysterectomy (removal of the uterus) or removal of both ovaries (verified by medical records), do not have to have pregnancy tests.

We will review test results with you at your next visit, or sooner if necessary. We will tell you about any results that are important to your health.

When we take blood, the amount will depend on the lab tests we need to do. It will be some amount between 15 mL and 325 mL (a little more than 1 tablespoon to a little more than 1 cup) depending on the visit. Your body will make new blood to replace the blood we take out. To compare, people who donate blood in the US can give a total of about 500 mL (about 2 cups) in an 8-week period.

Site: Of the following optional procedures for participants in Groups 1-3, include only those available to your participants and edit the information as needed to reflect how they will be done at the site/procedure facility.

12. **Optional samples: if you are willing and eligible, there are 2 optional procedures to collect more samples.**

We would like to collect these samples from about half of the participants in Groups 1-3. If you agree, we will collect one or two other samples to test your immune responses. Immune responses to vaccines involve cells in blood and in lymph nodes near the injection site. We would like to collect samples from these parts of the body to learn more about how the immune system responds to the study vaccines. We hope to see if we can detect any rare cells that respond to the study vaccines, and whether

these ways of making vaccines can stimulate the right kinds of cell responses. This information will guide the ways that vaccines are designed in the future.

Both types of samples will not be collected by every clinic participating in the study. Some of the sample collections will be done at other locations instead of this study clinic.

At the end of this form, we will ask if you agree to one or both of these sampling procedures. You can agree now and change your mind later. Even if you agree to give these samples, you may not be eligible to do so but you will remain in the study. You can decide not to give any of these samples and still be in the study.

Site: Sites must obtain copies of the informed consent form and any other educational materials that are available from the facilities where leukapheresis and FNA will be performed, so that they can be reviewed with potential participants during the study informed consent process

Leukapheresis procedure:

This procedure collects larger amounts of white blood cells than we could get in an ordinary blood sample. Blood is made up of red cells that carry oxygen, white cells that fight infection, platelets that help form clots, and plasma, which is the fluid left over when all the cells are removed. During leukapheresis, the white blood cells are removed, and the rest of the blood is put back into the body. To get the number of white blood cells we need for this research, this procedure should take about an hour.

The leukapheresis procedure will be done at a separate facility. Your eligibility to have this procedure will be decided by the study staff at our clinic and at the facility before you have the procedure. There will be another consent form for you to review and sign at the facility. It will provide additional details about the procedure and any risks involved.

For the procedure, a clinician will insert a sterile needle into a vein in each of your arms. The needles are attached to tubes. Your blood will go out of your body through one tube and into a machine that separates the blood and takes out the white blood cells. After the white blood cells are taken out, the rest of the blood will go back into your body through the tube going into your other arm. Sometimes the fluid lost during the procedure is replaced by a sterile salt water solution, or a solution containing a protein called albumin. This protein is normally found in human blood. An anticoagulant may be added to your blood during the procedure. Anticoagulants prevent blood from clotting.

It is normal to feel tired for up to 24 hours after having leukapheresis.

The leukapheresis procedure will be done about 2 weeks after your 2nd injection visit and again about 2 weeks after your 3rd injection visit.

Risks of leukapheresis

Generally, the risks of leukapheresis include pain, bruising, possibly fainting, and rarely, infection. Rarely, albumin can cause an allergic reaction. If the leukapheresis procedure has to be stopped, it could result in the loss of up to 1 cup of blood. Your body makes new blood within 2 weeks.

If you notice any symptoms during leukapheresis, please let the nurse know immediately. Usually the symptoms can be reversed quickly by adding fluid or by slowing down the procedure. If there are any problems, the staff will use the appropriate medical procedures to treat you.

Lymph node cell collection procedure:

The formal name for this procedure is a "Fine Needle Aspiration". This procedure collects cells from lymph nodes. Lymph nodes are one of the key places where immune responses develop, and they are located in several places on the human body. In this procedure, a doctor will use a very thin needle guided by ultrasound to collect cells from a lymph node on your arm or in your arm pit. This procedure allows researchers to understand the early immune responses that develop, before they can be seen in a blood sample.

The lymph node cell collection procedure will be done at a separate facility three times as shown in the shaded columns in the table above. Your eligibility to have this procedure will be decided by the staff at our clinic during screening, and again at the facility before you have the procedure. There will be another consent form for you to review, sign, and date at the facility. It will provide additional details about the procedure and any risks involved. The procedure usually takes about [#] hour(s).

Lymph node cell collection will happen about 6 weeks after your first injection visit and about 4 weeks after your second and third injection visits. In addition to having the procedure at a separate facility, you will also come to the study clinic at these 3 times to provide a blood sample and to answer study questionnaires.

To do the procedure, a clinician will find the appropriate lymph node by applying a cold gel to your skin and then pressing a hand-held ultrasound device against your skin. This device produces images of your lymph node that the clinician can watch on a computer monitor. If necessary, a small area near the lymph node may be shaved. Once the node is found, a cleaning solution will be applied to the skin in that area. The area will be numbed by an injection of a local anesthetic, such as lidocaine, which is like the numbing medicine used by dentists. The doctor will insert a very thin needle and use the images from the ultrasound to guide it into the lymph node. The needle may be moved up and down to collect cells from the lymph node. Fluid may also be collected through the needle into a syringe or bottle. Each cell collection lasts about 10-15 seconds. It usually takes about 4 separate cell collections to get enough cells.

After the procedure is done, the area will be cleaned with warm water. A band-aid will be used to cover the site where the needle was inserted.

Risks of lymph node cell collection:

The main risks of this procedure include pain, bruising, bleeding and infection where the needle is inserted. The cleaning solution or shaving the area may cause skin irritation. Injecting the local anesthetic may sting or burn for a little while until the numbing takes effect. The local anesthetic can rarely cause temporary arm numbness or weakness. Examining the lymph node area may be uncomfortable.

If discomfort, problems, or side effects happen during or after lymph node cell collection, the staff will use the appropriate medical procedures to treat you.

13. **We will counsel you about protecting yourself from HIV.**

    We will ask you questions about your HIV risk factors such as sexual behavior, alcohol, and drug use. We will talk with you about ways to keep your risk of getting HIV low.

    These vaccines are experimental and are not expected to protect you from getting HIV during this study.

14. **The HVTN will test your samples to see how your body, including your immune system, responds to the study vaccine.**

    We will send your samples (without your name or other identifying information) to labs approved by the HVTN for this study. In rare cases, some of your samples may be sent to labs in other countries for research related to this study.

    Researchers may also do genetic testing on your samples. Your genes are passed to you from your birth parents. They affect how you look and how your body works. This genetic testing will involve only some of your genes, not all of your genes (your genome). It will involve genes related to the immune system and HIV.

    If you get HIV, the researchers may look at all of the genes of the virus found in your samples. The researchers will use this information to learn more about HIV and how the virus is impacted by the study vaccine.

    In some cases, researchers may take cells from your samples and grow more of them over time, so that they can continue to contribute to this study.

    These tests done on your samples are for research purposes, not to check your health. The labs will not give the results to you or this clinic because their tests are not approved for use in making health care decisions. These labs are only approved to do research tests.

15. **When your samples are no longer needed for this study, the HVTN will continue to store them. When samples are no longer needed for this study, the HVTN wants to use them in other studies and share them with other researchers.**

    The HVTN calls these samples “extra samples”. The HVTN will only allow your extra samples to be used in other studies if you agree to this. You will mark your decision at the end of this form. If you have any questions, please ask.

Do I have to agree? No. You are free to say yes or no, or to change your mind after you sign this form. At your request, the HVTN will destroy all extra samples that it has. Your decision will not affect your being in this study or have any negative consequences here.

Where are the samples stored? Extra samples are stored in a secure central place called a repository. Your samples will be stored in the HVTN repository in the United States.

How long will the samples be stored? There is no limit on how long your extra samples will be stored.

Will I be paid for the use of my samples? No. Also, a researcher may make a new scientific discovery or product based on the use of your samples. If this happens, there is no plan to share any money with you. The researcher is not likely to ever know who you are.

Will I benefit from allowing my samples to be used in other studies? Probably not. Results from these other studies are not given to you, this clinic, or your doctor. They are not part of your medical record. The studies are only being done for research purposes.

Will the HVTN sell my samples and information? No, but the HVTN may share your samples with HVTN or other researchers. Once the HVTN shares your samples and information, it may not be able to get them back.

How do other researchers get my samples and information? When a researcher wants to use your samples and information, their research plan must be approved by the HVTN. Also, the researcher's institutional review board (IRB) or ethics committee (EC) will review their plan. IRBs/ECs protect the rights and well-being of people in research. If the research plan is approved, the HVTN will send your samples to the researcher's location.

What information is shared with HVTN or other researchers? The samples and information will be labeled with a code number. The key to the code will stay at this clinic. It will not be shared with the HVTN, other researchers, or with anyone else who does not need to know your name. Your name will not be part of the information. However, some information that the HVTN shares may be personal, such as your race, ethnicity, sex, health information from the study, and HIV status. The HVTN may share information about the study product you received and how your body responded to the study product.

What kind of studies might be done with my extra samples and information? The studies will be related to HIV, vaccines, the immune system and other diseases.

Researchers may also do genetic testing on your samples.

In some cases, researchers may take cells from your samples and grow more of them over time, so that they can continue to do research with them.

If you agree, your samples could also be used for genome-wide studies. In these studies, researchers will look at all of your genes (your genome). The researchers compare the genomes of many people, looking for common patterns of genes that could help them understand diseases. The researchers may put the information from the genome-wide studies into a protected database so that other researchers can access it, but your name and other personal information will not be included. Usually, no one would be able to look at your genome and link it to you as a person. However, if another database exists that also has information on your genome and your name, someone might be able to compare the databases and identify you. If others found out, it could lead to discrimination or other problems. The risk of this is very small. There may be other unknown risks.

Who will have access to my information in studies using my extra samples?

People who may see your information are:

- Researchers who use your extra samples and information for other research
- Government agencies that fund or monitor the research using your extra samples and information
- The researcher's Institutional Review Board or Ethics Committee
- Any regulatory agency that reviews clinical trials
- The people who work with the researcher

All of these people will do their best to protect your information. The results of any new studies that use your extra samples and information may be published. No publication will use your name or identify you personally.

16. We will do our best to protect your private information.

All of your samples and most of your study records will be labeled with a code number. Samples and study records are kept in secure locations. When you provide information in the online symptom log after the injection visits, that information only has your code number. Your data goes directly from the online symptom log into your study record.

Site: Any change to the following boxed text requires approval from HVTN Regulatory Affairs. You can remove the box around the text.

We do need to share your name with the HVTN in case you need proof in the future that you participated in an HIV vaccine study. The HVTN will keep your name in a secure file with these items:

- The name of your study
- Your age or date of birth
- Your study ID number
- What study vaccines(s) you received

No HIV test results are kept in this file. The HVTN will not share any information that could identify you without your agreement. The HVTN will remove your name from the file if you do not want it there.

Clinic staff can see your study records. Your records may also be reviewed by groups that watch over this study to see that we are protecting your rights, keeping you safe, and following the study plan. These groups include:

- The US National Institutes of Health and its study monitors,
- The US Food and Drug Administration,
- Any regulatory agency that reviews clinical trials,
- Advarra IRB
- Moderna, Inc., Scripps Research, IAVI, and people who work for them,
- The HVTN and people who work for them,
- The HVTN Safety Monitoring Board, and
- The US Office for Human Research Protections.

All reviewers will keep your records private.

We cannot guarantee absolute privacy. If you have a medical condition that we are required to report by law, then some of your information may be shared. At this clinic, we have to report the following information:

Site: Include any public health or legal reporting requirements. Bulleted examples should include all appropriate cases (reportable communicable disease, risk of harm to self or others, etc.) If your site does not have public health or legal reporting requirements, you may delete the last sentence in the paragraph above, along with the bullets below.

- [Item 1]
- [Item 2]
- [Item 3]

To help protect your privacy, we have a Certificate of Confidentiality from the US government. With the certificate, we do not have to release information about you to someone who is not connected to the study, such as the courts or police. The Certificate of Confidentiality does not prevent our clinic from reporting certain medical conditions as required by law (see above). However, we cannot withhold information from the US government because it funds this research. You can still give information about yourself and your study participation to others. If you want your research information released to an insurer, medical care provider, or any other person not connected with the research, you must provide consent to allow the researchers to release it.

The results of this study may be published. No publication will use your name or identify you personally.

We may share information from the study with other researchers. We will not share your name or information that can identify you.

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. The website may include a summary of the study results. You can search this Web site at any time.

17. We may stop your injections even if you want to stay in the study and even if you were scheduled for more injections.

We will stop your injections if you become pregnant. We will encourage you to stay in the study, but it will be your choice. If you leave the study while you are pregnant, we will contact you after your due date to ask some questions about your pregnancy and delivery.

We will stop your injections if you get HIV. We will also take fewer samples, and we will help you get care and support. We will encourage you to stay in the study for up to 12 months if you choose. We will counsel you about having HIV and about telling your partner(s). We will not provide or pay for your HIV care.

We will stop your injections if you enroll in a different research study where you get another study product.

18. We may take you out of the study at any time.

We may take you out of the study if:

- you do not follow instructions,

- we think that staying in the study might harm you, or
- the study is stopped for any reason.

Other Risks

19. There are other risks to being in this study.

In addition to the risks of the study vaccine and the procedures that were described above, this section describes the other risks we know about. There may be other risks, even serious ones. We will tell you if we learn anything new that may affect your decision to stay in the study.

Risks of abnormal laboratory results

Minor changes in laboratory test results occasionally happen. This means that the test results can show something to be abnormal when it is not. If this happens, we will ask you to come back to the clinic to be retested. This may cause you to worry, and it may be inconvenient to come back to the clinic. If retesting confirms something to be abnormal, we will provide care or help you get the care you need.

Risks of routine medical procedures:

Routine medical procedures such as taking blood and giving injections can cause bruising, pain, fainting, soreness, redness, stinging, swelling, itching, a sore, bleeding, and rarely infection where you got the injection. Taking blood can cause a low blood cell count (anemia), making you feel tired.

Personal problems/discrimination/testing HIV antibody positive:

Some people report personal problems or discrimination because they joined an HIV vaccine study. Family or friends may worry, get upset, or assume that you have HIV. Rarely, someone has lost a job because the study took too much time away from work, or because their employer thought they had HIV.

Most vaccines cause the body to make antibodies to prevent infection. Your body may make antibodies to HIV because you received an HIV study vaccine. Those antibodies could cause you to test positive on some types of HIV tests, even if you do not have HIV. This is called vaccine-induced seropositivity (VISP). VISP means that after you get the study vaccine, a routine HIV test done outside this clinic is likely say you have HIV, even if you don't. For this reason, you should get HIV tests only at this clinic. Our tests can tell the difference between true HIV infection and a positive result caused by the study vaccine. If you have VISP, we can arrange free HIV testing for as long as you need it.

It is unlikely, but you could test antibody negative at the end of the study and then test positive sometime later, even though you don't have HIV.

Site: Modify the following paragraph if applicable. If someone believes you have HIV, you could face discrimination and other problems. In some countries, you could be denied medical or dental care, employment, insurance, a visa, or entry into the military. If you have VISP, you will not be able to donate blood or organs. Your family and friends may treat you differently. We will give you a brochure that tells you more about VISP, and how you can avoid some of these problems.

If you become pregnant during or after the study and have VISP, the antibodies might be passed to your baby. We know that this happens with some other vaccines. The antibodies are not a danger to the baby, and they go away, usually in about 6 months.

You should tell the delivery staff if you have VISP. However, you may still be tested for HIV using the antibody test when you deliver your baby. If your test is positive, your baby may be started on antiretroviral treatment when it is not needed. If this happens, we can arrange for you and the baby to have tests that can distinguish true HIV infection from VISP. If you or the baby continue to have VISP, we can arrange this testing for free for as long as it is needed.

Embarrassment/anxiety:

You may feel embarrassed when we ask questions about HIV risks, such as sex and using drugs. Also, waiting for HIV test results could make you feel anxious. You could feel worried if test results show that you have HIV. If you feel embarrassed or anxious, please tell us and we will try to help you.

Risks of disclosure of your personal information:

Although the risk is very low, it is possible that someone may see your personal information who should not. If that happens, you could face discrimination, stress, and embarrassment. We can tell you more about how we will protect your personal information.

Risks of genetic testing:

It is possible that genetic tests could show you may be at risk for certain diseases. If others found out, it could lead to discrimination or other problems. However, genetic test results are not part of your study record, so it is almost impossible for anyone to connect them to you personally.

Even if your genetic information somehow gets linked to your name, a federal law called the Genetic Information Nondiscrimination Act (GINA) helps protect you. GINA keeps health insurance companies and employers from seeing results of genetic testing when deciding about giving you health insurance or offering you work. GINA does not help or protect you against discrimination by companies that sell life, disability, or long-term care insurance.

This law generally will protect you in the following ways:

- Health insurance companies and group health plans may not request your genetic information that the sponsor will get from this research.

- Health insurance companies and group health plans may not use your genetic information when making decisions regarding your eligibility or premiums.

- Employers with 15 or more employees may not use your genetic information that the sponsor will get from this research when making a decision to hire, promote, or fire you or when setting the terms of your employment.

All health insurance companies and group health plans and all employers with 15 or more people must follow this law.

Unknown risks:

We do not know if the study vaccine will increase, decrease, or not change your risk of getting HIV if exposed. If you get HIV, we do not know how the study vaccine might affect your HIV infection or how long it takes to develop AIDS.

We do not know if getting this study vaccine will affect how you respond to any future approved HIV vaccine. Currently, no HIV vaccine has been approved for use.

We do not know how the study vaccine will affect a pregnant participant or a developing baby.

Benefits

20. The study vaccination will not benefit you.

We do not expect the study vaccine to benefit you in any way. However, being in the study might still help you in some ways. The counseling that you get as part of the study may help you avoid getting HIV. The lab tests and physical exams might detect health problems you don't yet know about.

When asked, most study participants say that participating in a study made them feel good about helping others, increased their knowledge about HIV, and improved their self- esteem.

This study may help in the search for a vaccine to prevent HIV. However, if the study vaccine later becomes approved for sale, there are no plans to share any money with you.

Your rights and responsibilities

21. If you join the study, you have rights and responsibilities.

We list these in the Bill of Rights and Responsibilities (BRR) for HIV Research. We will give you a copy of it.

Leaving the study

22. Tell us if you decide to leave the study.

You are free to leave the study at any time and for any reason. This will not affect your care at this clinic and your legal rights.

Previously collected information about you will remain in the study records and will be included in the analysis of results. Your information cannot be removed from the study records.

We will ask you to come to the clinic one last time for a physical exam. We may also ask to take some blood samples and ask you to answer some questions. If you were assigned female sex at birth, we may test you for pregnancy. Whether you come for this last visit is up to you.

Injuries

23. If you get sick or injured during the study, contact us immediately.

Your health is important to us. We will tell you about care that we can give here. For care that we cannot provide, we will explain how we will help you get care elsewhere.

If you become sick or injured in this study, the HVTN has a process to decide if this is related to the study vaccine and/or procedures. If it is, we call it a study-related injury. There are funds to pay for treatment of study-related injuries if certain conditions are met.

The study product provider has agreed to pay medical costs for study-related injuries that are determined to be caused by the study product. If provider funds are not enough, or if the injury is determined to be caused by study procedures, the HVTN has limited funds to pay medical costs that it determines are reasonable. If the injury is not study related, then you and/or your health insurance will be responsible for treatment costs.

Some injuries are not physical. For example, you might be harmed emotionally by being in an HIV vaccine study. Or you might lose wages because you cannot go to work. However, there are no funds to pay for these kinds of injuries, even if they are study related.

You may disagree with the decision about whether your injury is study related. If you wish, the HVTN will ask independent experts to review the decision. You always have the right to use the court system if you are not satisfied.

Questions

24. Whom to contact about this study

During the study, if you experience any medical problems, suffer a research-related injury, or have questions, concerns or complaints about the study, please contact the study at the telephone number listed on the first page of this consent document. If you seek emergency care, or hospitalization is required, alert the treating physician that you are participating in this research study.

An institutional review board (IRB) is an independent committee established to help protect the rights of research participants. If you have any questions about your rights as a research participant, and/or concerns or complaints regarding this research study, contact:

- By mail:
  Study Subject Adviser
  Advarra IRB
  6100 Merriweather Dr., Suite 600
  Columbia, MD 21044
- or call **toll free**: 877-992-4724
- or by **email**: adviser@advarra.com

Please reference the following number when contacting the Study Subject Adviser: Pro00056475.

Your permissions and signature

25. In Section 12 of this form, we told you about 2 other optional sample collection procedures. Please write your initials or make your mark in the boxes next to the procedure(s) that you agree to have done, or leave them blank if you do not want to provide these extra samples. You can change your mind after signing this form. There will also be an additional consent form to sign at the time the procedure is done.

Site: Include boxes below only for the procedures available to your participants in Groups 1-3.

☐ I agree to leukapheresis.

☐ I agree to lymph node cell collection.

26. In Section 15 of this form, we told you about possible other uses of your extra samples and information, outside this study. Please choose only one of the options below and write your initials or make your mark in the box next to it. Whatever you choose, the HVTN keeps track of your decision about how your samples and information can be used. You can change your mind after signing this form.

☐ I allow my extra samples and information to be used for other studies related to HIV, vaccines, the immune system, and other diseases. This may include genetic testing and keeping my cells growing over time.

OR

☐ I agree to the option above *and* also to allow my extra samples and information to be used in genome wide studies.

OR

☐ I do not allow my extra samples to be used in any other studies. This includes not allowing genetic testing, growing more of my cells, or genome wide studies.

27. If you agree to join this study, you will need to sign or make your mark below. Before you sign or make your mark on this consent form, make sure of the following:

- You have read this consent form, or someone has read it to you.
- You feel that you understand what the study is about and what will happen to you if you join. You understand what the possible risks and benefits are.
- You have had your questions answered and know that you can ask more.
- You agree to join this study.

You will not be giving up any of your rights by signing this consent form.

Participant's name (print)	Participant's signature or mark	Date	Time

Clinic staff conducting consent discussion (print)	Clinic staff signature	Date	Time

For participants who are unable to read or write, a witness should complete the signature block below:

Witness's name (print)	Witness's signature	Date	Time

*Witness is impartial and was present for the entire discussion of this consent form.

Appendix G Sample Addendum Informed Consent Form for follow-up of participants with unresolved study product-related urticaria-associated symptoms

Sponsor / Study Title:	**National Institutes of Health / "A phase 1, randomized, open-label clinical trial to evaluate the safety and immunogenicity of BG505 MD39.3, BG505 MD39.3 gp151, and BG505 MD39.3 gp151 CD4KO HIV trimer mRNA vaccines in healthy, HIV-uninfected adult participants"**
Protocol Number:	**HVTN 302**
Principal Investigator: (Study Doctor)	**«PiFullName»**
Telephone:	**«IcfPhoneNumber»**
Address:	**«PiLocations»**

You are a participant in HVTN 302, a research study that tests an experimental vaccine against HIV. Thank you for your participation.

We have new information to share with you. Previously we told you about 7 different participants (out of 108 participants enrolled) that developed itchy hives and other related skin symptoms after getting their first or second vaccination. You are 1 of the participants who continue to have these symptoms. In order to continue to monitor your health, we are asking you to continue in the study for up to 4 years for additional follow-up.

Please read this consent form or ask someone to read it to you. If you decide to join the follow-up part of the study, we will ask you to sign or make your mark on this form. We will offer you a copy to keep. We will ask you questions to see if we have explained everything clearly. You can also ask us questions about the study.

1. **Information about the follow-up part of the study**

We are extending the study to continue to monitor the health of a small number of participants (including you) who continue to have hives/itchy rashes. You will be followed until the symptoms go away completely, or for up to 4 years from now, whichever time period is shortest.

We are doing this follow-up to see whether these symptoms go away over time and how they affect your quality of life. Most of the extra study visits will be done remotely, such as by phone. You will be asked to come in for at least two clinic visits. If the study staff feel that it is needed, you will be asked to come to the clinic for ~~an~~ additional in-person visits.

2. **What happens now?**

We will contact you every 3 months during the coming year, twice a year for the second year, and once a year during the third and fourth years. If your symptoms go away sooner, you will have less contacts and your participation can end. You will come into the clinic to provide a blood sample at the 6 month visit, at any clinic visit scheduled to evaluate your symptoms (if needed), and your last visit. The blood samples you provide may allow us to see any changes in your immune system over time. The amount of blood collected at any one visit will be about 17 mL (a little more than a tablespoon). The table below shows the schedule of the visits and procedures.

Follow-up study schedule									
Procedure	**3 months**	**6 months**	**9 months**	**1 year**	**1½ years**	**2 years**	**3 years**	**4 years**	**Clinic visit due to symptoms**
Medical history	√	√	√	√	√	√	√	√	√
Brief physical exam*	√	√	√	√	√	√	√	√	√
Interview/questionnaire	√	√	√	√	√	√	√	√	√
Blood collection***		√						√**	√

* Only if an in-person clinic visit is needed.

** Your last blood collection may happen before the 4-year visit if your symptoms go away sooner.

*** In addition to the 6-month and 4-year blood collections, blood samples will also be collected at any clinic visit scheduled due to having symptoms.

During these visits, we will:

- Ask questions about your health, including medications you may be taking;
- Ask questions about your quality of life and current health in relation to your hives and other symptoms;
- Do a physical exam if an in-person clinic visit is needed.
- Collect a blood sample

3. **Some things from the main study remain the same.**

   Some information described in the original consent form that you signed remains the same, such as:

   - The potential risks and benefits of study participation that are not related to the study products;
   - Your rights and responsibilities as a study participant;
   - How your samples will be used;
   - How we will protect your private information and who can access your study records;
   - Reasons we might end your participation in the study;
   - What will happen if you get sick or injured during the study.

   As before, there is no cost to you for participating in the study. We will give you [Site: Insert compensation] for each study visit you complete.

   In the original consent form, you chose whether the HVTN could use your extra samples and information in other studies. The HVTN will continue to honor your choice. You can change your mind if you want to at any time. Your decision will not affect your being in this study or have any negative consequences.

   In the original consent form, we told you about the requirement to use contraception. Since enough time has passed since your last study injection, use of contraception is no longer required for the purpose of this study.

4. **You can continue or leave the follow-up study at any time.**

   You are still free to leave the study at any time and for any reason. If you want to leave, please tell us. If you choose to leave, your other care at this clinic and the benefits or rights you would normally have will not be affected.

5. **Study Contacts**

   During this follow-up part of the study, if you have any medical problems, have a research-related injury, or have questions, concerns, or complaints about the study, please contact the study staff at the telephone number listed on the first page of this consent form. If you seek emergency medical care or if hospitalization is required, alert the doctor treating you that you are participating in this research study.

   An institutional review board (IRB) is an independent committee that helps protect the rights of research participants. If you have any questions about your rights as a research participant, and/or concerns or complaints about this research study, contact:

- By mail:

  Study Subject Adviser
  Advarra IRB
  6100 Merriweather Dr., Suite 600
  Columbia, MD 21044

- or call **toll free**: 877-992-4724

- or by **email**: adviser@advarra.com

Please reference the following number when contacting the Study Subject Adviser: Pro00056475.

6. **If you agree to join this follow-up part of the study, you will need to sign or make your mark below. Before you do, make sure of the following:**

- You have read this consent form, or someone has read it to you.
- You feel that you understand what this new part of the study is about and what will happen to you if you join. You understand what the possible risks and benefits are.
- You have had your questions answered and know that you can ask more.
- You agree to join this study.

You will not be giving up any of your rights by signing this consent form.

Participant's name (print)	Participant's signature or mark	Date	Time

Clinic staff conducting consent discussion (print)	Clinic staff signature	Date	Time

For participants who are unable to read or write, a witness should complete the signature block below:

Witness's name (print)	Witness's signature	Date	Time

*Witness is impartial and was present for the entire discussion of this consent form.

Appendix H Low risk guidelines for US

The following are intended as guidelines for the investigator to help identify potential vaccine trial participants at "low risk" for HIV infection. These guidelines are based on behaviors within the last 6-12 months prior to enrollment; however, it may be appropriate to consider a person's behavior over a longer period of time than specified to assess the person's likelihood of maintaining low risk behavior. *Some volunteers may not be appropriate for enrollment even if they meet these guidelines.* These guidelines should be supplemented and interpreted with local epidemiologic information about HIV prevalence in your area and community networks. The investigator may review the risk level of any volunteer with the site PI and/or the Protocol Safety Review Team.

A volunteer may be appropriate for inclusion if he/she/they meets these guidelines:

A. For US volunteers NOT on stable Pre-exposure prophylaxis (PrEP)

1. **SEXUAL BEHAVIORS**
   In the **last 12 months** did not:
   A. Have oral, vaginal or anal intercourse with an HIV-infected partner, or a partner who uses injection drugs
   B. Give or receive money, drugs, gifts or services in exchange for oral, vaginal or anal sex

   AND

   In the **last 6 months** has abstained from penile/anal or penile/vaginal intercourse, OR

   In the **last 6 months** had 4 or fewer partners of the opposite birth sex for vaginal and/or anal intercourse, OR

   Is an MSM (person born male with partner(s) born male) who, in the **last 12 months**:

   - Had 2 or fewer MSM partners for anal intercourse and had no unprotected anal sex with MSM, OR
   - Had unprotected anal intercourse with only 1 MSM partner, within a monogamous relationship lasting at least 12 months (during which neither partner had any other partners). If the monogamous relationship ended, the volunteer may then have had protected anal intercourse with 1 other MSM partner (total 2 or fewer partners in the last 12 months), OR

   Is a transgender person, regardless of the point on the transition spectrum, having sex with men (born male) and/or other transgender persons, who in the **last 12 months**:

   - Had 2 or fewer partners for anal or vaginal intercourse, and had no unprotected anal or vaginal sex, OR
   - Had unprotected anal or vaginal intercourse sex with 1 partner only within a monogamous relationship lasting at least 12 months (during which neither partner had any other partners). If the monogamous relationship ended, may then have

had protected anal or vaginal sex with 1 other partner (total 2 or fewer partners in the last 12 months).

AND

Uses or intends to use condoms in situations which may include penile/anal or

penile/vaginal intercourse with new partners of unknown HIV status, occasional partners,

partners outside a primary relationship, and/or partners known to have other partners.

2. NON-SEXUAL BEHAVIORS

In the **last 12 months** did not:

- Inject drugs or other substances without a prescription
- Use cocaine, methamphetamine, or excessive alcohol, which in the investigator's judgment, rendered the participant at greater than low risk for acquiring HIV infection.

  The investigator's judgment should consider local epidemiologic information about HIV prevalence in the area and community networks.

A volunteer is NOT appropriate for inclusion if he/she:

Acquired an STI (ie, new infection) in the **last 12 months**:

- Syphilis
- Gonorrhea
- Non-gonococcal urethritis
- Herpes Simplex Virus type 2 (HSV2)
- Chlamydia
- Pelvic inflammatory disease (PID)
- Trichomonas
- Mucopurulent cervicitis
- Epididymitis
- Proctitis
- Lymphogranuloma venereum
- Chancroid
- Hepatitis B

B. For US volunteers on Pre-exposure prophylaxis (PrEP)

1. PrEP ASSESSMENT

- Reports equal to or greater than six months of protective PrEP use
- Commits to maintaining protective PrEP use throughout trial

- Participant reports equal to or greater than 70% when asked the following:

"*Thinking about the past 4 weeks, what percent of the time were you able to take all your PrEP medications?"*

2. SEXUAL BEHAVIORS

Persons stably taking PrEP as described above for 6 months or longer are considered low risk of HIV infection, regardless of any sexual behavior that might otherwise be associated with high risk of HIV exposure.

3. NON-SEXUAL BEHAVIORS

In the **last 12 months** did not:

- Inject drugs or other substances without a prescription
- Use cocaine, methamphetamine, or excessive alcohol, which in the investigator's judgment, rendered the participant at greater than low risk for acquiring HIV infection.

The investigator's judgment should consider local epidemiologic information about HIV prevalence in the area and community networks.

Appendix I Approved birth control methods

Site: Any change to the following boxed text requires approval from HVTN Regulatory Affairs at vtn.core.reg@hvtn.org. You can remove the box around the text.

You should not become pregnant during the study because we do not know how the study vaccine could affect the developing baby.

You must agree to use effective birth control from 21 days before your first injection until 3 months after your last study injection.

Effective birth control means using any of the following methods every time you have sex:

- Birth control drugs that prevent pregnancy—given by pills, shots, patches, vaginal rings, or inserts under the skin;
- Male or female condoms, with or without a cream or gel that kills sperm;
- Diaphragm or cervical cap with a cream or gel that kills sperm;
- Intrauterine device (IUD); or
- Any other contraceptive method approved by the researchers.

You do not have to use birth control if:

- You are only having sex with a partner or partners who have had a vasectomy. (We will ask you some questions to confirm that the vasectomy was successful.);
- You have reached menopause, with no menstrual periods for one year;
- You have had a hysterectomy (your uterus removed);
- You have had your ovaries removed;
- You have a tubal ligation (your "tubes tied") or confirmed successful placement of a product that blocks the fallopian tubes;
- You are having sex only with a partner(s) assigned female sex at birth;
- You only have oral sex; or,
- You are sexually abstinent (no sex at all).

Remember: If you are having sex, male and female condoms are the only birth control methods that also provide protection against HIV and other sexually transmitted infections.

If you join the study, we will test you for pregnancy at some visits, including before each study injection.

Appendix J Case definition for Myocarditis and pericarditis

I. Brighton collaboration myocarditis case definition, by level of certainty – Level 1 (definitive), 2 (probable), and 3 (possible) (https://brightoncollaboration.us/myocarditis-case-definition-update/)

Myocarditis_Level of Certainty - 1 (Definitive Case)
Histopathologic examination of myocardial tissue (autopsy or endomyocardial biopsy) showed myocardial inflammation
OR
Elevated myocardial biomarkers (at least 1 of the findings below)
Troponin T
Troponin I
AND
Abnormal Imaging study
Abnormal Cardiac Magnetic Resonance Study (at least 1 of the findings below)
Edema on T2 weighted study, typically patchy in nature
Late gadolinium enhancement on T1 weighted study with an increased enhancement ratio between myocardial and skeletal muscle typically involving at least one non-ischemic regional distribution with recovery (myocyte injury).
OR
Abnormal Echocardiogram (at least 1 of the findings below)
New focal or difuse left or right ventricular function abnormalities (eg. decreased ejection fraction)
Segmental wall motion abnormalities
Global systolic or diastolic function depression/abnormality
Ventricular dilation
Wall thickness change
Intracavitary thrombi

Myocarditis_Level of Certainty - 2 (Probable Case)

Clinical Symptoms

Cardiac Symptoms (at least 1 finding below)

- Acute chest pain or pressure
- Palpitations
- Dyspnea after exercise, at rest, or lying down
- Diaphoresis
- Sudden death

OR

Non-Specific Symptoms (at least 2 findings below)

- Fatigue
- Abdominal pain
- Dizziness/syncope
- Edema
- Cough

OR

Infants and young children (at least 2 findings below)

- Irritability
- Vomiting
- Poor feeding
- Tachypnea
- Lethargy

AND

Testing supporting diagnosis (Biomarkers, ECHO, and EKG)

Elevated myocardial biomarkers (at least 1 of the findings below)

- Troponin T
- Troponin I
- CK Myocardial band

OR

Echocardiogram (ECHO) abnormalities (at least 1 of the findings below)

- New focal or difuse left or right ventricular function abnormalities (eg. decreased ejection fraction)
- Segmental wall motion abnormalities
- Global systolic or diastolic function depression/abnormality
- Ventricular dilation
- Wall thickness change
- Intracavitary thrombi

OR

Electrocardiogram (EKG) abnormalities that are new and/or normalize on recovery(at least 1 of the findings below)

- Paroxysmal or sustained atrial or ventricular arrhythmias (premature atrial or ventricular beats, and/or supraventricular or ventricular tachycardia, interventricular conduction delay, abnormal Q waves, low voltages)
- AV nodal conduction delays or intraventricular conduction defects (atrioventricular block (grade I-III), new bundle branch block)
- Continuous ambulatory electrocardiographic monitoring that detects frequent atrial or ventricular ectopy

AND

No alternative diagnosis for symptoms

Myocarditis_Level of Certainty - 3 (Possible Case)

- **Clinical Symptoms**
  - **Cardiac symptoms (at least 1 finding below)**
    - **Acute chest pain or pressure**
    - **Palpitations**
    - **Dyspnea after exercise, at rest, or lying down**
    - **Diaphoresis**
    - **Sudden death**
  - **OR**
  - **Non-Specific Symptoms (at least 2 findings below)**
    - **Fatigue**
    - **Abdominal pain**
    - **Dizziness/syncope**
    - **Edema**
    - **Cough**
  - **OR**
  - **Infants/Young children (at least 2 findings below)**
    - **Irritability**
    - **Vomiting**
    - **Poor feeding**
    - **Tachypnea**
    - **Lethargy**
- **AND**
- **Biomarkers supporting evidence of inflammation (at least 1 finding below)**
  - **Elevated CRP**
  - **Elevated ESR**
  - **Elevated D-Dimer**
  - ***AND***
  - **Non-Specific Electrocardiogram (EKG) Abnormalities that are new and/or normalize on recovery (at least 1 finding below)**
    - **ST-segment or T-wave abnormalities (elevation or inversion)**
    - **PACs and PVCs**
- **AND**
- **No alternative diagnosis for symptoms**

II. Brighton collaboration pericarditis case, by level of certainty – Level 1 (definitive), 2 (probable), and 3 (possible) (https://brightoncollaboration.us/myocarditis-case-definition-update/)

Pericarditis_Level of Certainty - 1 (Definitive Case)		
	Histopathologic examination of pericardial tissue (autopsy or pericardial biopsy) showed pericardial inflammation	
	OR	
	Abnormal testing need at least 2 of 3 of the following:	
	Evidence of abnormal fluid collection or pericardial inflammation by imaging (Echo, MR, cMR, CT)	
		OR
	Electrocardiogram (EKG) Abnormalities that are new and/or normalize on recovery (must have all findings)	
		Diffuse concave-upward ST-segment elevation
		ST-segment depression in aVR
		PR-depression throughout the leads without reciprocal ST-segment changes
		OR
	Physical examination findings: at least 1 finding	
		Pericardial friction rub
		Distant heart sounds (infant/children)
		Pulsus paradoxus

Pericarditis_Level of Certainty - 2 (Probable Case)		
Clinical Symptoms		
	Clinical Cardiac Symptoms (at least 1 finding below)	
		Acute chest pain or pressure
		Palpitations
		Dyspnea after exercise, at rest, or lying down
		Diaphoresis
		Sudden death
	OR	
	Infants/Children (at least 2 findings below)	
		Irritability
		Vomiting
		Poor feeding
		Sweating
AND		
Physical examination findings: (at least 1 findings)		
	Pericardial friction rub	
	Pulsus paradoxus	
	OR	
Evidence of abnormal fluid collection or pericardial inflammation by imaging (Echo, MR, cMR, CT)		
	OR	
Electrocardiogram (EKG) Abnormalities that are new and/or normalize on recovery (at least 1 finding below)		
	Diffuse concave-upward ST-segment elevation	
	ST-segment depression in aVR	
	PR-depression throughout the leads without reciprocal ST-segment changes	
AND		
No alternative diagnosis for symptoms (e.g. MI, PE, mediastinitis, etc)		

Pericarditis_Level of Certainty - 3 (Possible Case)
Clinical Symptoms
Clinical Cardiac symptoms (at least 1 finding below)
New onset cardiac chest pain or pressure
Palpitations
Dyspnea after exercise, at rest, or lying down
AND
Non-Specific Symptoms (at least 1 finding below)
Cough
Weakness
Gastrointestinal - nausea, vomiting diarrhea
Shoulder/upper back pain
Cyanosis
Low grade intermittent fever
Altered Mental Status
Edema
Fatigue
OR
Infants/Children (at least 2 findings below)
Irritability
Vomiting
Poor feeding
Back Pain
Tachypnea
Lethargy
AND
Abnormal testing
Abnormal chest radiograph showing enlarged heart
OR
Electrocardiogram (EKG) abnormalities
Non-specific changes that are new and/or normalize in recovery
AND
No alternative diagnosis for symptoms (e.g. MI, PE, mediastinitis, etc)

III. CDC working case definition of myocarditis and pericarditis and myopericarditis (54)

Condition	Definition	
	Probable case	**Confirmed case**
Acute myocarditis	Presence of ≥1 new or worsening of the following clinical symptoms:* • chest pain, pressure, or discomfort • dyspnea, shortness of breath, or pain with breathing • palpitations • syncope	Presence of ≥1 new or worsening of the following clinical symptoms:* • chest pain, pressure, or discomfort • dyspnea, shortness of breath, or pain with breathing • palpitations • syncope
	OR, infants and children aged <12 years might instead have ≥2 of the following symptoms: • irritability • vomiting • poor feeding • tachypnea • lethargy	**OR,** infants and children aged <12 years might instead have ≥2 of the following symptoms: • irritability • vomiting • poor feeding • tachypnea • lethargy
	AND ≥1 new finding of • troponin level above upper limit of normal (any type of troponin) • abnormal electrocardiogram (ECG or EKG) or rhythm monitoring findings consistent with myocarditis§ • abnormal cardiac function or wall motion abnormalities on echocardiogram • cMRI findings consistent with myocarditis¶	**AND** ≥1 new finding of • Histopathologic confirmation of myocarditis† • cMRI findings consistent with myocarditis¶ in the presence of troponin level above upper limit of normal (any type of troponin)
	AND • No other identifiable cause of the symptoms and findings	**AND** • No other identifiable cause of the symptoms and findings
Acute pericarditis**	Presence of ≥2 new or worsening of the following clinical features: • acute chest pain†† • pericardial rub on exam • new ST-elevation or PR-depression on EKG	

Condition	Definition
Myopericarditis	• new or worsening pericardial effusion on echocardiogram or MRI This term may be used for patients who meet criteria for both myocarditis and pericarditis.

Abbreviations: AV = atrioventricular; cMRI = cardiac magnetic resonance imaging; ECG or EKG = electrocardiogram.

* Persons who lack the listed symptoms but who meet other criteria may be classified as subclinical myocarditis (probable or confirmed).

† Using the Dallas criteria (Aretz HT, Billingham ME, Edwards WD, et al. Myocarditis. A histopathologic definition and classification. Am J Cardiovasc Pathol 1987; 1:3–14). Autopsy cases may be classified as confirmed clinical myocarditis on the basis of meeting histopathologic criteria if no other identifiable cause.

§ To meet the ECG or rhythm monitoring criterion, a probable case must include at least one of 1) ST-segment or T-wave abnormalities; 2) Paroxysmal or sustained atrial, supraventricular, or ventricular arrhythmias; or 3) AV nodal conduction delays or intraventricular conduction defects.

¶ Using either the original or the revised Lake Louise criteria. https://www.sciencedirect.com/science/article/pii/S0735109718388430?via%3Dihubexternal icon

** https://academic.oup.com/eurheartj/article/36/42/2921/2293375external icon

†† Typically described as pain made worse by lying down, deep inspiration, or cough, and relieved by sitting up or leaning forward, although other types of chest pain might occur.

Appendix K Protocol team

Protocol leadership

Chair	Jesse Clark University of California, Los Angeles 310-825-3543 jlclark@mednet.ucla.edu
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Other contributors to the original protocol

Core medical monitor	Nicole Grunenberg HVTN LOC, Fred Hutch
Study product developer representative	William Schief Scripps Research
Study product provider representative	Antu Dey IAVI, New York
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Regulatory affairs associate	Megan Brandon HVTN LOC, Fred Hutch
Clinic coordinator	Stacey Edick University of Pittsburgh
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DAIDS protocol pharmacist	Shawn Chiambah DAIDS, NIAID 301-761-7336
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Technical editor	Anders McConachie CoVPN LOC, Fred Hutch
Clinical safety specialist	Maija Anderson HVTN LOC, Fred Hutch
Clinical trials manager	Shelly Ramirez HVTN LOC, Fred Hutch
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SDMC Associate director of lab science	April Randhawa HVTN SDMC, Fred Hutch
DAIDS Product Lead	Michael Eller DAIDS, NIAID
Protocol development managers	Kajari Mondal Daciana Margineantu HVTN LOC, Fred Hutch
Community engagement unit representative	Gail Broder HVTN LOC, Fred Hutch
Community educator/recruiter	Christopher Blades University of California, Los Angeles

Appendix L Version history

The Protocol Team may modify the original version of the protocol. Modifications are made to HVTN protocols via clarification memos, letters of amendment, or full protocol amendments.

The version history of, and modifications to, Protocol HVTN 302 are described below.

Protocol history and modifications

Date: July 7, 2023

Protocol version: Version 3.0
Protocol modification: Full Protocol Amendment 2

Item 1 Revised in Section 1.6, Duration per participant; Section 2.8.1, Potential risks; Section 8.6, Extended follow-up for participants with unresolved study product-related urticaria-associated symptoms; Section 8.9, Early termination visit; Section 9.5, Total blood volume; Appendix C, Schedule of procedures for extended follow-up for participants with unresolved study product-realted urticaria-associated symptoms; Appendix G, Sample Addendum Informed Consent Form for follow-up of participants with unresolved study product-realted urticaria-associated symptoms: Language related to adding serum collection for participants in extended follow-up

Item 2 Updated in Appendix K, Protocol Team: name and contact information for DAIDS Medical Officer and DAIDS protocol pharmacist

Item 3 Updated Title page; and Appendix L, Version History: date, version number and contents of this amendment

Item 4 Updated per Protocol Version 2.0, Clarification Memo 1, dated January 12, 2022

Item 5 Updated per Protocol Version 2.0, Letter of Amendment 1, dated March, 29, 2022

Item 6 Updated per Protocol Version 2.0, Letter of Amendment 2, dated May 6, 2022

Item 7 Updated per Protocol Version 2.0, Letter of Amendment 3, dated June 27, 2022

Item 8 Updated per Protocol Version 2.0, Clarification Memo 2, dated August 16, 2022

Item 9 Updated per Protocol Version 2.0, Clarification Memo 3, dated September 16, 2022

Item 10 Updated per Protocol Version 2.0, Letter of Amendment 4, dated November 18, 2022

Item 11 Updated per Protocol Version 2.0, Letter of Amendment 5, dated March 16, 2023

Item 12 Minor editorial and formating changes have been made throughout the protocol

Date: March 16, 2023

Protocol version: Version 2.0
Protocol modification: Letter of Amendment 5

Item 1 Updated in Section 1.6, *Duration per participant*; Section 1.7, *Estimated total study duration;* new Section 8.6, *Extended follow-up for participants with unresolved study product-related urticaria-associated symptoms*; Section 9.1*, Adverse events;* new Appendix C, *Schedule of procedures for following up participants with unresolved study product-related urticaria-associated symptoms;* Appendix D, *Visit windows for the main study;* new Appendix E*, Visit windows for follow-up of participants with unresolved study product-related urticaria-associated symptoms* and Appendix G, *Sample Addendum Informed Consent Form for follow-up of participants with unresolved study product-related urticaria-associated symptoms:* language related to following up participants with unresolved study product-related urticaria-associated symptoms

Item 2 Updated in Appendix K; *Protocol team*: Name and contact information for protocol team Leader and Core medical monitor

Item 3 Updated throughout the protocol: Section numbering and cross references

Date: November 18, 2022

Protocol version: Version 2.0
Protocol modification: Letter of Amendment 4

Item 1 Added in Section 2.8.1, *Potential risks* and Appendix D, *Sample informed consent form*: updated information related to observed skin reactions

Item 2 Updated in Appendix H, *Protocol team*: Name and contact information for Community Advisory Board (CAB) member

Date: September 16, 2022

Protocol version: Version 2.0
Protocol modification: Clarification Memo 3

Item 1 Updated in Section 5.2.1, *Delaying vaccinations for a participant*: considerations for timing of receipt of SARS-CoV-2 vaccines and monkeypox vaccines

Date: August 16, 2022

Protocol version: Version 2.0
Protocol modification: Clarification Memo 2

Item 1 Added in Section 9.5, *Total blood volume*: alternate laboratory specimen tube types allowed for research samples upon HVTN laboratory center approval

Date: June 27, 2022

Protocol version: Version 2.0
Protocol modification: Letter of Amendment 3

Item 1 Updated in Section 2.8.1, *Potential risks*; Section 12, *Literature cited*; and Appendix D, *Sample informed consent form*: information due to new observed skin reactions

Item 2 Updated in Appendix D, *Sample informed consent form*: language related to payment for study related injury in section 23

Item 3 Corrected in Section 6.1.1, *Power calculations for immunogenicity* and Section 6.3, *Statistical analysis:* language related to statistical analysis plan

Date: May 6, 2022

Protocol version: Version 2.0
Protocol modification: Letter of Amendment 2

Item 1 Updated in Appendix A, Schedule of procedures for participants undergoing LN FNA and/or leukapheresis and Appendix B, Schedule of procedures for participants undergoing neither LN FNA nor leukapheresis: collection of PBMCs during vaccination 2 and vaccination 3 visits

Item 2 Updated in Appendix A, Schedule of procedures for participants undergoing LN FNA and/or leukapheresis and Appendix B, Schedule of procedures for participants undergoing neither LN FNA nor leukapheresis: footnote #9 related to the pregnancy testing

Item 3 Deleted in Appendix A, Schedule of procedures for participants undergoing LN FNA and/or leukapheresis and Appendix B, Schedule of procedures for participants undergoing neither LN FNA nor leukapheresis: row and footnote specifying plasma harvest

Item 4 Clarified in Section 8.3, Reactogenicity Assessments: details regarding vital signs collection

Date: March 29, 2022

Protocol version: Version 2.0
Protocol modification: Letter of Amendment 1

Item 1 Added in Section 5.1.1, *Inclusion criteria*: Language related to participants enrolled in other clinical trials

Item 2 Revised in Section 5.1.2, *Exclusion criteria and Acronyms and abbreviations*: Language related to participants who have received SARS-CoV-2 vaccination before enrollment

Item 3 Revised in Appendix E, *Low risk guidelines for US* and Section 5.1.1, *Inclusion criteria*: Language to remove the requirement for PSRT consultation

Item 4 Added in Section 5.2.1, *Delaying vaccinations for a participant*: Language to describe guidance for scheduling licensed vaccinations

Item 5 Revised in Section 7.3, *Product preparation*: Language to specify details about vaccine Dose Preparation and Administration Worksheets

Item 6 Clarified in Section 1.5, *Study Plan and schema table* and Section 9.6, *Sentinel Safety Reviews*: Number of participants in sentinel safety groups

Item 7 Corrected in Section 4*, Laboratory Strategy*: URL for the description of the standard HVTN laboratory assays

Item 8 Updated in Appendix H, *Protocol team*: Name and contact information for Laboratory lead

Date: January 12, 2022

Protocol version: Version 2.0
Protocol modification: Clarification Memo 1

Item 1 Updated in Section 7.3, *Product Preparation*: needle size for product administration

Item 2 Updated in Section 9.4.1, *Protocol Safety Review Team (PSRT)*: abnormal ALT monitoring and reporting process

Item 3 Updated in Appendix A, *Schedule of procedures for participants undergoing LN FNA and/or leukapheresis* and Appendix B*, Schedule of procedures for participants undergoing neither LN FNA nor leukapheresis:* blood storage volume for additional safety labs

Item 4 Corrected in Appendix D, *Sample informed consent form:* formatting error affecting sections 14 and 15

Date: September 30, 2021

Protocol version: Version 2.0
Protocol modification: Full Protocol Amendment 1

Item 1 Added in Section 2.8.2, *Myocarditis and Pericarditis with mRNA COVID-19 vaccines,* Section 3.1, *Objectives and endpoints,* Section 5.1.2, *Exclusion criteria,* Section 8.4, *Cardiac symptom management,* Section 9.1, *Adverse events,* Section 9.2, *AESIs,* Section 9.3.1, *Expedited reporting of adverse events to DAIDS*, Section 9.7, *Safety pause and prompt PSRT AE review*, Appendices A through D and Appendix G: Information on myocarditis and pericarditis following COVID-19 mRNA vaccines and risk mitigation and management approaches in response to the comments received from the FDA dated 17 August 2021.

Item 2 Added in Section 2.2.2.2, *In non human primates (NHPs*): Rationale for evaluating BG505 MD39.3 mRNA vaccine in response to the comment received from FDA dated September 10, 2021

Item 3 Added in Section 8.8, *Early termination visit*, Appendix A, *Schedule of procedures for participants undergoing LN FNA and/or leukapheresis,* Appendix B, *Schedule of procedures for participants undergoing neither LN FNA nor leukapheresis* and Appendix D*, Sample informed consent form:* Social impact assessment and Social impact assessment questionnaire

Item 4 Added in Section 8.3, *Reactogenicity Assessment and* Appendix D*, Sample informed consent form*: Additional information regarding electronic diary and paper diary

Item 5 Revised Appendix D, *Sample informed consent form:* to include changes requested upon Central IRB review of Protocol Version 1.

Item 6 Updated Acronyms and abbreviations

Item 7 Updated in Title page, Table of contents, Appendix 12, *Literature cited*, and Appendix I, *Version history*: date, version number and contents of this amendment

Item 8 Minor editorial and formatting changes have been made throughout the protocol

Date: July 8, 2021

Protocol version:1.0
Protocol modification: Not applicable

Original protocol