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A Phase 2 Randomized, Open-Label, Multisite Trial to Evaluate the Immunogenicity of Dose Reduction Strategies of the MVA-BN Vaccine

DMID Protocol Number: 22-0020

Sponsor: Division of Microbiology and Infectious Diseases (DMID)

Version Number: 2.0

22 September 2022

STATEMENT OF COMPLIANCE

Each institution engaged in this research will hold a current Federalwide Assurance (FWA) issued by the Office of Human Research Protection (OHRP) for federally funded research. The IRB/IEC must be registered with OHRP as applicable to the research.

The study will be carried out in accordance with the following as applicable:

- United States Code of Federal Regulations (CFR) 45 CFR Part 46: Protection of Human Subjects
- Food and Drug Administration (FDA) Regulations: 21 CFR Part 50 (Protection of Human Subjects), 21 CFR Part 54 (Financial Disclosure by Clinical Investigators), 21 CFR Part 56 (Institutional Review Boards), 21 CFR Part 11 (Electronic Records and Electronic Signatures), and 21 CFR Part 312 (Investigational New Drug Application), and/or 21 CFR 812 (Investigational Device Exemptions)
- The International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) E6(R2) Good Clinical Practice (GCP), and the Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research, Report of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research
- The policies and procedures of National Institutes of Health (NIH) Office of Extramural Research and DMID
- The National Institute of Allergy and Infectious Diseases (NIAID) Terms of Award
- Any additional Federal, State, and Local Regulations and Guidance

The signature below provides the necessary assurance that this study will be conducted according to all stipulations of the protocol including statements regarding confidentiality, and according to local legal and regulatory requirements, US federal regulations, and ICH E6(R2) Good Clinical Practice (GCP) guidelines.

Site Investigator Signature:

Signed: _____ Date: _____

Print Name, Credentials
Print Title

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1. PROTOCOL SUMMARY

1.1 Synopsis

Rationale

Since the first case was identified four months ago, monkeypox has rapidly spread to 85 non-endemic countries, marking the first time monkeypox has spread widely outside Central and West Africa. To prevent further spread of this outbreak, MVA-BN vaccine is being offered to individuals at high risk. However, there is a global vaccine shortage and increasing vaccine supply will take time. This study will evaluate dose sparing strategies to extend the vaccine supply during this global public health crisis.

Study Design

This study is a Phase 2 randomized, open-label, non-placebo controlled, multi-site clinical trial that will evaluate the immunogenicity and safety of two, dose sparing strategies including one-fifth ($2x10^7$) and one-tenth ($1x10^7$) of the standard dose of MVA-BN (JYNNEOS) administered intradermally (ID) on Day 1 and 29 (Arm 1 and 2, respectively), compared with the standard, licensed regimen of $1x10^8$ MVA-BN administered subcutaneously (SC) on Day 1 and 29 (Arm 3). At least 210 healthy, vaccinia-naïve adults 18 to 50 years of age will be enrolled and randomized 1:1:1 to one of three study arms. There will be eight study visits (plus an optional screening visit) with the last visit at approximately one year after the first study vaccination. Participants reporting monkeypox-like illness and who have a laboratory-confirmed monkeypox (i.e., a positive diagnostic test for monkeypox) will be seen at an ad hoc sick visit.

Primary Objectives

- To determine if peak humoral immune responses following an ID regimen of 2 x 10^7 $TCID_{50}$ MVA-BN are non-inferior to the licensed regimen of 1 x 10^8 MVA-BN administered SC.
- To determine if peak humoral immune responses following an ID regimen of 1 x 10^7 $TCID_{50}$ MVA-BN are non-inferior to the licensed regimen of 1 x 10^8 MVA-BN administered SC

For a full list of Objectives/Endpoints, see corresponding section of the protocol.

Inclusion Criteria

See inclusion criteria in corresponding section of the protocol.

Exclusion Criteria

See exclusion criteria in corresponding section of the protocol.

Study Phase

Phase 2

Study Population

The study population will be healthy, vaccinia-naïve, non-pregnant, non-breastfeeding adults 18 to 50 years of age. Participants with stable chronic medical conditions including those with

controlled HIV infection can participate. The study will attempt to enroll a representative study population that is proportional to those affected by the disease and demographically diverse.

Sites
Up to 10 US clinical research sites.

Study intervention
There will be three study arms in which JYNNEOS will be administered by either ID or SC injection at Day 1 and 29. JYNNEOS is live vaccine produced from the strain Modified Vaccinia Ankara-Bavarian Nordic (MVA-BN), an attenuated orthopoxvirus, that is non-replicating in human cells. It is FDA-approved and licensed as a smallpox and monkeypox vaccine in the United States.

Study Duration
This study will take approximately 15 months to conduct from study activation to the final participant's last study visit.

Participant Duration
It will take approximately one year for a participant to complete their participation in the study.

Safety
The trial will use study and individual pausing rules. See corresponding section for details. This trial will use a Data Safety Monitoring Board (DSMB) for objective oversight of the study and review for any study pause.

1.2 Schedule of Activities (SoA)

The trial will include the following activities and procedures at each study visit. Only procedures that contribute to participant eligibility and study objectives and endpoints are included. Please refer to Manual of Procedures (MOP) for detailed description of study activities.

Table 1. Schedule of Activities (SoA)

Procedures	Optional Screening Visit Day -7 to -1	Enrollment / Baseline Visit 1 Day 1	Visit 2 Day 15 +/- 3 day	Visit 3 Day 29 +/- 3 days	Visit 4 Day 43 +/- 3 days	Visit 5 Day 57 +/- 3 days	Visit 6 Day 90 +/- 5 days	Visit 7 Day 181 +/- 7 days	Visit 8 Day 365 +/- 14 days	Sick/Unscheduled Visit [6]
Informed consent	X	X[1]								
Demographics	X	X[1]								
Medical history	X	X[1]	X	X	X	X	X	X	X	X
Randomization		X								
Vaccine administration		X		X						
Targeted physical exam	X	X[1]	X[2]	X[2]	X[2]	X[2]	X[2]	X[2]	X[2]	X[2]
Vital signs	X	X[1]	X[2]	X[2]	X[2]	X[2]	X[2]	X[2]	X[2]	X[2]
Concomitant medication review	X	X[1]	X	X	X	X	X[3]	X[3]		X
SAE/ MAAEs [4]		All SAE/MAAEs, Days 1 through 57					Only related SAE/MAAEs Days 58 -181			X[4]
Unsolicited AEs[4]		Day 1 through Day 29 and Day 29 through Day 57								X[4]
Solicited (Local and Systemic) AEs		Day 1 through Day 15		Day 29 through Day 43						X[4]
Tolerability Assessment			X		X					
Pregnancy test [5]	X	X[1]		X						
Blood volume per visit (ml)		32	20	20	32	20	20	20	20	20
Blood volume total (ml)		32	52	72	104	124	144	164	184	204

[1] If there is no screening visit, these activities should be conducted at the Day 1 visit prior to randomization. Temperature should be collected on Day 1 and 29 pre-vaccination for all participants.
[2] A targeted (symptom-driven) physical exam will be done at screening (i.e., screening visit or Day 1), on Days 15, 29, and 43 (at least assessment of vaccination site and presence of skin discoloration and nodules will be done), and as needed in all other visits if interim change in medical status reported by participant. Vital signs will be done at screening and on Day 1 and 29 prior to vaccination and on an as needed basis during all other visits.
[3] After Day 57, only concomitant medications that are associated with a related SAE or MAAE (detected through Day 181) and clinically relevant to report, will be recorded in the CRF. An exception to this rule for recording concomitant medications is for sick visits. During sick visits, concomitant medications will be collected.
[4] If SAE/MAAE or unsolicited AE are reported during a sick visit and within the reporting window, it will be reported as AE. See more information about collection of AEs in the safety section of the protocol.

[5] A pregnancy test will be done at the screening visit (if conducted) and on Day 1 prior to randomization. If there is no screening visit, then pregnancy test done will be done on Day 1 only. Participants of childbearing potential must have negative serum or urine pregnancy test in the 24 hours prior to Day 1 and 29 study vaccinations and results confirmed prior to randomization.
[6] Participants reporting monkeypox-like illness and who have a laboratory-confirmed monkeypox will be seen at an ad hoc sick visit. An unscheduled visit may also occur for reasons including, but not limited to, AE follow up, blood draw, or early termination final visit.

1.3 Study Schema

Table 2. Study Schema

Arm	Dose of JYNNEOS (MVA-BN)	Route of Administration*	Vaccination Day	
			Day 1	Day 29
1	2 x 10^7 $TCID_{50,}$ (0.1 mL)	Intradermal	X	X
2	1 x 10^7 $TCID_{50,}$ (0.05 mL)	Intradermal	X	X
3	1 x 10^8 $TCID_{50,}$ (0.5 mL)	Subcutaneous	X	X

*Subcutaneous is administered in the deltoid region, intradermal is administered in the volar aspect (inner side) of the forearm.

2. INTRODUCTION

2.1 Study Rationale

On July 23, 2022, WHO declared the 2022 monkeypox outbreak a public health emergency of international concern (PHEIC). Since the first case of monkeypox was detected in the UK in a returning traveler from Nigeria on May 6, 2022, as of September 2022 the outbreak has rapidly spread to >100 countries or locations worldwide, including 96 that are non-endemic, marking the first time monkeypox has spread widely outside Central and West Africa. In August, the number of confirmed monkeypox cases in the U.S. surpassed that of any other country. To mitigate the consequences of this outbreak, monkeypox vaccine is being offered to individuals considered to be at high risk. However, there is a global vaccine shortage and increasing the supply will take time. This study will evaluate vaccine dose sparing strategies to extend the limited supply during this global public health crisis.

2.2 Background

Monkeypox is a reemerging infectious disease caused by Monkeypox virus (MPXV), a large double-stranded DNA virus belonging to the genus, Orthopoxvirus.[1] MPXV is a zoonotic orthopoxvirus, primarily transmitted to humans via contact with infected animals, and causing disease similar to smallpox, although with substantially lower mortality.[1-3] MPXV is endemic to West and Central Africa, where the incidence of human monkeypox cases has increased as much as 20-fold since the end of the smallpox vaccination campaign in 1980.[4,5] Outbreaks in non-endemic countries have been related to the exotic pet trade[6] and international travel.[7-9] Prior to the current global monkeypox outbreak, secondary human-to-human transmission in non-endemic countries was rare, and documented only twice since 2018, both cases involving

travelers returning from Nigeria. The first case involved transmission between the index patient and a healthcare provider[10] and second case involved transmission to another adult and an infant within the household of the index case.[11,12] Monkeypox reemerged in Nigeria in 2017 after more than 40 years with no reported cases.[13,14]

On May 18, 2022, the first U.S. case of monkeypox was detected as part of the larger global 2022 monkeypox outbreak. The U.S. was the fourth non-endemic country to detect a case in a returning traveler. On August 4, the U.S. Department of Health and Human Services declared the U.S. monkeypox outbreak to be a public health emergency with 7,510 confirmed cases of monkeypox detected in all U.S. states, D.C, and Puerto Rico. As of August 15, 2022, there have been 36,589 confirmed monkeypox cases in 92 countries; most cases have been from 85 non-endemic countries including 11,890 confirmed cases in the United States.

Vaccination is being used as a mitigation strategy in the current monkeypox outbreak for those exposed to and those deemed to be at higher risk of being exposed to monkeypox. There is one FDA-approved vaccine for monkeypox: JYNNEOS Modified Vaccinia Ankara-Bavarian Nordic (MVA-BN). A second vaccine, ACAM2000 is likely effective but is not FDA-approved for monkeypox and associated with moderate risk of myocarditis and pericarditis (5.7 cases per 1,000 vaccinated) as well as encephalomyelitis, progressive vaccinia, generalized vaccinia, and ocular complications. ACAM2000 should not be given to people with weakened immune system, eczema, cardiac disease, infants <12 months, or pregnant women. Given these attributes, JYNNEOS is the preferred vaccine during the current outbreak and there is a limited supply of the vaccine globally with production unlikely to keep up with the growing demand as the outbreak continues to spread. Given the limited supply of MVA-BN, dose sparing strategies have been suggested. On August 9, 2022, the U.S. Food and Drug Administration (FDA) issued an Emergency Use Authorization (EUA) for the emergency use of a lower (2×10^7) ID dose of JYNNEOS for individuals 18 years of age and older determined to be at high risk for monkeypox infection.

There are several potential dose sparing strategies we considered. Use of a reduced dose administered SC is unlikely to be of comparable immunogenicity to the licensed SC regimen. There were several Phase 1 and 2 trials that evaluated 2-dose regimens of MVA-BN administered SC four weeks apart (Table 3). Although the 2-dose 5×10^7 TCID50 SC regimen may elicit GMT similar to ACAM2000, the GMT will likely be below that of the licensed 2-dose 1×10^8 MVA-BN regimen.

Table 3. Prior Subcutaneous Dosing Trials

Reference		1 X10^7 TCID$_{50}$	2 X10^7 TCID$_{50}$	5 X10^7 TCID$_{50}$	1 X10^8 TCID$_{50}$	Assay
Vollmar et al, Vaccine, 2006 [15]	Liquid formulation	6.4 (CI not reported)	--	--	29.3 (CI not reported)	IHD-J PRNT GMT
Frey at al. Vaccine, 2007	Lyophilized formulation	--	347.2 (161.9, 744.7)	551.5 (321.5, 946.0)	914.5 (528.0, 1584)	MVA PRNT GMT

von Krempelhuber Vaccine, 2010	Lyophilized formulation	--	5.5 (3.2, 9.6)	10.3 (5.8, 18.4)	19.4 (11.1, 34.2)	IHD-J PRNT GMT

A single 1 x 10^8 MVA-BN dose has also been suggested for dose sparing, but in our assessment of the published literature, it is unlikely to be immunogenically equivalent to the standard licensed 2-dose regimen. The pivotal Phase 3 trial demonstrated that a single dose is likely to provide a significantly lower GMT than the licensed 2-dose MVA-BN regimen, 16.9 [13.7, 20.8] as compared to 153.5 [134.3, 175.6], respectively (Table 4).

Table 4. MVA-BN Compared to ACAM2000

	MVA-BN (N=185)			**ACAM2000 (N=186)**			**GMTs ratio MVA/ ACAM**	
Visit Week	n	GMT	95% CI	n	GMT	95% CI	Ratio	95% CI
Plaque Reduction Neutralization Test								
Week 0	185	1.0	[1.0, 1.1]	186	1.0	[1.0, 1.0]	1.008	[0.97, 1.05]
Week 2	184	16.2	[13.0, 20.1]	184	16.2	[13.1, 20.0]	0.997	[0.74, 1.35]
Week 4	185	16.9	[13.7, 20.8]	186	79.3	[67.1, 93.8]	0.213	[0.16, 0.28]
Week 6	185	153.5	[134.3, 175.6]	181	64.7	[54.9, 76.2]	2.372	[1.92, 2.93]

NIAID conducted a Phase 2 trial comparing the standard (now licensed) 2-dose 1 x 10^8 TCID50 MVA-BN SC regimen (liquid formulation) and a 2-dose 2 x 10^7 TCID50 MVA-BN ID regimen (liquid formulation) and found peak vaccinia virus, Western reserve strain (VV-WR) GMT of 49.5 (40.0, 61.3) and 59.6 (48.1, 74.0), respectively (Table 5).[16] The 2-dose 2 x 10^7 TCID50 ID regimen was non-inferior to the 2-dose 1 x 10^8 TCID50 SC regimen and utilized only one-fifth of the dose. These results are supported by a trial evaluating another MVA vaccine (ACAM3000) which showed ID vaccination at 1x10^7 TCID50 had similar VV-WR GMT to 1x10^8 TCID50 administered SC or IM.[17]

Table 5. Prior Intradermal Dosing Compared to Subcuteanous

Study visit day	**Group**		
	Lyophilized SC 1x10^8 (N=145) GMT [95% CI]	**Liquid SC 1x10^8 (N=149) GMT [95% CI]**	**Liquid ID 2x10^7 (N=146) GMT [95% CI]**
Day 0	7.5 [,]	7.7 [7.4, 8.0]	7.7 [7.4, 7.9]
Day 14	10.9 [9.9, 12.0]	10.0 [9.0, 11.1]	10.3 [9.3, 11.3]
Day 28	10.8 [9.9, 11.9]	9.6 [8.7, 10.6]	10.8 [9.9, 11.9]
Day 42	77.6 [62.3, 96.7]	45.2 [36.4, 56.2]	54.4 [43.7, 67.8]
Peak post vaccination 2	87.8 [71.2, 108.3]	49.5 [40.0, 61.3]	59.6 [48.1, 74.0]

Even with the EUA for intradermal MVA-BN being available, this trial is designed to evaluate several unique issues:

- As vaccine supply is not anticipated to meet the demand in the next year, this trial will evaluate dose reduction strategies focused on the ID route, that is, confirming the previously studied ID dose and evaluating one tenth dose of MVA-BN (1x10^7).

- This trial will also assess differences in safety and tolerability of the ID versus the licensed SC regimen as issues of tolerability have been raised by the manufacturer and some in the medical community.

2.3 Risk/Benefit Assessment

2.3.1 Known Potential Risks

The potential risks to the participants in the study are those related to venipuncture and reactogenicity as well as possible reactions to the vaccine.

Venipuncture

Venipuncture may cause transient mild discomfort and/or fainting. Bruising at the site of the venipuncture may occur but can be prevented or lessened by applying pressure for several minutes after the injection. Infection at the site is possible but highly unlikely with the use of aseptic technique.

Adverse Reactions

MVA-BN administered SC was approved by the FDA as the vaccine JYNNEOS for smallpox and monkeypox on September 24, 2019.[18] At the time of licensure, the clinical development program for JYNNEOS included 22 studies involving 7,093 smallpox vaccine-naïve and 766 smallpox vaccine-experienced participants who received at least 1 dose of JYNNEOS and were monitored for safety for at least 6 months following the first vaccination. There were mostly local and systemic reactogenicity and rare cases of allergy/hypersensitivity observed. Within 8 days of administration of a first or second dose of JYNNEOS, the most common solicited local (injection site) reactions among 2,943 smallpox vaccine-naïve healthy adult participants included pain (84.9%), redness (60.8%), swelling (51.6%), induration (45.4%), and itching (43.1%). The most common solicited systemic adverse reactions were muscle pain (42.8%), headache (34.8%), fatigue (30.4%), nausea (17.3%), and chills (10.4%). In a large clinical trial involving 3,003 participants who received at least one dose of MVA, the majority of solicited local and systemic adverse reactions had a median duration of 1 to 6 days, and there was a similar frequency of reported adverse reactions after the first and second dose of JYNNEOS except for site pain which was more common after the first dose (79.3% versus 69.9%).[19]

An analysis of serious adverse events (SAEs) among the 7,093 smallpox vaccine-naïve and 766 smallpox vaccine-experienced participants who received at least 1 dose of JYNNEOS identified only four SAEs (0.05%) in which a causal relationship could not be ruled out. All were non-fatal SAEs and included Crohn's disease, sarcoidosis, extraocular muscle paresis, and throat tightness (angioedema). The trial that had the related angioedema had three other post-vaccination hypersensitivity reactions which were non-serious and judged to related to the vaccination.

As with all injectable vaccines, there is a risk of an allergic reaction or an anaphylactic event. To mitigate the risk, individuals with known allergy or history of anaphylaxis or other serious adverse reaction to a vaccine or vaccine products will not be eligible to participate in the study. In addition, sites must observe participants for 30 minutes after each vaccination and will have appropriate medical treatment available to manage possible anaphylactic reactions. Participants

who have an allergic reaction that is temporally associated with vaccination and that the PI believes to be related, will not receive any more study product. However, the participant will be encouraged not to withdraw from the study so that they may be followed for safety and immunogenicity until the end of the study.

Intradermal Route of Administration
In a Phase 2 clinical trial that compared the ID and SC administration of the liquid formation of MVA-BN, no participants in the Liquid SC dose group experienced a severe systemic reaction and 20.4% experienced a moderate systemic reaction following the first vaccination, and 1.9% of participants experienced severe systemic reactions and 16.6% experienced moderate systemic reactions following the second vaccination.[16] In the Liquid ID dose group, 3.1% of participants experienced a severe systemic reaction and 24.1% experienced moderate systemic reactions following the first vaccination, and 0.7% experienced a severe systemic reaction and 15.0% experienced moderate systemic reactions following the second vaccination. Fatigue was the most common systemic reaction reported in both groups after both vaccinations, reported by 35.9% in the Liquid SC dose group and 41.4% in the Liquid ID dose group after the first vaccination, and 33.1%, and 29.4% of participants in the Liquid SC, and Liquid ID dose groups, respectively after the second vaccination.

Pain at the injection site occurred in 65.4% and 91.0% of participants in the Liquid ID group and the SC group, respectively. Itchiness was the most prevalent local reaction in the Liquid ID group, reported by 89.0% of participants after either vaccination compared to 48.5% in the Liquid SC group. Vaccine site discoloration (hyperpigmentation) was seen in 122 (63.9%) participants in the Liquid ID group and all but three of these events were graded as mild (Grade 1). In contrast, only seven (4.2%) participants in the Liquid SC group had discoloration at the vaccine site and all but one event was Grade 1. Nodule formation at the vaccine site occurred at a similar frequency of participants in the Liquid ID and Liquid SC group; 35 (18.3%) and 34 (20.4%) participants developed a nodule, respectively.

The original protocol (and report) used a more conservative grading scale for erythema and induration than the FDA's current guidance document, “Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials”. When NIAID re-analyzed the data using the FDA’s current toxicity grading scale, 12% of participants in the ID group and 7.2% in the SC group experienced moderate/severe erythema following the first vaccination, and 59.5% in the ID group and 31.2% in the SC group following the second vaccination. Systemic reactogenicity was similar across the two arms.

Risk During Pregnancy
There were four developmental toxicity studies that evaluated the effect of JYNNEOS on fetal and postnatal development and found no vaccine-related fetal malformations or adverse effects on postnatal development. JYNNEOS has not been evaluated in preclinical studies for carcinogenicity or mutagenicity.

No safety signals were observed in 29 pregnancies that occurred during the clinical development program of MVA-BN and the rate of spontaneous abortions observed was similar to the

published background incidence. However, there is insufficient data to assess the vaccine risk to the pregnant woman and her fetus and because of this, woman who are pregnant will not be eligible to participate in this study and females of reproductive potential will be asked to use effective birth control through Day 57.

Risk to Breast Feeding Infant
It is not known whether JYNNEOS is excreted in human milk. Data are not available to assess the effects of JYNNEOS in the breastfed infant or on milk production/excretion. For this reason, woman who are breastfeeding will not be eligible to participate in this study.

Risk to Children and Adolescents
Safety and immunogenicity of JYNNEOS have not been established in individuals less than 18 years of age. For this reason, this study will enroll only adults 18 to 50 years of age.

Risk to Individuals with Chronic Diseases
JYNNEOS does not replicate in human cell lines and therefore the vaccine can be safely administered to immunocompromised individuals. Safety has been demonstrated in clinical trials and studies involving severely immunocompromised animals[20,21] and humans with HIV infection.[22-24] The safety of JYNNEOS in HIV-infected individuals was evaluated in an open label trial that included 351 HIV-infected smallpox vaccine-naïve participants, 131 HIV-infected participants who previously received smallpox vaccine, 88 non-HIV-infected smallpox vaccine-naïve participants, and 9 non-HIV-infected participants who had previously received a smallpox vaccine.[22] HIV-infected participants had CD4 counts ≥ 200 and ≤ 750 cells/μL at study entry. Among smallpox vaccine-naïve participants, solicited local and systemic adverse reactions were reported at similar or lower frequencies in HIV-infected participants than in non-HIV-infected participants. Frequencies of other solicited local and systemic adverse reactions were similar to those reported in other trials.[23,24] In this study, individuals infected with HIV can participate in the study unless they had an AIDS defining illness within the last year or their viral load is not well-controlled with antiretroviral therapy (ART).

Individuals with atopic dermatitis (AD) are not able to receive replicating smallpox vaccines including ACAM2000. However, two clinical trials have shown that they can safely be given JYNNEOS.[25,26] The larger of the two studies was a multicenter, open-label clinical trial that evaluated the safety of JYNNEOS in 350 smallpox vaccine-naïve participants either with active or a history of AD and in 282 participants without AD.[26] The proportion of participants reporting solicited local and systemic adverse reactions was similar between the arms, except for redness (61.2% with AD vs. 49.3% without AD), swelling (52.2% with AD vs. 40.8% without AD), chills (15.9% with AD vs. 7.8% without AD) and headache (47.2% with AD vs. 34.8% without AD). However, most adverse events were mild to moderate in severity. In this study, individuals with AD are eligible to participate.

Cardiac Adverse Events of Special Interest (AESI)
During the 2002-2004 smallpox vaccination campaign initiated by the US Department of Defense (DoD) and Department of Health and Human Services (DHHS) to protect military personnel and civilian first-responders, there were high rates of pericarditis and myocarditis

reported among those vaccinated with Dryvax. Similarly, high rates were detected after receipt of ACAM2000.[27] Because of this, during the clinical development of MVA-BN, participants were monitored for cardiac-related signs or symptoms through at least 6 months after the last vaccination. In 22 studies, cardiac AESIs were reported to occur in 1.3% (95/7,093) of JYNNEOS recipients and in 0.2% (3/1,206) of placebo recipients who were smallpox vaccine naïve. The higher proportion of JYNNEOS recipients who experienced cardiac AESIs was driven by cases of asymptomatic post-vaccination elevation of troponin I, the clinical significance of which is unknown.

Among the cardiac AESIs reported, there were six, non-serious cases (0.08%) deemed causally related to JYNNEOS vaccination including tachycardia, electrocardiogram (ECG) T wave inversion, unspecified ECG abnormality, ECG ST segment elevation, ECG T wave abnormality, and palpitations. These data are consistent with findings from a randomized, double-blind, placebo-controlled Phase 3 trial that evaluated the immunogenicity and safety of three consecutive production lots of MVA-BN. In that trial, eight out of 3,003 (0.3%) participants who received at least one dose of MVA-BN had a cardiac AESI. Of these events, two of the eight were considered possibly related to MVA-BN vaccination; one participant had a right bundle branch block on ECG, and the other participant had clinical symptoms consistent with acute pericarditis but had a negative cardiac work-up. This same participant had positive serology to Coxsackie B virus suggesting that an acute viral infection may have been the cause of their symptoms. Given these data, we will not be monitoring for cardiac AESI during this study. However, we will be monitoring AE, MAAE, and SAE that will allow us to detect symptomatic cardiac conditions.

2.3.2 Known Potential Benefits

JYNNEOS was licensed in 2019 based on pre-clinical studies and clinical trial data showing that the vaccinia virus-specific (VV-WR) geometric mean titer (GMT) elicited by two doses of JYNNEOS was non-inferior to that elicited by ACAM2000; ACAM2000 was licensed based on non-inferiority to Dryvax. Participation in this study may result in protection against monkeypox during this current outbreak. However, participation may also provide no direct benefit to the participant if they develop antibodies to vaccinia virus and/or monkeypox virus that are not fully protective. Society may benefit from the identification of dose sparing regimens that could be used during the current global vaccine shortage. Additional supportive data on ID dosing may be used to inform the public health response to the current outbreak.

2.3.3 Assessment of Potential Risks and Benefits

JYNNEOS is a licensed product with a good safety profile. This study compares the standard 2-dose licensed SC dosing regimen with two, 2-dose ID regimens that use lower doses of the vaccine. We do not anticipate that the risks of participating in the trial and receiving this licensed vaccine will outweigh the benefits of participation and the value of the information gained from conducting this study. Risk to participants will be minimized by excluding groups of individuals identified to be at greatest risk for adverse reactions following vaccination with JYNNEOS.

3. OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS (OUTCOME MEASURES)
Primary	
To determine if peak humoral immune responses following an ID regimen of 2 x 10^7 $TCID_{50}$ MVA-BN are non-inferior to the licensed regimen of 1 x 10^8 MVA-BN administered SC	Vaccinia virus specific PRNT GMT at Day 43
To determine if peak humoral immune responses following an ID regimen of 1 x 10^7 $TCID_{50}$ MVA-BN are non-inferior to the licensed regimen of 1 x 10^8 MVA-BN administered SC	Vaccinia virus specific PRNT GMT at Day 43
Secondary	
To determine if individual peak humoral immune responses following each ID regimen are non-inferior to the licensed regimen administered SC	Individual peak GMT through Day 365
To evaluate humoral immune responses of each ID regimen (separately) compared to licensed SC regimen each study day.	Vaccinia virus specific PRNT GMT at Study Day 1, 15, 29, 43, 57, 90, 181, and 365
To evaluate the kinetics of the humoral immune responses of each ID regimen (separately) compared to licensed SC regimen through Day 365	Vaccinia virus specific PRNT half-life (t ½)
To compare relative safety among study arms as assessed by systemic and local reactogenicity for 14 days after each vaccination, unsolicited adverse events for 28 days after each vaccination, and serious adverse events (SAE) and medically attended events (MAAE) from Day 1 through Day 57, and related SAE/MAAEs through Day 181	Frequency, severity, and relatedness of solicited systemic and local AE for 14 days after each vaccination. Frequency severity, and relatedness of unsolicited AEs in each study arm for 28 days after each vaccination. Frequency and relatedness of MAAE and SAEs in each study arm. Frequency of withdrawals or discontinuation of vaccination in each study arm
Exploratory	
To evaluate other measures of the humoral immune responses for each regimen	Results from additional immunologic assays for vaccinia and other related viruses.
To evaluate humoral immune responses of each ID regimen (separately) compared to licensed dose administered SC to monkeypox virus.	Monkeypox virus specific PRNT GMT at Day 1 and 43.

4. STUDY DESIGN

4.1 Overall Design

This study is a Phase 2 randomized, open-label, non-placebo controlled, multi-site clinical trial that will evaluate two ID regimens for MVA-BN vaccine compared to the standard SC regimen in healthy, vaccinia-naïve adults 18 to 50 years of age, inclusive. At least 210 participants will be enrolled and randomized to one of three study arms. The two dose sparing strategies include one-fifth (2×10^7) and one-tenth (1×10^7) of the standard dose of MVA-BN administered ID on Day 1 and 29 (Arm 1 and 2, respectively). The comparator arm (Arm 3) will be the 2-dose standard (1×10^8) MVA-BN SC regimen.

The study will enroll a 1:1:1 randomization allocation. Participants will not be stratified by clinical trial site, demographic characteristics or HIV infection status; however, these data will be collected during screening and enrollment. Each participant may be screened either in a separate visit in the 7 days prior to Day 1 or on Day 1. Participants will be followed as outlined in the Schedule of Activities. The last follow-up visit will be at about 1 year after the first vaccination.

4.2 Scientific Rationale for Study Design

The rationale for each of the arms in this study is as follows:

1. **MVA-BN 2 x 10^7 ID (1/5th dose) on Days 1, 29 (Study Arm 1)** – This regimen matches the prior ID dosing and matches the dose authorized in the EUA. This regimen would allow for 5-fold increase in vaccine supply.
2. **MVA-BN 1 x 10^7 ID (1/10th dose) on Days 1, 29 (Study Arm 2)** – This regimen would test a larger dose conservation. This regimen would allow for 10-fold increase in vaccine supply.
3. **MVA-BN 1 x 10^8 SC on Days 1, 29 (Study Arm 3)** – This regimen is the licensed dose and route and will be used as a comparator.

4.3 Justification for Dose

This study will use the FDA-approved, licensed dose 1×10^8 administered SC on Day 1 and 29 as the comparator for the ID doses of MVA-BN. The ID regimens being evaluated in this study use lower doses of the licensed vaccine, and either match or are one dilution lower than the dose used in the prior ID dosing study (PMID 26143613). Therefore, it is anticipated this range is safe and immunogenic.

5. STUDY POPULATION

This study will enroll healthy, non-pregnant, non-breastfeeding adults 18 to 50 years old. Participants with stable medical conditions and well-controlled HIV infection can participate. The study will aim to enroll a population that has demographic features similar to the population being affected with monkeypox, yet also reflects the larger U.S. population to ensure broad applicability of the study findings. To this end, the goal will be to enroll:

- at least 30% female participants,
- at least 20% African American/Black participants,

- at least 20% Hispanic participants.

These are enrollment goals and will not be a deviation if these are not ultimately met. Sites will be chosen based on projected ability to enroll the representative study population. Enrollment will be monitored and adjusted at individual sites to pursue these goals while also enrolling this study quickly to inform public health decisions.

Participant Inclusion and Exclusion Criteria must be confirmed by an investigator named on the delegation log. If there is any uncertainty, the PI should make the decision on whether a potential participant is eligible for study enrollment. No exemptions are granted on Inclusion/Exclusion Criteria.

5.1 Inclusion Criteria

To be eligible to participate in this study, an individual must meet all the following criteria:

1. Individuals 18 - 50 years of age inclusive at the time of consent.
2. Able to read the written informed consent, states willingness to comply with all study procedures, and is anticipated to be available for all study visits.
3. Agreement to adhere to Lifestyle Considerations (defined in section 5.4) during the study.
4. Females of reproductive potential who have sexual intercourse with males must agree to use highly effective contraception for at least 1 month prior to signing ICF and through Day 57.

   Note: See MOP for definitions and list of highly effective contraception
5. In good general health as evidenced by medical history, physical examination, and clinical judgement of the investigator to be in stable state of health.

   Note: Participants with pre-existing stable chronic medical conditions defined as conditions not requiring significant change in therapy or hospitalization for worsening disease in the 4 weeks prior to enrollment can be included at the discretion of the investigator. This includes stable, well-controlled HIV positive individuals.
6. If HIV infected individual, they must be on suppressive ART for at least 6 months, report a CD4 count of greater than 350 cells/μL and no AIDS-defining illness in the last year.

5.2 Exclusion Criteria

An individual who meets any of the following criteria will be excluded from participation in this study:

1. Ever received a licensed or an investigational smallpox or monkeypox vaccine.

   Note: this includes Dryvax, Acam2000, LC 16 m8, MVA-based vaccine candidate or licensed vaccines, and Jynneos, Imvamune or Imvanex)
2. Any history of monkeypox, cowpox, or vaccinia infection.

3. Close contact of anyone known to have monkeypox in the 3 weeks prior to signing ICF
4. Immunocompromised as determined by the investigator
5. Recent or current use of any immunosuppressing medications in the 4 weeks prior to signing ICF.
   Note: topical, ophthalmic, inhaled, intranasal and intraarticular corticosteroids are acceptable, but receipt of ≥20 mg/day of prednisone or equivalent for ≥14 consecutive days in the 4 weeks prior to signing ICF is exclusionary.
6. Pregnant or breast feeding.
7. Received or plans to receive a live vaccine in the 4 weeks prior to signing ICF and 4 weeks after each vaccination
8. Received or plans to receive any other vaccine in the 2 weeks prior to signing ICF through Day 43.
9. Received experimental therapeutic agent or vaccine in the 3 months prior to signing ICF.
10. Has known allergy or history of anaphylaxis or other serious adverse reaction to a vaccine or vaccine products.
    Note: this includes individuals with history of severe allergic reaction to gentamicin, ciprofloxacin, chicken or egg protein.
11. Has tattoos, scars, or other marks which would, in the opinion of the investigator, interfere with assessment of the vaccination site.
12. Has any medical disease or condition that, in the opinion of the participating site PI or appropriate sub-investigator, precludes study participation.
    Note: this includes acute, subacute, intermittent, or chronic medical disease or condition that would place the participant at an unacceptable risk of injury, render the participant unable to meet the requirements of the protocol, or may interfere with the evaluation of responses or the participant's successful completion of this trial.

5.2.1 Exclusion of Specific Populations

This study will enroll healthy non-pregnant, non-breastfeeding adults 18 to 50 years old. Adults with a history of receiving smallpox vaccine as a child and those born before 1971 will be excluded as they are likely to have received smallpox vaccination during the smallpox eradication campaign. Because the effects on the fetus are not known, pregnant individuals will not be eligible for the trial (see Risk/Benefit Assessment Section). In addition, individuals who are breastfeeding will not be eligible because it is not known if JYNNEOS is excreted in the breastmilk. Adolescents and children will not be included in this trial because there is no safety or efficacy data on the ID route of administration in this population. Should the outcome of this trial be deemed acceptable, additional trials may be initiated, including those in other populations.

5.3 Inclusion of Vulnerable Participants

Not Applicable. This study will not enroll vulnerable participants including prisoners, cognitively impaired participants, adolescents, and children.

5.4 Lifestyle Considerations

During this study, participants are asked to:

- Follow public health guidance on preventing monkeypox infection and notify the clinical site if exposed to an individual with monkeypox.
- Contact the clinical site immediately if they develop signs and symptoms consistent with monkeypox to schedule an *ad hoc* study sick visit.
- Refrain from receiving a live vaccine from enrollment through Day 57.
- Refrain from receiving any other vaccine through Day 43.
- Decline participation in another study evaluating investigational vaccines through Day 181.
- Decline participation in another study evaluating an investigational MVA-based vaccine through end of trial.

Of note, participants may enroll in non-interventional, observational studies (e.g., natural history study of monkeypox). However, concurrent participation in this trial and observational studies can only occur if the recommended blood collection volumes are not exceeded.

5.5 Screen Failures

After the screening evaluations have been completed, the investigator or designee is to review the inclusion/exclusion criteria and determine the participant's eligibility for the study. The following information will be collected on screen failures: demographics (age, screen number, sex, ethnicity, and race) and reason(s) for ineligibility. Participants who are found to be ineligible will be told the reason for ineligibility.

A participant may be re-screened once if a protocol eligibility criterion that is not met at the initial time of screening, will be met by rescreening at a later date (e.g., a vaccine was received within exclusionary window at first screening that would not be in the exclusionary window at a later rescreen).

5.6 Strategies for Recruitment and Retention

5.6.1 Recruitment

Potential participants will learn about the study via IRB-approved recruitment strategies, which may include direct mailing, recruitment from an IRB-approved trial registry, and local flyers or advertisements. Recruiting may begin with a brief telephone call between study staff and the potential participant. Information about the study will be presented to potential participants, and questions about their health and ability to comply with the study visit schedule will be asked of potential participants to presumptively determine eligibility. Information about the participant may be recorded from interviews or medical records. Appointments will be made at the research

site for potential participants who are interested in the study for further screening procedures and additional protocol-specific information.

5.6.2 Retention

Study retention strategies will include education and explanation of the study schedule and procedures during screening and enrollment visits and restriction of enrollment to persons who can attend all study visits. Study participants will be reminded of subsequent visits during each visit, and study staff may contact participants prior to appointments. Study staff will contact participants who miss appointments to encourage them to return for completion of evaluations.

5.6.3 Compensation Plan for Participants

Participants may be compensated for their participation in this trial. Compensation will be in accordance with local institution requirements, and pursuant to IRB approval. Reimbursements will be disbursed at specific timepoints during the study with the amount contingent on completing study procedures.

5.6.4 Costs

There is no cost to participants for the research tests, procedures, and study product while taking part in this trial. Procedures and treatment for clinical care may be billed to the participant, participant's insurance or third party.

6. STUDY PRODUCT

6.1 Study Product(s) and Administration

6.1.1 Study Product Description

JYNNEOS is FDA-approved and licensed as a smallpox and monkeypox vaccine in the United States. The vaccine, when thawed, is a milky, light yellow to pale white colored suspension that is licensed for subcutaneous injection. JYNNEOS is a live vaccine produced from the strain Modified Vaccinia Ankara-Bavarian Nordic (MVA-BN), an attenuated, non-replicating orthopoxvirus. MVA-BN is grown in primary Chicken Embryo Fibroblast (CEF) cells suspended in a serum-free medium containing no material of direct animal origin, harvested from the CEF cells, purified, and concentrated by several Tangential Flow Filtration (TFF) steps including benzonase digestion. Each 0.5 mL dose is formulated to contain 0.5×10^8 to 3.95×10^8 infectious units of MVA-BN live virus in 10 mM Tris (tromethamine), 140 mM sodium chloride at pH 7.7. Each 0.5 mL dose may contain residual amounts of host-cell DNA (≤ 20 mcg), protein (≤ 500 mcg), benzonase (≤0.0025 mcg), gentamicin (≤0.163 mcg), and ciprofloxacin (≤0.005 mcg).

6.1.2 Dosing and Administration

In this trial, a licensed vaccine is being evaluated (i.e., JYNNEOS) and there is no placebo arm. Use of the contralateral arm is an option if there is any residual local reaction at Day 29 or if participant requests that this be done.

Table 6. Study Arms

Arm	Dose of JYNNEOS (MVA-BN)	Route of Administration*	Vaccination Day	
			Day 1	Day 29
1	2 x 10^7 $TCID_{50,}$ (0.1 mL)	Intradermal	X	X
2	1 x 10^7 $TCID_{50,}$ (0.05 mL)	Intradermal	X	X
3	1 x 10^8 $TCID_{50,}$ (0.5 mL)	Subcutaneous	X	X

*Subcutaneous is administered in the deltoid region, intradermal is administered in the volar aspect (inner side) of the forearm.

6.1.3 Dose Escalation

Not Applicable.

6.1.4 Dose Modifications

Not Applicable.

6.1.5 Criteria for Redosing

If study product administration is unsuccessful, another administration will not be attempted.

6.2 Preparation/Handling/Storage/Accountability

6.2.1 Acquisition and Accountability

Product: JYNNEOS (MVA-BN vaccine)
The JYNNEOS (MVA-BN vaccine) will be stored and provided by DMID Repository. Study product will be shipped to the clinical research site upon request and approval from DMID.

Accountability
The participating site PI is responsible for study product distribution and disposition and has ultimate responsibility for study product accountability. The participating site PI may delegate to the participating site's research pharmacist responsibility for study product accountability. The participating site's research pharmacist will be responsible for maintaining complete records and documentation of study product receipt, accountability, dispensation, storage conditions, and final disposition of the study products.

Time of vaccine administration to the participant will be recorded on the appropriate data collection form (DCF). All study product(s), including the amount of JYNNEOS (MVA-BN vaccine), and whether administered or not, must be documented on the appropriate study product accountability record or dispensing log. The sponsor's monitoring staff will verify the participating site's study product accountability records and dispensing logs per the DMID-approved clinical monitoring plan (CMP).

Once all participant dosing is complete, the pharmacy staff should retain or dispose of used study products and complete study product accountability procedures in accordance with site-specific standard operating procedures (SOPs). This applies to:

- used and unused JYNNEOS (MVA-BN vaccine) vials
- JYNNEOS (MVA-BN vaccine) cartons

All used vials and cartons may either be sequestered from the unused supplies and retained until study conclusion or until study product accountability has occurred by the monitor and written notification stating retention is no longer required is received. Used syringes should be discarded per usual procedure.

Destruction
After the study treatment period has ended or as appropriate over the course of the study after study product accountability has been performed, disposition of unused and used JYNNEOS (MVA-BN vaccine) vials can be destroyed on-site following applicable site procedures. A certificate of destruction or documentation of destruction should be provided to the sponsor and retained in the Pharmacy Binder once completed.

6.2.2 Formulation, Appearance, Packaging, and Labeling

Product: JYNNEOS (MVA-BN vaccine)
JYNNEOS is supplied in a single-dose vial with turquoise and white label for SC injection. Each 0.5 mL dose is formulated to contain 0.5 x 10^8 to 3.95 x 10^8 infectious units of MVA-BN live virus in 10 mM Tris (tromethamine), 140 mM sodium chloride at pH 7.7. Each 0.5 mL dose may contain residual amounts of host-cell DNA (≤ 20 mcg), protein (≤ 500 mcg), benzonase (≤0.0025 mcg), gentamicin (≤0.163 mcg), and ciprofloxacin (≤0.005 mcg). JYNNEOS is a sterile vaccine formulated without preservatives. The vial stoppers are not made with natural rubber latex.

6.2.3 Product Storage and Stability

Product: JYNNEOS (MVA-BN vaccine)
JYNNEOS should be kept frozen at -25°C to -15°C (-13°F to +5°F) and stored in the original package to protect from light. JYNNEOS should not be re-frozen once a vial has been thawed. Once thawed, the vaccine may be kept at +2°C to +8°C (+36°F to +46°F) for 12 hours. The package insert provides additional instructions for the handling and storage of JYNNEOS.

6.2.4 Preparation
Each dose (0.5 mL) is supplied in a single-dose vial. Sites should allow the vaccine to thaw and reach room temperature before use (about 15 to 30 minutes). After thawing, the total time stored at +2°C to +8°C (+36°F to +46°F) should not exceed 12 hours. Once the vial is punctured and a dose is withdrawn, if it is not used in its entirety, it should be stored at +2°C to +8°C (+36°F to +46°F) and discarded within 8 hours of the first puncture. If the unpunctured vial is initially shipped/stored at 2-8C then it is stable for up to 8 weeks. Sites should not refreeze the vaccine. JYNNEOS may be kept frozen until the expiration date.

JYNNEOS is a light yellow to pale white colored, milky suspension when thawed. Sites will visually inspect the product for particulate matter and discoloration prior to administration. If either condition is present, the vaccine will not be administered. Study product administrators will gently swirl the vial for at least 30 seconds.

For the ID injection (Arm 1), 0.1 mL of vaccine will be withdrawn from the vial into a sterile syringe and injected intradermally into the volar aspect (inner side) of the forearm.

For the ID injection (Arm 2), 0.05 mL of vaccine will be withdrawn from the vial into a sterile syringe and injected intradermally into the volar aspect of the forearm.

For the SC injection (Arm 3), 0.5 mL of vaccine will be withdrawn from the vial into a sterile syringe and injected into the fatty tissue over the deltoid area.

See the protocol-specific MOP for detailed information on the preparation, labeling, storage, and administration for each arm.

6.3 Measures to Minimize Bias: Randomization and Blinding

6.3.1 Treatment Assignment Procedures

The randomization procedure will be described in the MOP.

6.3.2 Randomization

There will be a 1:1:1 randomization allocation. Randomization will not be stratified. Randomized treatment assignments will be generated by a statistician at The Emmes Company, LLC, the Data and Statistical Coordinating Center (SDCC) for this study. Participants will be registered using a web-based application developed by The Emmes Company, LLC. Upon entry of demographic data and confirmation of eligibility for the trial, the participant will be enrolled and randomized. The randomization procedure will be described in more detail in the MOP.

6.3.3 Blinding and Masking Procedures

This study will not utilize blinding or masking procedures as it is an open-label, non-placebo-controlled trial. Research laboratories will be blinded to participant study arm for the purposes of immunogenicity/efficacy evaluations.

6.4 Study Intervention Compliance

Each dose of JYNNEOS will be administered by a member of the clinical research team that is qualified and licensed to administer the study product. Administration and date, time, and location of injection will be recorded on the appropriate eCRF.

6.5 Concomitant Therapy

At screening (i.e., the screening visit or Day 1 visit if the screening visit is not conducted), participants will be asked about receipt of immunosuppressive therapy, experimental therapeutic agents and vaccines, other medications, and vaccines. At each subsequent study visit through Day 57, new concomitant medication(s) and changes to existing medications will be recorded as well as receipt of a vaccine. For this study, concomitant medications to be reported in the Case Report Form (CRF) are prescription drugs, over-the-counter medications, and supplements. Concomitant medications taken and vaccines received in the 4 weeks prior to providing informed consent will be recorded in the CRF. Any drug taken or vaccine received by the participant during the trial should be recorded on the appropriate CRF through Day 57. After Day 57, only concomitant medications that are associated with a related SAE or MAAE through Day 181 and clinically relevant to report will be recorded in the CRF. An exception to this schedule for recording concomitant therapy, is during sick visits for those who have laboratory-confirmed monkeypox (illness). During sick visits, concomitant medications and treatment will be collected.

Medications that might interfere with the evaluation of the immune response to MVA-BN should not be used by the participant during the study-reporting period unless clinically indicated as part of the participant's health care for a condition diagnosed after enrollment. This would include any systemic immunosuppressant medication that may impact the immunogenicity endpoints. In the event medical conditions dictate the use of these medications, participants are encouraged to obtain adequate care, comply with the course of therapy as prescribed by their physician and inform the study Investigator as soon as practical.

6.5.1 Rescue Medicine

Not applicable.

7. STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 Halting Criteria and Discontinuation of Study Intervention

7.1.1 Study Halting Criteria

The study will be paused for safety analysis if any of the following events occur:

1. One (1) participant experiences a SAE after the administration of the vaccine that is considered related to the vaccine.
2. One (1) participant experiences a Grade 4 solicited AE after the administration of the vaccine that is considered related to the vaccine.
3. Three (3) or more participants experience a Grade 3 or higher unsolicited AE coded to the same Preferred Term based on the medical dictionary for Regulatory Activities (MedDRA) that are deemed related to the vaccine.

In the event a halting rule is met, an unscheduled safety analysis by the DSMB will be required for approval of further enrollment. Further administration of the vaccine, including a second dose, is suspended for all participants until an assessment by the DSMB takes place.

7.1.1.1 Sentinel Participant Halting Rules

Not Applicable.

7.1.1.2 Cohort Halting Rules

Not Applicable.

7.1.2 Individual Halting Criteria

If a single participant meets an individual halting criterion, this will not halt the whole study but halts the participant's involvement in terms of the administration of the second dose of the vaccine. However, while the participant is discontinued from receipt of vaccine, they should be encouraged to remain in the study to monitor safety and evaluate immunogenicity until the end of the study.

1. Any participant experiences laryngospasm, bronchospasm or anaphylaxis within 24 hours after the administration of the vaccine that is considered related to the vaccine within the 24 hours after vaccination.
2. Any participant experiences generalized urticaria (defined as urticarial lesions occurring at more than two body parts) within 3 days after the administration of the vaccine.
3. Any participant experiences any Grade 4 solicited AE or an SAE after the administration of the vaccine that is deemed related to the vaccine.
4. Any participant that experiences ulceration, abscess or necrosis at the injection site that is considered related to the vaccine administration.

A participant may be removed from the study if the Investigator or the medical monitor deems it in the best interest of the participant. The participant could be withdrawn from the study at any time during the trial.

7.1.3 Follow up for participants that discontinued study intervention

Discontinuation from vaccination administration does not mean discontinuation from the study, and remaining study procedures should be completed as indicated by the study protocol. If a clinically significant finding is identified (including, but not limited to changes from baseline) after enrollment, the investigator or qualified designee will determine if any change in participant management is needed.

The second vaccination will not be given, but other follow up visits will continue, if the participant:

- Becomes pregnant.
- Develops monkeypox (symptomatic disease) after the first vaccination

- Meets an exclusion criterion for participation in the study (either newly developed or not previously recognized) that precludes further study participation.

  If a participant meets an exclusion criterion after enrollment/randomization and first dose, it does not necessarily mean the participant cannot receive the second dose of JYNNEOS. The decision of what "precludes further study participation" depends on what exclusion criteria are met, with a focus on what is best for participant safety. The most likely exclusion criteria to be met are participants that receive a licensed vaccine after randomization (i.e., exclusion criteria 7 and 8). If a participant receives an off-study vaccine during the study-defined restricted time periods (i.e., as defined in exclusion criteria and Section 5.4), receipt of the off-study vaccine should be reported as a deviation. The participant can receive the second study vaccination if the investigator determines that the participant is otherwise eligible to receive the second MVA-BN in this trial.

There is no reactogenicity threshold for administration of the second dose of vaccine, that is the second dose may be administered even if there is residual erythema, induration or other reactogenicity from the first vaccination. Participants may decline the second vaccination due to residual reactogenicity at the Day 29 visit and will be discontinued from the study intervention. However, the participant will remain in the study and followed for safety and immunogenicity through Day 365 unless they withdraw from the study.

The data to be collected at the time of study intervention discontinuation will include:

- Reason for discontinuation study vaccination including, but not limited to, unacceptable (intolerable) reactogenicity, new medical condition identified that places participant at risk, participant unwillingness to have second vaccination.
- Confirmation of willingness to remain in the study and understanding of study schedule.

7.2 Participant Withdrawal from the Study and Replacement

Participants are free to withdraw from participation in the study at any time upon request. An investigator may withdraw a participant from the study if the participant:

- Has an AE or other medical condition that continued participation in the study would not be in the best interest of the participant.
- Is lost to follow-up

The reason for participant discontinuation or withdrawal from the study, whether the decision was made by investigator or participant, and the extent of the withdrawal (i.e., whether participant withdrew from all components of research study or just the study intervention) will be recorded on the CRF. If the participant agrees, every attempt will be made to follow all AEs through resolution (return to baseline) or until defined as stable. The investigator will inform the participant that already collected data will be retained and analyzed for this study even if the participant withdraws from this study. Biospecimens collected will still be used to analyze the study endpoints.

Participants who withdraw, or are withdrawn from this study, or are lost to follow-up after signing the ICF and administration of the study product will not be replaced.

7.3 Lost to Follow-Up

A participant will be considered lost to follow-up if he or she fails to return for two scheduled visits and is unable to be contacted by the study site staff after three attempts. Extensive effort (i.e., generally three documented contact attempts via telephone calls, e-mail, etc., made on separate occasions) will be made to locate or recall the participant, or at least to determine the participant's health status. These efforts will be documented in the participant's study file.

8. STUDY ASSESSMENTS AND PROCEDURES

8.1 Screening Procedures

In this study, screening assessments can occur in an optional screening visit on Day –7 to –1, or at Visit 1 on Day 1 before the first vaccination. At the time of the screening assessment, the participant will be provided with detailed study information and written informed consent will be obtained. The following assessments are performed to determine eligibility requirements as specified in the inclusion and exclusion criteria:

- Obtain demographic data.
- Obtain monkeypox exposure history in the 3 weeks prior to signing ICF.
- Review vaccination history, including any experimental vaccines, and determine receipt of exclusionary vaccine(s):
    - Live vaccine in the prior 4 weeks or an inactivated vaccine in the prior 2 weeks.
    - Licensed or investigation smallpox or monkeypox vaccine.
- Obtain medical history. Ask specifically about worsening of any chronic medical condition or hospitalizations in the prior 4 weeks, and whether they have the following medical conditions:
    - HIV infection
    - History of monkeypox, cowpox or vaccinia infection
    - History of anaphylaxis or severe adverse reaction to a vaccine or vaccine product
    - History of allergy to gentamicin, ciprofloxacin, chicken or egg protein
    - History of keloid scar formation
- Review medications and therapies up to 4 weeks prior to signing ICF and record on the appropriate CRF and determine receipt of exclusionary medication(s):
    - Experimental therapeutic agent (or vaccine) in the 3 months prior to consent
    - Use of immunosuppressing drugs prior to consent.
- Perform targeted physical examination and inspect arms for smallpox vaccination scar
- Measure vital signs (HR, BP, and oral temperature).
- Review of birth control history with female participants.
    - Counsel participants to use adequate birth control methods required during the trial to avoid pregnancy.
    - Do serum or urine pregnancy test (in participants of childbearing potential)
- Review inclusion and exclusion criteria.

The overall eligibility of the participant to participate in the study will be assessed once all screening values are available. The screening process can be suspended prior to complete assessment at any time if exclusions are identified by the study team.
Study participants who qualify for inclusion will be enrolled.

8.1.1 Procedures to be Followed in the Event of Abnormal Clinical Laboratory Test Values or Abnormal Clinical Findings During Screening

A participant may be re-screened once if a protocol eligibility criterion that is not met at the initial time of screening, will be met by rescreening at a later date. No participant may be screened more than twice due to a screening failure result.

Participants will be provided the results of pregnancy testing and abnormal clinical findings necessitating follow-up at the discretion of the participating site PI or appropriate sub-investigator.

8.1.2 Sick Visit

Participants with signs and symptoms consistent with monkeypox (e.g., vesicular or pustular lesions, fever, chills, lymphadenopathy, malaise, myalgias, headache, or respiratory symptoms) during the study are asked to call the study site. Those who have laboratory-confirmed monkeypox will be evaluated at the clinical site; those who have not been tested will be asked to have a diagnostic test done. During the visit, the participant will be asked about the current illness (e.g., onset date, treatment received) and for information about the diagnostic testing that was done. Participants will have a targeted physical examination done, and a blood sample will be taken. For more information about this visit, see the MOP and the CRF.

8.2 Immunogenicity Assessments

8.2.1 Immunogenicity/Efficacy Evaluations

Prior comparator studies used the Bavarian Nordic (BN) Vaccina virus Western Reserve strain (VV-WR) plaque reduction neutralizing antibody (PRNT) assay and enzyme-linked immunosorbent assay (ELISA) to evaluate immunogenicity. [16,23,28] In this study, we will use the PRNT as the primary assay to determine immunogenicity and will test a minimum of 4 timepoints (i.e., at least Days 1, 29, 43, 90 from Days 1, 15, 29, 43, 57, 90, 181 and 365).
This study will also measure a monkeypox virus specific PRNT and will test a minimum of 2 timepoints (Days 1 and 43). The monkeypox PRNT assay is not yet developed, and therefore this endpoint is as an exploratory endpoint. Additional timepoints for each will be evaluated as able (given lab throughput and cost).

8.2.2 Exploratory Assessments

Additional assessments of humoral immunity including ELISAs may be performed on samples from this trial.

8.3 Safety and Other Assessments

Study procedures are specified in the SOA and more detailed information as needed is provided in the MOP. A study clinician, licensed to make medical diagnoses and listed on the Form FDA 1572 as the participating site PI or appropriate sub-investigator, will be responsible for all study-related medical decisions.

- Medical history
    - A complete medical history will be obtained by interview of participants at screening (i.e., the screening visit or on Day 1 if no screening visit is conducted).
    - An interim medical history will be obtained by interview of participants at subsequent study visits or ad hoc telephone call with participant. Any changes since the previous clinic visit will be noted. The interim medical history should include an assessment for adverse events and new medical conditions.
- Physical examination
    - A symptom-directed (targeted) physical exam will be performed if indicated by medical history.
- Reactogenicity Assessments
    - Reactogenicity assessments of solicited systemic reactogenicity occurring from the time of each vaccination through 14 days post each vaccination will include fever, chills, nausea, headache, fatigue, change in appetite, myalgia, and arthralgia.
    - Assessments of solicited local (injection site) reactogenicity occurring from the time of each vaccination through 14 days post each vaccination will include pain at injection site, erythema/redness, induration/swelling, and pruritis at site.
    - Any ongoing events at Day 15 will be followed at subsequent visits in order to note a resolution date. See Memory Aid section below on measurement of ongoing erythema/redness and/or induration/swelling.
- Memory Aid
    - All participants will complete a Memory Aid to document local and systemic reactogenicity from time of each vaccination through 14 days post each vaccination (Days 1-15 for the first vaccination and Days 29-43 for the second vaccination).
    - Memory Aids will be reviewed with the participants for any solicited reactions, as well as unsolicited AEs and SAEs.
    - The vaccination injection site will be examined at all visits until symptoms resolve.
    - For those with no residual systemic or local reactogenicity at the Day 15 or Day 43 visit, no more data is collected by the participant in the Memory Aid.
    - For those with ongoing systemic and/or local reactogenicity at Day 15 or Day 43 visit, they will be asked to do the following:
        - Measure erythema/redness and/or induration/swelling and record in the Memory Aid until it has resolved, if applicable.
        - Record end date of all other signs and symptoms
    - Nodules and hyperpigmentation are AEs, and not collected in the Memory Aid to avoid double counting these events which tend to develop at end of second week as a post-inflammatory reaction.
- Assessment of tolerability

  - On Day 15 and Day 43, the participants will be asked several global assessment questions to evaluate how well the vaccine is tolerated.
- Vital signs
  - Vital sign measurements will include systolic and diastolic BP, HR, and oral temperature. Vital signs will be measured at timepoints specified in the SOA.
- Clinical laboratory evaluations
  - Serum or urine pregnancy test will be performed locally by the site laboratory at the screening and must be negative within 24 hours prior to each vaccination on Days 1 and 29. This means that if the screening visit is conducted more than 24 hours prior to vaccination, pregnancy test will need to be done on Day 1. Pregnancy tests may be done as needed at interim or unscheduled visits for all women of childbearing potential.
  - Results must be confirmed as negative prior to enrollment on Day 1 and administration of each vaccination.

8.3.1 Procedures to be Followed in the Event of Abnormal Clinical Laboratory Test Values or Abnormal Clinical Findings During Conduct of the Trial

In this study, there will be no clinical laboratory screening or safety tests done except for pregnancy testing. Participants will be notified of the results of the pregnancy test.

8.4 Adverse Events and Serious Adverse Events

8.4.1 Definition of Adverse Events (AE)

Adverse event means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)). An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of medicinal (investigational) product.

Any medical condition that is present at the time the participant is screened will be considered as baseline and not reported as an AE. However, if the severity (i.e., grade) of any pre-existing medical condition increases, it should be recorded as an AE.

Adverse events can be further divided into solicited adverse events and unsolicited adverse events. Solicited adverse events are those for which the study team will specifically query the participant whether they occurred. Unsolicited adverse events are those events that the participant report occurring without being queried about the specific event.

8.4.1.1 Solicited Adverse Events

Reactogenicity will be especially important to document because of prior studies demonstrating more local (measurement) reactogenicity among participants who received the ID MVA-BN vaccine injection. In this study, we will collect the following solicited adverse events based on prior studies. This includes local (pain at the site of the injection, erythema/redness, induration/swelling, pruritis) and systemic (fever, chills, nausea, headache, fatigue, change in appetite, myalgia [exclusive of the injection site], and arthralgia) reactogenicity. In addition, we expect that some participants will develop skin discoloration and nodules which will be reported as AEs and not collected on the memory aid and followed through to resolution. For this study, we will use the following toxicity grading scale:

> "*FDA Guidance for Industry, Toxicity Grading Scale for Healthy Adults and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials*" as a reference.

Of note, pruritis/itching, arthralgia, chills, and change in appetite are not included in the FDA toxicity table. However, the FDA Guidance allows for addition of other signs and symptoms as follows:

Mild:	No interference with activity
Moderate:	Some interference with activity
Severe:	Prevents daily activity
Potentially Life Threatening:	ER visit or hospitalization

8.4.1.2 Unsolicited Events

Unsolicited events are all AEs spontaneously reported by the participant and/or in response to an open question from study personnel or revealed by observation, physical examination or other diagnostic procedures.

8.4.2 Definition of Serious Adverse Events

An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes (21 CFR 312.32 (a)):

- death
- a life-threatening adverse event*
- inpatient hospitalization or prolongation of existing hospitalization
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- a congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or participant 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, convulsions that do not result in inpatient hospitalization, etc.

* An adverse event is considered “life-threatening” if, in the view of either the investigator or sponsor, its occurrence places the patient or participant at immediate risk of death. It does not include grade 4 severity unless the adverse event might have caused death.

8.4.2.1 Definition of Medically Attended Adverse Events (MAAE)

A medically attended adverse event (MAAE) is an unsolicited AE that results in unscheduled medical attention such as a hospitalization for less than 24 hours, an emergency room visit, or an otherwise unscheduled healthcare visit for any reason. All SAEs and MAAEs will be collected from Day 1 through Day 57, and all SAE/MAAEs related to the vaccine through Day 181.

8.4.2.2 Definition of Suspected Unexpected Serious Adverse Reactions (SUSAR)

A SUSAR is a SAE that is considered related to study product and unexpected.

Unexpectedness to study product will be determined by what is listed in the JYNNEOS package insert.

8.4.3 Classification of an Adverse Event

The determination of seriousness, severity, and causality will be made by an on-site investigator or other staff as listed on the delegation log.
Any potential grade 3 or 4 AE should be evaluated by someone qualified to provide a medical evaluation of AEs. This includes but is not limited to physicians, physician assistants, and nurse practitioners.

8.4.3.1 Severity of Event

All AEs or SAEs will be assessed for severity, according to the toxicity grading scale, *FDA Guidance for Industry, Toxicity Grading Scale for Healthy Adults and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials*. For adverse events (AEs) not included in the protocol-defined grading scale, the following guidelines will be used to describe severity.

> Mild (Grade 1): Events that are usually transient and may require only minimal or no treatment or therapeutic intervention and generally do not interfere with the participant's usual activities of daily living.
> Moderate (Grade 2): Events that are usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research participant.
> Severe (Grade 3): Events interrupt usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention. Severe events are usually incapacitating.
> Life-threatening (Grade 4): Extreme limitation in activity, significant assistance required; significant medical intervention/therapy required, hospitalization or care provided.

8.4.3.2 Relationship to Study Intervention

For each reported adverse event or reaction, the Principal Investigator or designee must assess the relationship of the event to the study product using the following guideline:

> Related – The AE is known to occur with the study intervention, there is a reasonable possibility that the study intervention caused the AE, or there is a temporal relationship between the study intervention and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study intervention and the AE.
> Not Related – There is not a reasonable possibility that the administration of the study intervention caused the event, there is no temporal relationship between the study intervention and event onset, or an alternate etiology has been established.

8.4.4 Time Period and Frequency for Event Assessment and Follow-Up

For this study:

- Systemic and local solicited adverse events will be collected from Day 1 through Day 15, and Day 29 through Day 43. However, participants with ongoing systemic and/or local reactogenicity at Day 15 or Day 43 visit, will be asked to measure erythema/redness and/or induration/swelling until it has resolved, if applicable, and record end date of all other signs and symptoms.

- Unsolicited adverse events will be collected from Day 1 through Day 29, and Day 29 through Day 57.
- All Serious adverse events (SAEs) and MAAEs will be collected from Day 1 through Day 57, and all SAE/MAAEs related to the vaccine through Day 181.
- All SAEs will be followed through resolution or until the site investigator deems the event to be chronic or the participant is stable.
- AEs will be followed through resolution.

8.4.5 Adverse Event Reporting

8.4.5.1 Investigators Reporting of AEs

All clearly related signs, symptoms, and results of diagnostic procedures performed because of an AE should be grouped together and recorded as a single diagnosis or AE term, if available. If the AE is a laboratory abnormality that is part of a clinical condition or syndrome, it should be recorded as the syndrome or diagnosis rather than the individual laboratory abnormality. Each AE will also be described in terms of duration (start and stop date), severity, association with the study product, action(s) taken, and outcome.

AEs characterized as intermittent require documentation of onset and duration of each episode. All AEs will be assessed for severity, relatedness to study vaccine (see Section 8.4.3), and seriousness (see section 8.4.2) by the study PI or sub-investigator.

All AEs will be captured on the appropriate data collection form. Information to be collected for AEs includes event term or description, date of onset, assessment of severity, relationship to study product, and alternate etiology (assessed only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as an investigator), date of resolution, seriousness, and outcome. AEs occurring during the trial collection and reporting period will be documented appropriately regardless of relationship.

8.4.5.2 Special Reporting of Adverse Events

Not applicable.

8.4.6 Serious Adverse Event Reporting

8.4.6.1 Investigators Reporting of SAEs

Any AE that meets a protocol-defined serious criterion must be submitted immediately (within 24 hours of site awareness) on an SAE form to the DMID Pharmacovigilance Group, at the following address:

DMID Pharmacovigilance Group
Clinical Research Operations and Management Support (CROMS)
6500 Rock Spring Dr. Suite 650
Bethesda, MD 20817, USA
SAE Hot Line: 1-800-537-9979 (US) or 1-301-897-1709 (outside US)

SAE FAX Number: 1-800-275-7619 (US) or 1-301-897-1710 (outside US)
SAE Email Address: PVG@dmidcroms.com

In addition to the SAE form, select SAE data fields must also be entered into the SDCC system. Please see the protocol-specific MOP for details regarding this procedure.

Other supporting documentation of the event may be requested by the DMID Pharmacovigilance Group and should be provided as soon as possible. The DMID Medical Monitor will review and assess the SAE for regulatory reporting and potential impact on study participant safety and protocol conduct.

At any time after completion of the study, if the site principal investigator or appropriate sub-investigator becomes aware of an SAE that is suspected to be related to study product, the site principal investigator or appropriate sub-investigator will report the event to the DMID Pharmacovigilance Group.

8.4.6.2 Regulatory Reporting of SAEs

Following notification from the site Principal Investigator or appropriate sub-investigator, DMID, as the IND sponsor, will report any SUSAR to the FDA as an IND safety report. DMID will report an AE as a suspected unexpected adverse event only if there is evidence to suggest a causal relationship between the study intervention and the AE.

The Sponsor must ensure the event meets all three of the definitions:

- Suspected adverse reaction.
- Serious.
- Unexpected.

Both serious and unexpectedness are important, but the event will be reported only if there is evidence to suggest a causal relationship between the drug and the adverse event, such as:

- A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure.
- One or more occurrences of an event that is not commonly associated with drug exposure but is otherwise uncommon in the population exposed to the drug.
- On review as an aggregate analysis when the occurrence is higher than the historical control.

DMID will report to the FDA any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than 7 calendar days after the sponsor's initial receipt of the information. If the event is not fatal or life-threatening the IND safety report will be submitted within 15 calendar days after the sponsor determines that the information qualifies for reporting as specified in 21 CFR Part 312.32. Relevant follow up information to an IND safety report will be submitted as soon as the information is available. Upon request from FDA, DMID will submit to the FDA any additional data or information that the agency deems necessary, as soon as possible, but in no case later than 15 calendar days after receiving the request.

DMID will submit an IND safety report to the FDA and will notify all participating site Principal Investigators (i.e., all Principal Investigators to whom the sponsor is providing drug under its IND(s) or under any Principal Investigator's IND(s) of potential serious risks from clinical studies or any other source, as soon as possible. SAEs that are not SUSARs will be reported to the FDA at least annually in a summary format which includes all SAEs.

8.4.7 Reporting Events to Participants

Participants have the right to be informed of any new findings of AEs or SAEs that may affect their safety or influence their choice to participate or continue participating in the study.

8.4.8 Adverse Events of Special Interest (AESI)

There are no pre-specified AESI for this trial.

8.4.9 Reporting of Pregnancy

Pregnancy is not an AE. However, any pregnancy that occurs during study participation (through Day 365) should be reported to the sponsor on the appropriate DCF. Pregnancy should be followed for safety and outcome. This follow-up will include pregnancy outcome (termination, pre-term birth, term birth) and newborn outcome (live birth, fetal demise, stillbirth; presence of any congenital anomalies). No in-person visits will be required for pregnancy outcome determination.

8.5 Unanticipated Problems

8.5.1 Definition of Unanticipated Problems (UP)

The Department of Health and Human Services Office for Human Research Protections (OHRP) considers unanticipated problems (UPs) involving risks to participants or others to include, in general, any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied; and
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Incidents arising from noncompliance with study procedures should be reported as protocol deviations (see Section 10.1.10). An incident that qualifies as both a UP and a protocol deviation should be reported as both.

8.5.2 Unanticipated Problem Reporting

The investigator will report unanticipated problems (UPs) to the study sponsor, the reviewing Institutional Review Board (IRB) and to the Data and Statistical Coordinating Center (SDCC).

The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs that are serious adverse events (SAEs) will be reported to the IRB and to the SDCC/study sponsor within 24 hours of the investigator becoming aware of the event.
- Any other UP will be reported to the IRB and to the SDCC/study sponsor within a timeline defined according to the relevant policies.
- UPs will be collected through the end of the study.

8.5.3 Reporting Unanticipated Problems to Participants

Participants will be informed of any UPs that will potentially influence their participation in this trial.

9. STATISTICAL CONSIDERATIONS

9.1 Statistical Hypotheses

The primary hypothesis involves a two-step hierarchical process. The study will first test non-inferiority of the 2 x 10^7 ID regimen relative to 1 x 10^8 SC (standard dose regimen). If the 2 x 10^7 ID regimen is non-inferior to the standard dose regimen, hypothesis testing will proceed to test non-inferiority of the 1 x 10^7 ID regimen relative to the standard dose regimen.

Primary Hypothesis 1

- At Day 43 the humoral immune response of the 2 x 10^7 MVA-BN ID regimen will be non-inferior to the standard 1 x 10^8 MVA-BN SC regimen, as assessed by PRNT GMT.

Primary Hypothesis 2

- At Day 43 the humoral immune response of the 1 x 10^7 MVA-BN ID regimen will be non-inferior to standard 1 x 10^8 MVA-BN SC regimen, as assessed by PRNT GMT.

Secondary hypotheses

- At Day 365 the humoral immune response of the 2 x 10^7 MVA-BN ID regimen will be non-inferior to the standard 1 x 10^8 MVA-BN SC regimen, as assessed by PRNT GMT.
- At Day 365 the humoral immune response of the 1 x 10^7 MVA-BN ID regimen will be non-inferior to the standard 1 x 10^8 MVA-BN SC regimen, as assessed by PRNT GMT.
- The humoral immune responses, as assessed by PRNT GMT, for each ID regimen will be similar to the SC regimen at all study days. [Note, this corresponds to two hypotheses, one for each ID study arm.]
- The humoral immune responses, as assessed by vaccinia specific PRNT half-life, for each ID regimen will be similar to the SC regimen. [Note, this corresponds to two hypotheses, one for each ID study arm.]
- The humoral immune responses, as assessed by monkeypox specific PRNT GMT, for each ID regimen will be similar to the SC regimen at Day 1 and 43. [Note, this corresponds to two hypotheses, one for each ID study arm.]

9.2 Sample Size Determination

Approximately 210 evaluable (mITT) participants (70 per study arm; 140 total for each test of non-inferiority) will provide ample power for both tests of non-inferiority with a type I error rate of 0.05 (Table 7), and assumes, conservatively, that both arms have a standard deviation of 0.6.

A sample size of 140 total for the test of non-inferiority will provide 84% power with the non-inferiority (NI) margin of 0.5 and two-sided type I error rate of 0.05. Power calculations conservatively assume a standard deviation of 0.6 in both arms (Table 8 and 9). Data from prior studies by Pittman and Frey suggest that the standard deviation will be lower than 0.6 at later expected peak times, in which case power should be higher for the non-inferiority tests. Note that the power is expected to be lower for the comparison of the 1 x 10^7 ID regimen and the standard dose regimen for two reasons: 1) the hypothesis test is conditional on rejecting the null hypothesis comparing the 2 x 10^7 ID arm to the standard regimen arm, and 2) the true GMT for the 1 x 10^7 ID may be less than the standard SC dose.

In a prior study that compared ID and SC routes of MVA-BN[16], 10% of the randomized participants did not receive the second vaccination. Therefore, this trial will enroll up to 231 participants (210 x 1.1) to have 210 evaluable participants.

Table 7: Power calculations

Power calculations for a non-inferiority test of means using unequal variances and two-sided type one error rate of 0.05. Non-inferiority margin of 0.5. Assumed standard deviation (on log10 scale) of 0.6.

NI margin	Sample Size per arm	Total sample size for NI test	Power
0.5 $Log_{10}(0.5)$ =-0.301	60	120	0.78
0.5	70	140	0.84
0.5	80	160	0.88
0.5	85	170	0.90
0.5	90	180	0.92

Table 8: Standard deviation estimates

Standard deviation estimates computed from Table S4 of Pittman et al, 2019[29]

	MVA-BN				ACAM2000				Ratio of GMTs	95% CI
Week	N	GMT	95% CI	SD (for log_{10} GMT)	N	GMT	95% CI	SD (for log_{10} GMT)		
2	184	16.2	[13.0, 20.1]	0.65	184	16.2	[13.1, 20.0]	0.63	0.997	[0.738, 1.348]
4	185	16.9	[13.7, 20.8]	0.63	186	79.3	[67.1, 93.8]	0.51	0.213	[0.163, 0.278]
6	185	153.5	[134.3, 175.6]	0.41	181	64.7	[54.9, 76.2]	0.49	2.372	[1.922, 2.928]
8	179	118.2	[102.9,135.8]	0.41	183	67.1	[56.9, 79.0]	0.49	1.763	[1.422, 2.185]
Peak	185	153.5	[134.3, 175.6]	0.41	186	79.3	[67.1, 93.8]	0.51	1.935	[1.562, 2.397]

Table 9: Standard deviation estimates

Standard deviation estimates computed from Frey et al, 2015[16]

Study visit day	Liquid SC $1x10^8$ N=149		Liquid ID $2x10^7$ N=146	
	VV-WR GMT [95% CI]	SD	VV-WR GMT [95% CI]	SD
Day 14	10.0 [9.0, 11.1]	0.28	10.3 [9.3, 11.3]	0.27
Day 28	9.6 [8.7, 10.6]	0.27	10.8 [9.9, 11.9]	0.23
Day 42	45.2 [36.4, 56.2]	0.59	54.4 [43.7, 67.8]	0.59
Peak post 2^{nd} vaccination	49.5 [40.0, 61.3]	0.58	59.6 [48.1, 74.0]	0.59

9.3 Statistical Analyses

9.3.1 General Methodology

Hypothesis testing will be stepwise, starting with the non-inferiority test of the 2 x 10^7 ID regimen relative to standard regimen, with an unequal-variance, two-sample t-test statistic and 95% two-sided confidence intervals. The ID-dose regimen will be considered non-inferior if the lower bound of the confidence interval (original scale) is no less than half that of the standard dose, giving a non-inferiority (NI) margin of 0.5 (NI= -0.301 log10 scale). The selection of this NI margin was based on the pivotal Phase 3 trial by Pittman et al., in 2019 comparing one dose of ACAM2000 and the now licensed 2-dose standard MVA-BN regimen, which provided an estimated relative (peak) immune response of approximately 2-times higher for MVA-BN (Table 8). An NI margin of 0.5 will correspond an immune response of the lower-dose MVA-BN at least as high as what would be expected for ACAM2000.

If the null hypothesis from analysis 1 is rejected in favor of non-inferiority (i.e., the lower limit of the 95% CI of the difference in log of GMTs for 2 x 10^7 MVA-BN ID compared to 1 x 10^8 MVA-BN SC is greater than log(0.5) which is equal to -0.301), testing will proceed with a non-inferiority test of the 1 x 10^7 MVA-BN ID regimen relative to the standard dose regimen, using an unequal variance, two-sample t-test statistic with 95% confidence interval.

9.3.2 Timing of Analyses

9.3.2.1 Interim Safety Analyses

Given the need for rapid review and dissemination of study data for public health reasons, AEs and SAEs may be reviewed as necessary outside of DSMB reviews. The DSMB may not need to meet (unless halting rules are met), and materials will be provided electronically. Documentation of review and any concerns noted will be solicited electronically.

The DSMB will review separate cumulative AE data reports for all participants within each arm. Given the safety database known for authorized/approved vaccines, there is no routine mandatory review by the DSMB unless halting rules are triggered.

9.3.2.2 Interim Immunogenicity Analyses

Interim data review of immunogenicity may be performed after all participants have completed Day 43 (i.e., 14 days after the second vaccine for the last participant). Analyses will be performed as needed to inform public health decisions. It is recognized that the data may not be frozen, but the risk of introducing bias at that point in the trial is considered small. The analyses will be provided to the study team. There is no blinded data in this trial. However, research laboratories will be blinded to participant study arm for the purposes of immunogenicity/efficacy evaluations.

Data may be disseminated to public health officials and partners as needed and included in publications and presentations to inform the global scientific community.

9.3.2.3 Final Analyses

The final efficacy/immunological/safety outcome(s) may be analyzed after all data for the primary and secondary outcome(s) have been collected and locked.

9.3.3 Populations for Analyses

There will be three analysis populations in this study. The safety analysis population includes all enrolled participants who received at least one dose of study vaccine. Analyses for the safety population will include safety reported through the end of the study. The modified intent-to-treat (mITT) population includes all participants who received at least one dose of vaccine and contributed both pre- and at least one post-vaccination venous blood sample for immunogenicity testing for which valid results were reported.

In the final analysis, protocol deviations will be reviewed to determine which protocol deviations may affect the analysis. The per protocol (PP) population will then be defined – and this includes all participants in the mITT subset with the following exclusions:

- Data from all available visits for participants found to be ineligible at baseline.
- Data from all visits after the protocol deviations that are considered to affect the science (as determined by the sponsor at an ad hoc meeting).
- Data from any visit that occurs substantially out of window.

For the PP and mITT analyses, participants will be analyzed according to the study product that they received.

9.3.4 Baseline Characteristics and Participant Disposition

Summaries of demographic characteristics of the study population including age, sex, ethnicity, and race will be presented by vaccination arm and overall. In addition, this descriptive analysis will be done by reported HIV infection status.

9.3.5 Immunogenicity Analyses

Descriptive summaries of immunogenicity data will be presented for the mITT population. If there are protocol deviations which may affect the analysis, a per-protocol (PP) analysis may also be performed.

Geometric Mean Titers (GMT) and Geometric Mean Fold Rise (GMFR) from baseline of VV-WR PRNT will be calculated, along with 95% CIs, for all groups, at each timepoint. Summaries will also be displayed graphically. Geometric Mean Titer Ratio, defined as the ratio of each ID arm to the control SC arm, will also be reported at each timepoint, along with 95% CIs.
There are no imputations planned for missing data.

A Statistical Analysis Plan (SAP) will be developed and will detail the full planned analysis.

9.3.6 Safety Analyses

The safety outcomes for the three study arms will be evaluated including systemic reactogenicity for 14 days after each vaccination, local reactogenicity for 14 days after each vaccination, and unsolicited adverse events for 28 days after each vaccination. All SAEs and MAAE will be evaluated from Day 1 through Day 57 of the trial as compared to licensed dose, and all related SAE/MAAEs will be evaluated through Day 181.

9.3.6.1 Reactogenicity

The number, percentage (observed rate) and exact two-sided 95% CI for participants reporting each solicited local (injection site) reaction and solicited systemic reaction following vaccination will be summarized by study arm after first and second vaccination, and overall (i.e., after any vaccination). In addition, the most severe local and systemic reactions recorded during the follow-up period (i.e., maximum severity) for each participant will be determined and summarized by study arm following each vaccination (as well as overall) and the resulting number and percentage of participants will be summarized by severity grade (none, mild, moderate, severe, life-threatening). Last, the daily severity of local and systemic reactions will be determined for each participant and the proportion of participants having a sign/symptom each day will summarized by grade and presented by study arm following each vaccination. Rates for any severe (Grade 3 or 4) solicited local or systemic reaction following any vaccination will be compared between study arms.

9.3.6.2 Other Adverse Events

The number of associated unsolicited AEs, MAAEs and SAEs following vaccine administration is expected to be small. A complete listing of all events will therefore be provided, including the nature of the event, timing relative to vaccination, severity, duration, and the investigator's assessment of the likely relationship to vaccination. For each type of AE, the observed rate and exact two-sided confidence intervals will be computed for each study arm and listed by toxicity grade. AEs will be MedDRA® coded for preferred term and system organ class. Event rates and exact 95% confidence intervals for each study arm and the entire study cohort will be presented, in aggregate and by MedDRA® categories. Related unsolicited AEs, MAAE and SAEs rates will be compared between the study arms.

The number, percentage (observed rate) for participants that withdrew or discontinued their vaccination series due to safety or reactogenicity events will be tabulated. Rates of withdrawal/discontinuation will be compared between study arms using Fisher's exact test.

Refer to the SAP for additional information.

10. SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 Regulatory, Ethical, and Study Oversight Considerations

This study will be conducted in conformity with the principles set forth in The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Participants of Research (US National Commission for the Protection of Human Participants of Biomedical and Behavioral Research; April 18, 1979), and the federal policy for the Protection of Human Participants codified in 45 CFR Part 46, 21 CFR Part 50 (Protection of Human Participants), and the ICH E6(R2).

An OHRP-registered IRB will review and approve this protocol, associated informed consent documents, recruitment materials, and handouts or surveys intended for the participants, prior to the recruitment, screening and enrollment of participants. The IRB review shall be in accordance with 45 CFR 46 and 21 CFR 50, 21 CFR 56 (IRBs), and other federal, state, and local regulations and policies, as applicable.

Each institution engaged in this research will hold a current Federalwide Assurance (FWA) issued by the Office for Human Research Protections (OHRP) for federally funded research. and provide the FWA number to DMID. Each site principal investigator will obtain IRB approval for this protocol and any amendments to be conducted at his/her research site(s).

Any amendments to the protocol or informed consent documents will be approved by the IRB before they are implemented. IRB review and approval will occur at least annually throughout enrollment and follow-up of participants, in accordance with applicable regulations and the requirements of the IRB/IEC. The participating site PI will notify the IRB of deviations from the protocol and reportable SAEs, as applicable to the IRB policy.

DMID must receive the documentation that verifies IRB/IEC approval for this protocol, informed consent documents and associated documents, prior to the recruitment, screening and enrollment of participants, and any IRB approvals for continuing review or amendments as required by DMID.

10.1.1 Informed Consent Process

Informed consent is a process that is initiated prior to an individual agreeing to participate in a trial and continuing throughout the individual's trial participation. Before any study procedures are performed, informed consent will be obtained and documented. Participants will receive a concise and focused presentation of key information about the clinical trial, verbally and with a written consent form. The key information about the purpose of the study, the procedures and experimental aspects of the study, study interventions/products, probability for random assignment to treatment groups, risks and discomforts, the expected duration of the participant's participation in the trial, any expected benefits to the participant, and alternative treatments and procedures that may be available to the participant. The explanation will be organized and presented in lay terminology and language that facilitates understanding why one might or might not want to participate.

Participants will receive an explanation as to whether any compensation and any medical treatments are available if injury occurs, and, if so, what they consist of, or where further information may be obtained. Participants will be informed of the anticipated financial expenses,

if any, to the participant for participating in the trial, as well as any anticipated prorated payments, if any, to the participant for participating in the trial. They will be informed of whom to contact (e.g., the investigator) for answers to any questions relating to the research project. Information will also include the foreseeable circumstances and/or reasons under which the participant's participation in the trial may be terminated. The participants will be informed that participation is voluntary and that they are free to withdraw from the study for any reason at any time without penalty or loss of benefits to which the participant is otherwise entitled.
Participants will be informed that records identifying the participant will be kept confidential, and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available and, if the results of the trial are published, the participant's identity will remain confidential. Participants will be informed whether private information collected from this research and/or specimens will be used for additional research, even if identifiers are removed.
Participants will be informed that the monitor(s), auditors(s), IRB, NIAID, and regulatory authority(ies) will be granted direct access to the participant's original medical records for verification of clinical trial procedures and/or data without violating the confidentiality of the participant, to the extent permitted by the applicable laws and regulations, and that, by signing a written informed consent form, the participant is authorizing such access.
Participants will be allowed sufficient time to consider participation in this research trial and can discuss this trial with their family, friends or legally authorized representative, or think about it prior to agreeing to participate.

Informed consent forms will be IRB-approved, and participants will be asked to read and review the consent form. Participants must sign the informed consent form prior to starting any study procedures being done specifically for this trial. Once signed, a copy of the informed consent form will be given to the participant(s) for their records.
New information will be communicated by the site principal investigator to participants who consent to participate in this trial in accordance with IRB requirements. The informed consent document will be updated, and participants will be re-consented per IRB requirements, if necessary.

10.1.1.1 Requirements for Permission by Parents/Guardians and Assent by Children (in case of a minor)

Not applicable.

10.1.1.2 Other Informed Consent Procedures

Participants will be asked for consent to collect blood for the use of residual specimens and samples for secondary research. This extra/residual blood and corresponding serum will be used as back-up specimens for PP defined assays or designated for secondary research use and stored indefinitely at a designated storage facility. Collection of extra/residual samples during the study will help facilitate rapid follow-on analyses, if warranted, to provide more comprehensive scientific insights into the impact (safety and immunological) of the vaccine on the host response to vaccination. To maintain statistical power in follow-on analyses it is important that extra blood collection and secondary research use be included in as many participants as possible, due to the limited sample size per treatment arm. For this reason, if participants choose not to provide

permission for extra blood and secondary research use, they will not be eligible for enrollment into the study.

The stored samples will be labeled with barcodes to maintain confidentiality. Research with identifiable samples and data may occur as needed, however, participant confidentiality will be maintained as described for this protocol and with IRB approval. Samples designated for secondary research use may be used for additional immunological assessments that may include but are not limited to antibody epitope mapping, B and T cell repertoire determination, non-traditional immune assay development, determination of innate immune factors and the ability of vaccine-induced antibodies to cross-react to different proteins and virus strains. These blood samples might be used in new or different immunological laboratory tests, to provide information for the development of new vaccines or therapeutics, or for the studies of monkeypox virus or other related viruses. Samples will not be sold for commercial profit. Although the results of any future research may be patentable or have commercial profit, participants will have no legal or financial interest in any commercial development resulting from any future research. There are no direct benefits to the participant for extra specimens collected or from the secondary research. No results from secondary research will be entered into the participant's medical record. Incidental findings will not be shared with the participant, including medically actionable incidental findings, unless required by law.

Risks are associated with the additional volume of blood collected, such as anemia. Risks for loss of privacy and confidentiality are described below. Participants may withdraw permission to use samples for secondary use at any time. They will need to contact the participating site and the samples will be removed from the study repository after this study is completed and documentation will be completed that outlines the reason for withdrawal of permission for secondary use of samples. Participants who withdraw consent before the last visit will not have the extra blood drawn for secondary use.

Participants will be asked to consent specifically on primary and secondary research samples. The consent process will include an explanation of the potential risks to the individual participants associated with data submitted to an NIH data repository and subsequent sharing. Data that may potentially identify human participants will not be released in unrestricted databases. Participants will be informed that analytical methods are associated with the risk of re-identification, even when specimens are de-identified. The consent will include whether individual participant data will be shared through a NIH controlled access data repository.

10.1.2 Study Termination and Closure

Section 7, Study Intervention Discontinuation and Participant Discontinuation/Withdrawal, describes the temporary halting of the study.
This study may be prematurely terminated if there is sufficient reasonable cause, including but not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Results of interim efficacy or futility analysis
- Insufficient compliance to protocol requirements

- Data that are not sufficiently complete and/or evaluable
- Regulatory authorities' determination
- Sponsor's determination

If the study is prematurely terminated, the Principal Investigator (PI) will promptly inform study participants and the Institutional Review Board (IRB) and regulatory authorities as applicable. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule. The PI will assure appropriate follow-up for the participants, as necessary. The sponsor will notify regulatory authorities as applicable.

10.1.3 Confidentiality and Privacy

Participant confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover clinical information relating to participants, test results of biological samples and genetic tests, and all other information generated during participation in the study. No identifiable information concerning participants in the study will be released to any unauthorized third party. Participant confidentiality will be maintained when study results are published or discussed in conferences.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

All source records including electronic data will be stored in secured systems in accordance with institutional policies and federal regulations. All study data and research specimens that leave the site (including any electronic transmission of data) will be identified only by a coded number that cannot be linked to a participant.

As this research is funded by the NIH, it is covered by NIH policy which effectively issues the research a Certificate of Confidentiality. By this policy, researchers cannot be forced to disclose or provide, in any Federal, State, or local civil, criminal, administrative, legislative, or other proceeding, the name of such individual or any such information, document, or biospecimen that contains identifiable, sensitive information about the individual and that was created or compiled for purposes of the research, unless such disclosure or use is made with the consent of the individual to whom the information, document, or biospecimen pertains.

The Certificate cannot be used to resist a demand for information from personnel of the United States Government that is used for auditing or evaluation of federally funded projects, like this study, or for information that must be released in order to meet the requirements of the Federal Food and Drug Administration (FDA).

A Certificate of Confidentiality does not prevent the participant from voluntarily releasing information about themselves or their involvement in this research. If any person or agency

obtains a written consent to receive research information, then the researchers may not use the Certificate to withhold that information.

The Certificate of Confidentiality does not prevent the researchers from reporting without the participant's consent, information that would identify the participant as a participant in the research project regarding matters that must be legally reported including child and elder abuse, sexual abuse, or wanting to harm themselves or others.

The release of individual private information or specimens for other research will only occur if consent was obtained from the individual to whom the information, document, or biospecimen pertains, *or* for the purposes of other research that the release is in compliance with applicable Federal regulations governing the protection of human participants in research.

10.1.4 Secondary Use of Stored Specimens and Data

10.1.4.1 Samples for Secondary Research

Repository Research Sample
To participate in this study, participants will be asked for consent for storage of samples for secondary use. Samples will be stored indefinitely at a DMID-designated storage facility. Each sample will be encoded (labeled) only with a barcode and a unique tracking number to protect participant confidentiality. Secondary research with coded samples and data may occur, however, participant confidentiality will be maintained as described for this protocol. An IRB review of the secondary research using coded specimens is required.

Repository Research Samples, upon written request and approval from DMID and any approvals required by the site, may be shared for secondary research with investigators at the participating site, with researchers at other sites or other institutions, or company-designated research laboratories. The samples will not be sold or used directly for production of any commercial product. DMID will authorize shipment from the repository.

Reports from secondary research will not be kept in the participants' health records or shared with participants, unless required by law. Reports will not be sent to the specimen repository. The participant's decision for secondary research can be changed at any time by notifying the study doctors or nurses in writing. If the participant changes his/her decision, the samples will be destroyed if the samples have not been used for research or released for a specific research project.

10.1.4.2 Data Sharing for Secondary Research

Data from this study may be used for secondary research. Individual participant data collected during the trial that are sufficient to reanalyzed primary and secondary endpoints will be made available after deidentification. The Statistical Analysis Plan and Analytic Code will also be made available. This data will be available following publication, with no end date. The data will be made available to researchers who provide a methodologically sound proposal. The data will

be available for any purpose outlined in the approved proposal. Proposals should be directed to DMID. To gain access, data requestors will need to sign a data access agreement.
The investigator may request removal of data on individual study participants from NIH data repositories in the event that a research participant withdraws or changes his or her consent. However, data that have been distributed for approved research use cannot be retrieved.

10.1.5 Key Roles and Study Governance

This is a multi-site clinical trial and as such, all study team members and roles are listed in the Manual of Procedures (MOP).

10.1.6 Safety Oversight

Safety oversight will be conducted by a DSMB that is an independent group of experts that monitors participant safety and advises DMID. The DSMB members will be separate and independent of study personnel participating in this trial and should not have scientific, financial or other conflict of interest related to this trial. The DSMB will consist of members with appropriate expertise to contribute to the interpretation of the data from this trial. The DSMB will operate under the rules of a DMID-approved charter that will be written at the organizational meeting of the DSMB. Procedures for DSMB reviews/meetings will be defined in the charter. Reports may include enrollment and demographic information and solicited and unsolicited AE/SAEs. The DSMB will review SAEs on a regular basis and ad hoc during this trial. The DMID Medical Monitor will be responsible for reviewing SAEs in real time.

The DSMB will conduct the following reviews:

- Approximately Day 22-29 after study start. Given the planned short enrollment period (3-4 weeks) and second dose of vaccine given on day 29, there is a narrow window for review. As such the review will focus on AEs utilizing real time reports where able.
- Ad hoc meeting:
    - When trial-level halting criteria are met
    - At the request of DMID to review a potential safety concern identified in the trial.
- Interim and final data will be shared with the DSMB, but the DSMB does not need to meet to review the final data.

Additional data may be requested by the DSMB. The DSMB may receive data in aggregate and presented by treatment arm. The DSMB may also be provided with expected and observed rates of the expected AEs in an unblinded fashion and may request the treatment assignment be unblinded for an individual participant if required for safety assessment. The DSMB will review grouped and unblinded data in the closed session only. As an outcome of each review/meeting, the DSMB will make a recommendation as to the advisability of proceeding with study interventions (as applicable), and to continue, modify, or terminate this trial.

The DSMB will operate under the rules of a DMID-approved charter that will be written at the organizational meeting of the DSMB. At this time, each data element that the DSMB needs to assess will be clearly defined. Procedures for DSMB reviews/meetings will be defined in the charter. The DSMB will review applicable data to include, but not limited to, study progress and

participant, clinical, safety and safety related data. Reports may include enrollment and demographic information, medical history, concomitant medications, physical assessments, clinical laboratory values, dosing compliance, and solicited and unsolicited AE/SAEs.

DMID or the DSMB chair may convene the DSMB on an ad hoc basis according to protocol criteria or if there are immediate concerns regarding observations during the course of this trial. The DMID Medical Monitor is empowered to stop enrollment and study product administration if AEs that meet the halting criteria are reported. The DMID Medical Monitor will be responsible for reviewing SAEs in real time. The DSMB will review SAEs on a regular basis and ad hoc during this trial.

10.1.7 Clinical Monitoring

Clinical site monitoring is conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable. Clinical Monitoring ensures that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), International Council for Harmonisation (ICH), Good Clinical Practice, and with applicable regulatory requirement(s) and sponsor requirements.

Monitoring for this study will be overseen by NIAID/DMID and conducted by NIAID/DMID contractors. Details of clinical site monitoring are documented in a Clinical Monitoring Plan (CMP). The CMP describes in detail who will conduct the monitoring, at what frequency monitoring will be done, at what level of detail monitoring will be performed, and the distribution of monitoring reports. Monitoring visits will include, but are not limited to, review of regulatory files, investigational product administration and accountability records, eCRFs, informed consent forms, medical and laboratory reports, site study storage records, training records, and protocol and GCP compliance. Site monitors will have access to each participating site, study personnel, and all study documentation according to the NIAID or DMID-approved site monitoring plan. Study monitors will meet with site principal investigators (or designee) to discuss any problems and outstanding issues and will document site visit findings and discussions.

10.1.8 Quality Assurance and Quality Control

Quality control (QC) and quality assurance (QA) activities are essential to the safety of participants, and the reliability of study data. Each site participating in the study is responsible for integrating quality control checks throughout the study and implementing a QA Checklist or Clinical Quality Management Plan (CQMP). The site will conduct the following:

1. Routine internal QC and QA activities for the purposes of measuring, documenting and reporting study conduct, protocol adherence, human participants' protections, and reliability of the protocol-driven data collected.
2. Processes for addressing data quality issues (i.e., collecting, recording), and reporting findings in a timely manner.

3. Identify systemic issues (e.g., protocol or human participant protections non-compliance), and implementation and evaluation of Corrective and Preventative Action Plan (CAPA) procedures, if applicable.

10.1.9 Data Handling and Record Keeping

10.1.9.1 Data Collection and Management Responsibilities

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

10.1.9.2 Data Coordinating Center / Biostatistician Responsibilities

The data coordinating center for this study will be responsible for data management, quality review, analysis, and reporting of the study data. Adverse events and medical history will be coded according to the MedDRA dictionary. Concomitant medications will be coded according to the WHO Drug dictionary. At the end of the study, a copy of all datasets including annotated case report forms and data dictionary will be provided to DMID.

A separate Study Data Standardization Plan (SDSP) will be developed which describes the technical recommendations for the submission of human study data and related information in a standardized electronic format throughout product development.

10.1.9.3 Data Capture Methods

Clinical research data, including, but not limited to, AE/SAEs, concomitant medications, medical history, physical assessments will be abstracted from the source documentation and/or collected on source document worksheet by study personnel then entered into an eCRFs via a 21 CFR Part 11-compliant internet data entry system provided by the study data coordinating center. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate.

The IND Sponsor is responsible for review of data collection tools and processes, and review of data and reports.

Study data will be collected on paper CRFs and then entered into the eCRF or data will be entered directly into the eCRF.

10.1.9.4 Study Record Retention

Study related records, including the regulatory file, study product accountability records, consent forms, participant source documents and electronic records should be maintained for a period of 2 years following the date a marketing application is approved for the investigational product for the indication for which it is being investigated; or, if no application is to be filed or if the application is not approved for such indication, until 3 years after the investigation is

discontinued and FDA is notified. These documents should be retained for a longer period, however, if required by local policies or regulations. No records will be destroyed without the written consent of DMID. Consent forms with specimen retention linked to identifiable specimens will be maintained for as long as the specimens remain in identifiable format, and a minimum of three years after use of the identifiable specimens in nonexempt human participant research.

10.1.9.5 Source Records

Source data are all information, original records of clinical findings, observations, or other activities documented in a clinical trial necessary for the reconstruction and evaluation of the trial. Each participating site will maintain appropriate medical and research records for this trial, in compliance with ICH GCP, regulatory, and institutional requirements. Data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded on the source documents. Data entered directly into the eCRFs will be considered the source document. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Interview of participants is sufficient for obtaining medical history. Solicitation of medical records from the participant's primary care provider is not required.

10.1.10 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, any process that is noted in the protocol and refers to details in the MOP, or GCP requirements.

The noncompliance may be either on the part of the participant, the investigator, or the study site staff. Following a deviation(s), corrective actions should be developed by the site and implemented promptly. All individual protocol deviations will be addressed in participant study records.

It is the responsibility of the site principal investigator and personnel to use continuous vigilance to identify and report deviations within five working days of identification of the protocol deviation, or within five working days of the scheduled protocol-required activity. All deviations must be promptly reported to DMID per the protocol deviation reporting procedures. Protocol deviations must be sent to the local IRB/IEC per their guidelines. The site principal investigator and personnel are responsible for knowing and adhering to their IRB requirements.

10.1.11 Publication and Data Sharing Policy

10.1.11.1 Human Data Sharing Plan

All NIH protocols are required to have a Data Sharing Plan describe provisions for protecting the participants' privacy and the confidentiality of the data. This study will be conducted in accordance with the following publication and data sharing policies and regulations:
National Institutes of Health (NIH) Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-

reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

As of January 2018, all clinical trials supported by the NIH must be registered on ClinicalTrials.gov, no later than 21 days after the enrollment of the first participant. Results of all clinical trials supported by the NIH, generally, need to be submitted no later than 12 months following the primary completion date. As part of the result posting, a copy of this protocol and a copy of the Statistical Analysis Plan will be posted on ClinicalTrials.gov.

10.1.11.2 Genomic Data Sharing Plan

Not Applicable.

10.1.11.3 Publication

This study will comply with the NIH Public Access Policy, which ensures that the public has access to the published results of NIH funded research. As such, the final peer-reviewed journal manuscripts will be accessible to the public on PubMed Central no later than 12 months after publication.

10.1.12 Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. DMID has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

10.2 Additional Considerations

10.2.1 Research Related Injuries

For any potential research related injury, the site principal investigator or designee will assess the participant. The site principal investigator should then determine if an injury occurred as a direct result of the tests or treatments that are done for this trial. As needed, referrals to appropriate health care facilities will be provided to the participant. No financial compensation will be provided by the NIAID, NIH, or the federal government to the participant, for any injury suffered due to participation in this trial.

10.2.2 PREP Act

The study vaccine and the efforts for this clinical trial are covered under the Public Readiness and Emergency Preparedness Act (PREP Act). The PREP Act applies under the terms of the PREP Act declaration in place for smallpox/orthopoxvirus countermeasures for activities

conducted pursuant to federal agreements or federal programs (ref: www.federalregister.gov/documents/2015/12/09/2015-31092/smallpox-medical-countermeasures-amendment). JYNNEOS is used under an IND for monkeypox pursuant to a federal agreement/program and is therefore covered under the PREP Act. The PREP Act covers persons (such as manufacturers, distributers, program planners, and other qualified persons who prescribe, administer or dispense study product) are immune from liability from the administration, or use of a covered countermeasure, such as JYNNEOS. The PREP Act provides immunity for covered persons from liability, unless the injury was caused by willful misconduct.

The PREP Act also established the Countermeasures Injury Compensation Program (CICP) to provide compensation for serious injuries or death that occur as the direct result of the administration or use of certain countermeasures. Any requests for compensation must be filed within one year of the administration or use of the covered countermeasure. Requests for Benefits must be made to the Health Resources and Services Administration's (HRSA) Countermeasures Injury Compensation Program (http://www.hrsa.gov/cicp/) by filing a Request for Benefits Form and all required medical records and supporting documentation. Additional information on filing a Request for Benefits is available on the CICP's website at http://www.hrsa.gov/cicp/. Compensation may then be available for reasonable and necessary medical benefits, lost wages and/or death benefits to eligible individuals for certain injuries in accordance with regulations published by the Secretary of HHS (found at 42 CFR part 110).

If an individual suffers a serious physical injury or death from the administration or use of a covered countermeasure in this study, the individual, the individual's legal or personal representative, the administrator/executor of a deceased individual's estate, or certain survivors may request benefits from the CICP. A serious physical injury means an injury that warranted hospitalization (whether or not the person was actually hospitalized) or that led to a significant loss of function or disability. The CICP is the payer of last resort. This means that it only covers expenses or provides benefits that other third-party payers (such as health insurance, the Department of Veterans Affairs, or Workers' Compensation programs) do not have an obligation to pay.

If the Secretary of HHS does not make a final determination on the individual's request within 240 days, or if the individual decides not to accept the compensation, the injured individual or his representative may pursue a tort claim in the US District Court for the District of Columbia, but only if the claim involves willful misconduct and meets the other requirements for suit under the PREP Act. Any award is reduced by any public or private insurance or worker's compensation available to the injured individual. Awards for non-economic damages, such as pain, suffering, physical impairment, mental anguish, and loss of consortium are also limited. If the individual accepts compensation, or if there is no willful misconduct, then the individual does not have a tort claim that can be filed in a US Federal or a State court.

10.3 Abbreviations

AE	Adverse Event
ANCOVA	Analysis of Covariance
BLA	Biologics License Application
CAPA	Corrective and Preventative Action Plan
CFR	Code of Federal Regulations
CI	Confidence Interval
CIOMS	Council for International Organizations of Medical Sciences
CLIA	Clinical Laboratory Improvement Amendments
CMP	Clinical Monitoring Plan
CMS	Clinical Material Services
COC	Certificate of Confidentiality
CONSORT	Consolidated Standards of Reporting Trials
CQMP	Clinical Quality Management Plan
CRF	Case Report Form
CRO	Contract Research Organization
CSR	Clinical Study Report
SDCC	Data and Statistical Coordinating Center
DHHS	Department of Health and Human Services
DMID	Division of Microbiology and Infectious Diseases
DSMB	Data and Safety Monitoring Board
EC	Ethics Committee
eCRF	Electronic Case Report Form
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FWA	Federalwide Assurance
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
GWAS	Genome-Wide Association Studies
HAART	Highly Active Antiretroviral Therapy
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IDE	Investigational Device Exemption
IEC	Independent or Institutional Ethics Committee
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISO	International Organization for Standardization
ITT	Intention-To-Treat
MedDRA	Medical Dictionary for Regulatory Activities

MOP	Manual of Procedures
MSDS	Material Safety Data Sheet
N	Number (typically refers to participants)
NDA	New Drug Application
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
OHRP	Office for Human Research Protections
OHSR	Office for Human Participants Research
PHI	Protected Health Information
PI	Principal Investigator
PK	Pharmacokinetics
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SDSP	Study Data Standardization Plan
SMC	Safety Monitoring Committee
SOA	Schedule of Activities
SOC	System Organ Class
SOP	Standard Operating Procedure
UP	Unanticipated Problem
US	United States
WHO	World Health Organization

10.4 Protocol Amendment History

Version	Date	Description of Change	Brief Rationale

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