A Phase 1, Placebo-Controlled, Double-Blinded Study to Assess the Safety and Pharmacokinetics of Single Ascending Doses of EV68-228-N ins Healthy Adult Volunteers

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STATEMENT OF ASSURANCE

Each Institution will hold a current Federal Wide Assurance (FWA) issued by the Office of Human Research Protections (OHRP) for federally-funded human participants research. Each FWA will designate at least one Institutional Review Board (IRB)/Independent Ethics Committee (IEC) registered with OHRP, for which the research will be reviewed and approved by the IRB/IEC and will be subject to continuing review [45 CFR 46.103(b)]. The IRB/IEC designated under an FWA may include an institution's IRB/IEC, an independent IRB/IEC, or an IRB/IEC of another institution after establishing a written agreement with that other institution.

STATEMENT OF COMPLIANCE

The study trial will be carried out in accordance with Good Clinical Practice (GCP) and as required by the following:

- United States Code of Federal Regulations (CFR) 45 CFR Part 46: Protection of Human Subjects
- Food and Drug Administration (FDA) Regulations, as applicable: 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, and 21 CFR Part 312 (Investigational New Drug Application), 21 CFR 812 (Investigational Device Exemptions)
- International Conference on Harmonisation: Good Clinical Practice (ICH E6); 62 Federal Register 25691 (1997); and future revisions
- 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
- National Institutes of Health (NIH) Office of Extramural Research, Research Involving Human Subjects, as applicable
- National Institute of Allergy and Infectious Diseases (NIAID) Clinical Terms of Award, as applicable
- Applicable Federal, State, and Local Regulations and Guidance

SIGNATURE PAGE

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

I agree to conduct the study in compliance with GCP and applicable regulatory requirements.

I agree to conduct the study in accordance with the current protocol and will not make changes to the protocol without obtaining the sponsor's approval and IRB/IEC approval, except when necessary to protect the safety, rights, or welfare of participants.

Site Investigator Signature:

Signed: _____ Date: _____

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LIST OF ABBREVIATIONS

ADA	Anti-drug Antibodies
AE	Adverse Event/Adverse Experience
AFM	Acute Flaccid Myelitis
ALT	Alanine Transaminase
AUC	Area under the serum concentration-time curve
BLA	Biologics License Applications
BMI	Body Mass Index
BP	Blood Pressure
CAP	Central Assay Plan
CFR	Code of Federal Regulations
CI	Confidence Interval
CIOMS	Council for International Organizations of Medical Sciences
CMS	Clinical Material Services
CNS	Central Nervous System
CONSORT	Consolidated Standards of Reporting Trials
Cr	Creatinine
CRF	Case Report Form
CRO	Contract Research Organization
CS	Clinically Significant
CSF	Cerebrospinal Fluid
CSR	Clinical Study Report
CTCAE	Common Terminology Criteria for Adverse Events
DBP	Diastolic Blood Pressure

DHHS	Department of Health and Human Services
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH, DHHS
DMPA	Depot Medroxyprogesterone Acetate
DSMB	Data and Safety Monitoring Board
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
EV-D68	Enterovirus D68
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act
FWA	Federal Wide Assurance
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HED	Human Dose Equivalent
Hgb	Hemoglobin
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IDCRC	Infectious Diseases Clinical Research Consortium
IDE	Investigational Device Exemption

IDES	Internet Data Entry System
IEC	Independent or Institutional Ethics Committee
IgE	Immunoglobulin E
IND	Investigational New Drug Application
IP	Intraperitoneal
IRB	Institutional Review Board
IV	Intravenous
IVIg	Intravenous Immunoglobulin
JAMA	Journal of the American Medical Association
Kg	Kilogram
LARC	Long-acting Reversible Contraception
MAAE	Medically Attended Adverse Event
mAb	Monoclonal Antibody
MedDRA®	Medical Dictionary for Regulatory Activities
MFD	Maximum Feasible Dose
µg	Microgram
Mg	Milligram
mL	Milliliter
mM	Millimolar
MOP	Manual of Procedures
MRI	Magnetic Resonance Imaging
MTD	Maximum Tolerated Dose
N	Number (typically refers to participants)
NDA	New Drug Application
NF	National Formulary

NEJM	New England Journal of Medicine
NIAID	National Institute of Allergy and Infectious Diseases, NIH, DHHS
NIH	National Institutes of Health
NOAEL	No Observed Adverse Event Limit
NOCMC	New Onset Chronic Medical Condition
OHRP	Office for Human Research Protections
OHSR	Office for Human Subjects Research
PHI	Protected Health Information
PI	Principal Investigator
PK	Pharmacokinetics
Plt	Platelet
PSRT	Protocol Safety Review Team
PVX	Potato Virus X
QA	Quality Assurance
QC	Quality Control
RAMP	Rapid Antibody Manufacturing Platform
RNA	Ribonucleic Acid
SAE	Serious Adverse Event/Serious Adverse Experience
SBP	Systolic Blood Pressure
SDCC	Statistical and Data Coordinating Center
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
T bili	Total Bilirubin
TVCV	Turnip Vein Clearing Virus

US	United States
USP	United States Pharmacopeial
VTEU	Vaccine Treatment and Evaluation Unit
WBC	White Blood Cells
WHO	World Health Organization

PROTOCOL SUMMARY

Title:	A Phase 1, Placebo-Controlled, Double-Blinded Study to Assess the Safety and Pharmacokinetics of Single Ascending Doses of EV68-228-N in Healthy Adult Volunteers
Design of the Study:	This is a Phase 1, randomized, placebo-controlled, double-blinded study to assess the safety and pharmacokinetics (PK) of EV68-228-N administered as a single intravenous (IV) infusion in healthy adult volunteers. The study will enroll three cohorts that will be randomized to EV68-228-N (n=30) or placebo (n=6) arms. Cohorts will be dosed sequentially with a single dose of EV68-228-N at increasing dose levels. All participants will be monitored for solicited adverse events (AE) following infusion through Day 3 and abnormal safety laboratory results through Day 8. Additional safety follow-up will occur through the end of study participation. Safety data for each cohort will be reviewed by a Protocol Safety Review Team (PSRT) with concurrence provided by the Safety Monitoring Committee (SMC) before advancing to the next cohort.
Study Phase:	1
Study Population:	36 healthy, nonpregnant, nonlactating adult participants ages 18 to 49 years, inclusive
Number of Sites:	Two
Description of Study Product or Intervention:	A single IV infusion of either 3, 10, or 30 milligram(mg)/kilogram(kg) of EV68-228-N

Study Objectives:	Primary: • To evaluate the safety of a single IV infusion of either 3, 10, or 30 mg/kg of EV68-228-N when administered to healthy adults Secondary: • To characterize the PK of single ascending doses of EV68-228-N for approximately four months following the infusion • To measure the occurrence of ADAs elicited following a single IV infusion of EV68-228-N in healthy adults
Duration of Individual Participation:	Approximately four to six months
Estimated Time to Last Participant/Last Study Day:	10 months

Table 1:Single Ascending Dose Cohorts and Dose Regimens

Dosing for each cohort will begin with two sentinel participants. There will be a minimum of 2 days of observation following dosing of the sentinels before dosing of additional participants in each cohort is authorized. All participants within a cohort will be actively monitored for AEs and safety laboratory data following dosing through Day 8. These safety data will be reviewed by the PSRT with electronic review and concurrence provided by the SMC before advancing to the next cohort. Electronic review of the safety data by the SMC is required prior to the cohort dose escalation when halting rules are met or there are any safety concerns.

Cohort	Arm	Number of Participants	Study Product
1	1	10	3 mg/kg of EV68-228-N
	2	2	Placebo
2	1	10	10 mg/kg of EV68-228-N
	2	2	Placebo
3	1	10	30 mg/kg of EV68-228-N
	2	2	Placebo

1 KEY ROLES

Lead Principal Investigator:	C. Buddy Creech, MD, MPH Director, Vanderbilt Vaccine Research Program Edie Carell Johnson Chair and Professor Department of Pediatrics Division of Pediatric Infectious Diseases Vanderbilt University Medical Center
DMID Clinical Project Manager:	Gabriele Malone, RN, MSN NIAID/DMID/VB
Statistical and Data Coordinating Center:	The Emmes Company, LLC

2 BACKGROUND AND SCIENTIFIC RATIONALE

2.1 Background

Enterovirus D68 (EV-D68) is a re-emerging picornavirus associated with severe respiratory infections and neurologic diseases, such as acute flaccid myelitis (AFM). AFM is a subtype of myelitis characterized by flaccid weakness in at least one limb and magnetic resonance imaging (MRI) changes in grey matter indicating demyelination within the spinal cord. AFM occurs in ~1% of EV-D68 infections in children with an average age of onset of 5-7 years (1, 2). A majority of AFM patients experience other symptoms of upper respiratory illness within 5-10 days prior to onset of limb weakness, and neurological sequelae typically peak within several days (≤10) of onset (1, 3). Studies have identified enterovirus antibodies in the cerebrospinal fluid of patients with AFM, and more recently EV-D68 ribonucleic acid (RNA) and protein were identified in anterior horn motor neurons of the cervical spinal cord from a 5-year-old boy who died of an AFM-like illness in 2008 (4).

AFM outbreaks have occurred worldwide and have been well characterized in the U.S., with peaks in incidence occurring in August through October every other year since 2014. The outbreak in 2014 remains the largest ever recorded EV-D68 outbreak, with 1,152 confirmed cases spanning all states except for Alaska. Over half of the hospitalized patients with respiratory disease were admitted to intensive care units, with 80% receiving supplemental oxygen, 23% requiring non-invasive ventilation, 8% requiring intubation and mechanical ventilation, and 1% died (3). Since that time, incidence has steadily increased. The biennial domestic AFM outbreaks have been associated with EV-D68 circulation, and there is now strong evidence of causality for enteroviruses and AFM. In 2020 and 2022, AFM incidence was lower than in previous years (5), likely due to social distancing measures from the ongoing COVID pandemic; however, it is likely that EV-D68 will re-emerge in years to come, leading to sustained AFM incidence.

Currently, no agent is approved in the U.S. for the treatment of severe EV-D68 infection or AFM, and the current standard of care is limited to supportive treatment and polyclonal intravenous immunoglobulin (IVIg). Commercial lots of IVIg contain varying levels of antibodies to EV-D68, and the efficacy of IVIg administration is unknown.

The human monoclonal antibody 228 was derived from patients recovering from EV-D68 infection (6). This monoclonal antibody (mAb) was found to have potent *in vitro* neutralizing activity against multiple clinical EV-D68 strains across multiple years (2014, 2016, and 2018 epidemic years). The 228 antibody has subsequently been produced by KBio, Inc., using genetically engineered plasmids in a *Nicotiana benthamiana* (tobacco plant) based rapid antibody manufacturing platform (RAMP) (product name: EV68-228-N) (7). It has been tested

against EV-D68 *in vitro* and *in vivo* and has demonstrated therapeutic effects when administered soon after the onset of neurological symptoms in a murine model of EV-D68 infection.

In a murine neurologic model of infection that mimics human AFM, mAb EV68-228-N treatment eliminated detectable virus from the blood within 24 hours of administration; improved survival and neurologic disease when given up to 48 hours after infection; and improved clinical response when given as late as 72 hours after infection. In a separate *in vivo* murine model of EV-D68 induced AFM, mAb EV68-228-N treatment effectively prevented progression of paralysis and improved histopathologic evidence of disease in tissues of the central nervous system (CNS) when treatment was initiated after the onset of limb weakness; most critically, EV68-228-N treatment conferred a protective effect on CNS motor neurons following EV-D68-infection induced paralysis. These data support the hypothesis that mAb EV68-228-N is a potent neutralizing antibody *in vivo* and can result in clinical improvement in murine models of EV-D68 infection, even when administered after the onset of neurologic disease (7).

While the pathogenesis of AFM is incompletely understood, targeting the replicating virus with a neutralizing monoclonal antibody appears to be a reasonable next step based on recent human data. Vogt, Wright, and Hickey reported on a 5-year-old boy who died of an AFM-like illness in 2008 (4). EV-D68 was detected in the cerebrospinal fluid (CSF) and using preserved formalin-fixed and paraffin-embedded autopsy tissue, the authors were able to detect EV-D68 RNA and protein in anterior horn motor neurons and their axons using in situ hybridization and immuno-histochemistry. In addition, Vogt et al identified CD8+ T-cell and CD68+ macrophage infiltration in EV-D68-infected regions along with up-regulated inflammatory gene transcripts in inflamed tissues. Not only do these data support the causal association between EV-D68 and AFM, but they also support the hypothesis that the clinical presentation of AFM includes a combination of the direct effects of viral infection of spinal cord motor neurons and damage resulting from local inflammation. Taken together, the data support ongoing efforts to develop antiviral and neutralizing monoclonal antibody treatment options that may aid in viral clearance and possible immunomodulation.

Given the therapeutic potential of EV68-228-N and the exceptional safety profile of fully human monoclonal antibodies, further development is warranted. This Phase 1 clinical trial will evaluate the safety and pharmacokinetics of EV68-228-N in healthy adult volunteers to support future evaluations of its efficacy as a treatment for AFM in pediatric patients.

2.2 Scientific Rationale

2.2.1 Purpose of Study

The dose of EV68-228-N mAb required to confer protective efficacy in human pediatric AFM patients is currently unknown. This phase 1 clinical trial will evaluate the safety and

pharmacokinetics of EV68-228-N after a single IV infusion in healthy adult male and female volunteers. Safety and PK data from the Phase 1 study of EV68-228-N will be used to support identification of a target dose for future efficacy trials in AFM patients.

2.2.2 Rationale for Dosage and Dosing Regimen

The dose of EV68-228-N mAb required to provide protective efficacy in human AFM patients is currently unknown. The proposed 3-30 mg/kg dose range for the Phase 1 study of EV68-228-N in healthy adult volunteers mirrors the most commonly used IV dosing strategies for other antiviral human pediatric mAbs (8, 9), and is designed to bracket the anticipated dose range required to provide saturating EV-D68 viral inhibition over the proposed duration of treatment. The 3-30 mg/kg dose range is supported by several *in vitro* and *in vivo* lines of evidence as described below.

The proposed Phase I study dose range is supported by nonclinical studies that indicate that IV dosing up to 100 mg/kg in rats is safe and well-tolerated, and that IP dosing at 1 mg/kg in mice provides complete protective efficacy against mortality and protection against neurologic disease.

Intravenous EV68-228-N administration at up to 100 mg/kg was well tolerated by male and female Sprague Dawley rats in two separate toxicology and toxicokinetic studies, including a Good Laboratory Practice (GLP) repeat-dose study evaluating the safety of five sequential 100 mg/kg doses of EV68-228-N administered over 21 days. No target organ effects were identified, and no adverse effects were observed in EV68-228-N-treated animals. A maximum tolerated dose (MTD) for EV68-228-N could not be determined but is considered to be greater than 100 mg/kg; the no observed adverse effect level (NOAEL) could not be determined but is considered to be at least 100 mg/kg.

The 100 mg/kg dose level used in nonclinical safety studies of EV68-228-N was identified as a maximum feasible dose (MFD) due to dosing constraints in rodents and formulation of the test article at 20 mg/mL. The multiple of exposure to be achieved in the GLP toxicology study in comparison to the 3-30 mg/kg clinical dose range proposed for the Phase 1 study of EV68-228-N was calculated on a cumulative exposure basis as follows:

(100 mg/kg/dose in rat) x (5 total doses administered in rat)
= 500 mg/kg total exposure in rat

= 16x the maximum proposed 30 mg/kg single-dose clinical exposure

Using allometric scaling as described by Jacob et al (10), the scaled HED of a 100 mg/kg NOAEL in rats is approximately 16 mg/kg, and the scaled human dose equivalent (HED) of the 500 mg/kg total exposure in rat is approximately 80 mg/kg. The scaled NOAEL HED of 16 mg/kg provides a 5x exposure margin over the starting clinical dose of 3 mg/kg. On a cumulative basis, the scaled NOAEL HED of 80 mg/kg provides a 26x exposure margin over the starting clinical dose of 3 mg/kg. This approach is consistent with nonclinical safety studies performed to support the approval of other IV antiviral antibody products (reference INMAZEB BLA 761169) where it is not always feasible to achieve a ≥10x exposure margin on an individual dose basis. This approach is also consistent with FDA's Guidance for Industry: *Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers* (11), which states that safety factors smaller than 10 may be justified when the NOAEL was determined based on toxicity studies of longer duration compared to the proposed clinical schedule in healthy volunteers, and when the candidate therapeutic is a member of a well-characterized class. The United States Food and Drug Administration (FDA) was consulted on this approach prior to initiation of the GLP toxicology study for EV68-228-N.

Concentration of EV68-228-N needed for EV-D68 neutralization *in vitro*: Microneutralization experiments indicate that EV68-228-N is broadly neutralizing against clinical EV-D68 isolates with an observed IC_{50} of ≤ 150 nanogram/mL (7).

Efficacious dose of EV68-228-N in murine models of severe EV-D68 infection: Murine EV-D68 challenge studies indicate that a single 10 mg/kg intraperitoneal (IP) dose of EV68-228-N given up to 48 hours after infection can protect mice from infection-associated mortality and weight loss and reduce blood viral levels. Furthermore, these studies indicated that a 10 mg/kg IP dose of EV68-228-N given after the onset of neurological symptoms halted and reversed elevated neurological scores caused by infection. Using allometric scaling as previously described (Jacob et al, 2022), a 10 mg/kg dose in mice provides a human dose equivalent (HED) of approximately 0.8 mg/kg.

Dose-ranging murine EV-D68 challenge studies indicate that a single IP dose of EV86-228-N as low as 1 mg/kg given after the onset of limb weakness effectively halts progression of neurologic scores caused by EV-D68 infection of the central nervous system (CNS). Treatment of mice with 10, 3, or 1 mg/kg IP doses of EV68-228-N provided significant prevention of progression of neurological symptoms when compared to IVIg. Administration of EV68-228-N also significantly reduced viral titer in spinal cord and muscle when compared to placebo and IVIg. Using allometric scaling as previously described (10), a 1 mg/kg dose in mice provides a human dose equivalent (HED) of approximately 0.08 mg/kg.

In the non-GLP toxicokinetic study, mean plasma concentrations of EV68-228-N in rats who received three 25 mg/kg or 100 mg/kg intravenous doses ranged from 10 to 3,000 micrograms(μg)/mL. The observed group C_{max} was 1,050 μg/mL in male and female rats at the

lower dose of 25 mg/kg, and 2,983 ± 44.8 (males) and 3,260 ± 90 (females) at the higher dose of 100 mg/kg. Plasma exposure to EV68-228-N at the highest dose level studied (100 mg/kg) was 872 hr*mg/mL (males) and 903 hr*mg/mL (females). EV68-228 was eliminated with a terminal $t_{1/2}$ of approximately 200 hours at 25 mg/kg, and approximately 225 hours at 100 mg/kg.

In the GLP toxicokinetic study, mean C_{max} in rodents was approximately 500 µg/ml after a single 25 mg/kg dose of EV68-228-N and approximately 2,300 µg/ml after a single 100 mg/kg dose of EV68-228-N. The mean AUC_{last} was 8,908 hr*µg/ml (Males) and 6,089 hr*µg/ml (Females) after a single 25 mg/kg dose and 29,890 hr*µg/ml (Males) and 25,503 hr*µg/ml (Females) after a single 100 mg/kg dose. Exposure to EV68-228-N based on the AUC_{last} after administration of the highest dose, 100 mg/kg/day, 5 times over a period of 21 days was 51,452 hr*µg/ml (Males) and 42,369 hr*µg/ml (Females) after a dose of 100 mg/kg. Plasma exposure of EV68-228-N was dose-dependent with a 3.1 to 4.2-fold increment in AUC_{last} for a 4-fold dose increment from 25 to 100 mg/kg in both sexes on Days 1 and 21. EV68-228-N was eliminated with a long terminal $t_{1/2}$ ranging from 183 to 241 hr (~7-10 days) across both treatment groups on Day 21.

The goal of EV68-228-N clinical dosing for the proposed indication will be to achieve complete EV-D68 viral inhibition, *i.e.,* CNS concentrations of antibody ≥IC_{90}, throughout the course of clinical disease following treatment (≤10 days after presentation with limb weakness). A target clinical dose substantially in excess of the nonclinical efficacy HED is justified based on the low rate (~0.1%) of antibody penetration from circulation into the CNS (12), the excellent safety profile of human antibodies directed at foreign viral antigens and the heterogeneity of the anticipated patient population for EV68-228-N, which will consist primarily of pediatric patients aged 2-10 years old of variable body mass index (BMI). This dosing strategy will ensure that a saturating inhibitory effect will still be achieved in the event of dose variability to protect against genetic drift and the risk of viral escape.

2.2.3 Study Population

As this is the first time EV68-228-N will be tested in humans, the study population will be comprised of healthy males and non-pregnant, non-lactating females, ages 18-49 years inclusive at the time of consent, regardless of religion, sex, or ethnic background, who meet all of the inclusion and none of the exclusion criteria. Participants will be recruited from the general population of the participating Vaccine and Treatment Evaluation Unit (VTEU) sites. As this is a first-in-human, Phase 1 trial, minors and pregnant or lactating women will be excluded from study participation. A successful outcome of the current trial may lead to future evaluation of the study product which then may incorporate a broader participant population including children.

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

No clinical trials in humans have been conducted. The safety of the EV68-228-N mAb for IV administration has been evaluated in two pre-clinical safety studies, in which there were no indications of potential off-target reactivity of EV68-228-N administration and no observations of any adverse or toxicological effects related to EV68-228-N administration. The recommended dosing and administration are based on efficacy studies in non-clinical models of infection and previous clinical experience with monoclonal antibody therapeutics.

However, as this is a first-in-human study for EV68-228-N, unexpected AEs are possible in humans. Consideration of these potential risks was included in the design of both the EV68-228-N product and this first-in-human clinical study. EV68-228-N is a fully human mAb which reduces the risk of immunogenicity-related complications. EV68-228-N was produced in tobacco plants, eliminating the risk of mammalian virus transmission that might occur in mAbs produced in mammalian cell culture. Residual nicotine from the production platform may be present at levels of <2 μg/dose, and residual host cell DNA may be present at levels of less than 10 ng/dose. In addition, protein impurities, including product related mAb species (such as charge variants and oxidized species) and host cell proteins may be present in low quantities.

It is expected that the mAb comprising EV68-228-N produced by these methods carries a low risk of inducing serious toxicities for several reasons. Among these are the fully human sequence of the antibody, a glycosylation pattern also present in human immunoglobulin, and binding specificity against a non-human antigen (EV-D68 capsid protein).

Participants will receive only a single dose of EV68-228-N, further limiting the risk of developing anti-drug antibodies that are more likely to result from repeated exposures. A single dose will also limit exposure and allow reversibility of any treatment-related adverse effects. The initial fixed starting dose of 3 mg/kg allows for a 5-fold safety margin based on single dose exposure in a GLP toxicity study in rats (100 mg/kg/dose, with 16 mg/kg human equivalent dose) and a >25-fold safety margin based on GLP cumulative dose toxicity studies (11).

Participants will be screened to ensure enrollment of only healthy adult participants without significant underlying medical issues or history of hypersensitivity. Sentinel dosing will be employed for each dose level to limit the impact of unforeseen drug-related adverse safety outcomes. Risk of acute reactions will be managed through close monitoring of participants during and following infusion of EV68-228-N. Participants will remain in the clinical site for at least five hours after the infusion ends with follow-up visits on Days 2 and 3 to allow for monitoring of hypersensitivity and of laboratory and systemic adverse effects of EV68-228-N dosing. Eight planned outpatient visits will follow participants for approximately 120 days after dosing to provide an appropriate amount of time to observe delayed EV68-228-N-related AEs

prior to participant completion of the study. Review of cumulative safety data will be performed prior to dose escalations.

Potential theoretical risks associated with the clinical evaluation of EV68-228-N in humans include toxicity of or immune reactions to EV68-228-N or its excipients and unintended adverse effects related to the specificity of the antibody. Potential AEs may occur immediately following infusion or be delayed and may range from mild to severe, potentially fatal reactions.

As with other monoclonal antibodies, infusion reactions and hypersensitivity reactions, including anaphylaxis, may occur immediately or within a few hours of infusion. Infusion reactions during or immediately following infusions of mAb can occur but are generally mild. The frequency and intensity of the reaction may be controlled by using slower infusion rates. Potential cardiovascular reactions from infusion reactions will be monitored during administration.

Immediate hypersensitivity reactions are typically distinct from those that occur at later time points; events that occur early are often mediated by Immunoglobulin E (IgE), while later events may be triggered by antidrug antibodies (ADA). In contrast to anaphylaxis, symptom onset is hours to days after infusion, rather than upon immediate exposure. Antidrug antibody-mediated reactions are rare and often associated with monoclonal antibodies that target human proteins. EV68-228-N is a human monoclonal IgG1 antibody that targets enterovirus D68; in preclinical testing, no tissue cross-reactivity was found in a GLP study using rat and human tissue. Because EV68-228-N is a fully human antibody that targets viral proteins, we expect the risk of anaphylactic reactions and cytokine release syndrome to be very low.

Delayed hypersensitivity and immune responses secondary to immune complex formation or complement activation (e.g., serum sickness or complement activation-related pseudoallergy) typically have a subacute presentation. As a result, the association between administration of a protein therapeutic and non-acute reactions may be more difficult to establish. Clinical signs may include delayed onset of fever, rash, arthralgia, myalgia, hematuria, proteinuria, serositis, central nervous system complications, and hemolytic anemia in the face of an ongoing antibody response to the protein therapeutic.

Provisions for the treatment of hypersensitivity reactions should be available for immediate use in the event of an allergic reaction during administration of the infusion.

2.3.2 Potential Benefits

There are no direct medical benefits to the participant because of study participation. However, participation could benefit society by developing data about the safety and immunogenicity of this candidate treatment against EV-D68 infection. These data and PK data will be instrumental in further development of this product as an EV-D68 treatment for licensure. Development of an

efficacious treatment against EV-D68 would significantly improve the clinical outcomes of children who contract EV-D68 and develop AFM.

3 STUDY DESIGN, OBJECTIVES AND ENDPOINTS OR OUTCOME MEASURES

3.1 Study Design Description

This is a Phase 1, randomized, placebo-controlled, double-blinded study to assess the safety and pharmacokinetics of single IV administrations of EV68-228-N in healthy adult volunteers.

Three doses (3, 10 and 30 mg/kg) of EV68-228-N will be evaluated in three separate, sequential cohorts in this single dose escalation study. The cohorts will be randomized in a 5:1 randomization scheme. The first two participants in each cohort will serve as sentinels. Sentinel participants may be located at different sites. Sentinel safety data will be collected through Day 3 before submitting to the PSRT for review. The PSRT is comprised of the Principal Investigator (PI), the DMID Medical Monitor, and the DMID Medical Officer.

Data to be reviewed will include clinical data collected from Visits 1, 2 and 3, the results of laboratory testing conducted at these visits, solicited AEs and the passive reporting of adverse events through Day 3. From the time of infusion of the sentinels to at least 48 hours after infusion, no new participants will be given study product or placebo, but screening may continue. If no safety signal is detected in the sentinel group, and after approval from the DMID Medical Monitor, the remaining 10 participants in the cohort will be dosed following the overall 5:1 randomization scheme.

All participants will be actively monitored for AEs and safety laboratory data following dosing through Day 8. Data will be reviewed by the PSRT and discussed with the SMC for their concurrence before advancing to the next cohort. Electronic review of the safety data by the SMC is required prior to the cohort dose escalation when halting rules are met or there are any safety concerns.

Assuming no safety concerns are identified after review of the first cohort safety data through Day 8, enrollment of Cohort 2 will begin. The dose of EV68-228-N will be increased to 10 mg/kg for the second cohort. The same sentinel design and safety plan will be used to evaluate sentinel participants in Cohort 2 and determine whether to enroll the remaining participants in Cohort 2. In addition, the same sentinel design and safety plan will be used for Cohort 3, which will evaluate the 30 mg/kg dose.

Following informed consent, participants will be screened for eligibility, including medical history, physical examination, weight and height measurements, vital signs, screening laboratory tests, and a 12-lead electrocardiogram (ECG). Within 28 days of screening, eligible participants will be seen at the clinical research unit (Day 1) and be randomized to receive either a single intravenous dose of EV68-228-N or placebo (formulation buffer alone). Participants will remain

in the unit for at least 5 hours following infusion and return for assessments on Day 2 and Day 3. Participants will have subsequent follow-up clinic visits on Days 8, 15, 29, 61, 91, and 121.

Participants will be monitored and assessed for safety and the incidence of AEs at all visits beginning with the dosing visit. An electronic memory aid will be utilized from Day 1 through Day 3 to assist with collecting solicited AEs. Safety laboratory studies will be collected at screening and on Days 1, 2, 3, 8, and 29. Concomitant medications taken 28 days before and after dosing will be recorded.

Pharmacokinetic (PK) samples will be collected prior to infusion, end of infusion, 1, 3, 5, 24 and 48 hours after *end* of infusion; and on Days 8, 15, 29, 61, 91, and 121. The single dose PK parameters to be estimated include maximum observed serum concentration (C_{max}), time to C_{max} (T_{max}), area under the serum concentration-time curve (AUC) from time zero to time t (AUC_{0-t}), from time zero to 48 hours post infusion [$AUC_{(0-48)}$], from time zero to the last measurable concentration [AUC_{0-last}] and extrapolated to infinity [$AUC_{0-\infty}$], apparent serum terminal elimination phase half-life ($t_{1/2}$), total serum clearance (CL), and volume of distribution during the terminal phase (Vz). PK parameters will be calculated from serum EV68-228-N levels measured using an electrochemiluminescence (ECL) enzyme-linked immunosorbent assay (ELISA).

Samples will be collected prior to infusion on Day 1 and on Days 8, 15, 29, 61, 91 and 121 for serum levels of anti-EV68-228-N antibodies.

A sample will be collected pre-infusion on Day 1 for hypersensitivity testing in the event that the participant experiences an infusion reaction. These baseline samples will only be analyzed in the event of a hypersensitivity reaction related to the infusion. If a participant experiences anaphylaxis or an anaphylactoid event related to the infusion, three additional samples will be collected: 1) during onset, 2) 2 or more hours after onset, and 3) after resolution of symptoms.

The study dosing plan is shown in Table 1.

3.2 Study Objectives

3.2.1 Primary

- To evaluate the safety of a single IV infusion of either 3, 10, or 30 mg/kg of EV68-228-N when administered to healthy adults

3.2.2 Secondary

- To characterize the PK of single ascending doses of EV68-228-N for approximately four months following the infusion

- To measure the occurrence of ADAs elicited following a single IV infusion of EV68-228-N in healthy adults

3.3 Study Endpoints or Outcome Measures

3.3.1 Primary

- Proportion of participants experiencing solicited adverse events (AEs) through 48 hours post-infusion.
- Proportion of participants experiencing unsolicited adverse events (AEs) – including clinical and laboratory AEs – through Day 29.
- Proportion of participants experiencing serious AEs (SAEs), medically attended AEs (MAAEs), and new onset chronic medical conditions (NOCMCs) through the end of the study.

3.3.2 Secondary

After a single IV infusion of EV68-228-N:

- EV68-228-N concentrations in serum will be determined with a validated assay. The PK parameters will be estimated based on the concentration-time data, including area under the serum concentration-time curve (AUC) from time 0 to infinity ($AUC_{0-\infty}$), AUC from time 0 to time t (AUC_{0-t}), AUC from time 0 to 48 hours postdose (AUC_{0-48}), AUC from time 0 to the time of the last quantifiable concentration (AUC_{0-last}), maximum observed serum concentration (C_{max}), time of the C_{max} (T_{max}), apparent serum terminal elimination half-life ($t_{1/2}$), total serum clearance (CL), and volume of distribution during the terminal phase (Vz) calculated from serum EV68-228-N levels
- Incidence of anti-EV68-228-N antibodies as measured by the proportion of participants with detectable anti-EV68-228-N antibodies in serum

4 STUDY PRODUCT

4.1 Study Product Description

The EV68-228-N mAb is produced by expression of genetically engineered plasmids in a *Nicotiana benthamiana* based RAMP using proprietary plasmid vectors with plant viral sequences to reduce cost and improve the speed of production. The use of an engineered strain of *N. benthamiana* in which the plant specific glycosyltransferases, α1,3 fucosyltransferase and β1,2 xylosyltransferase, are mutated allows the RAMP-produced mAbs to have highly homogenous mammalian N-glycans and mitigates the potential for immunogenicity caused by carryover of plant glycans. See the IB for additional information regarding product manufacturing (7).

KBio uses an internal nomenclature system for identification of investigational products; the internal KBio nomenclature for EV68-228-N is "KB13A.1.1". This identification number will appear on the Certificate of Analysis as well as product labeling.

4.1.1 Formulation, Packaging, and Labeling

EV68-228-N: Study Product(s) and Components

The active pharmaceutical ingredient in EV68-228-N is a recombinant human monoclonal IgG1 antibody directed against enterovirus EV-D68 capsid protein which is produced in *Nicotiana benthamiana.* EV68-228-N study product is formulated at a target concentration of 20 (±2) mg/mL in Citrate, Glycine, Sucrose and Polysorbate 80 and filled into glass vials at 10 mL per vial.

The mAb in EV68-228-N study product is > 90% monomeric, with <5% high molecular weight species. Glycan profiling of EV68-228-N produced in the *N. benthamiana* platform at KBio identified the main class of N-glycans as the complex bi-antennary N-glycan G0, and the majority of the remaining detected glycans were high mannose species ranging from Man5 to Man9. These glycoforms are all typical of human glycoproteins.

Protein impurities, including product related mAb species (such as charge variants and oxidized species) and host cell proteins may be present in low quantities. Residual nicotine from the production platform may be present at levels of <2 μg/dose, and residual host cell DNA may be present at levels of less than 10 ng/dose and residual protein A may be present at ≤100 ng/mg. Endotoxin content is controlled throughout manufacturing following United States Pharmacopeial (USP) guidelines to ensure that treatment levels are below the pyrogenic threshold of 5 EU/kg/hr for intravenous administration.

The EV68-228-N study product is formulated as a sterile, preservative-free, concentrated aqueous solution for intravenous infusion. The EV68-228-N study product will be supplied as a 10 mL aqueous solution containing a targeted concentration of 20 (±2) mg/mL of mAb-EV68-228-N in formulation buffer (20 millimolar (mM) Citrate + 10 mM Glycine + 8% Sucrose + 0.01% Polysorbate 80, pH 5.5). All of the inactive formulation buffer components are approved for IV infusion at higher doses than the amounts which will be administered in a 30 mg/kg EV68-228-N dose. Drug Product will be supplied in SG EZ-fill® 10R ISO vials with 20 mm stoppers and 20 mm Flip-Off® seals. The product composition is presented in Table 2(7).

Table 2:Composition of the EV68-228-N Study Product Unit Dosage Form

Component	Quality Standard	Function	Target Concentration	Target Quantity per 10 mL
EV68-228-N Antibody Drug Substance	In-house standard	Active ingredient	20 mg/mL	200 mg
Citric Acid Anhydrous	USP or NF	Buffer	4.8 mM	9.25 mg
TriSodium Citrate Dihydrate	USP or NF	Buffer	15.2 mM	44.65 mg
Glycine	USP	Stabilizer	10 mM	7.507 mg
Sucrose	USP	Stabilizer	8% w/v	800 mg
Polysorbate 80	NF	Surfactant	0.01% w/v	1.0 µL (1.0 mg)

The study product will be labeled according to manufacturer or regulatory specifications and include the statement “Caution: New Drug – Limited by Federal Law to Investigational Use.”

Formulation Buffer: Placebo Comparator

The placebo is a sterile buffer solution formulated to match the EV68-228-N Drug Product. The formulation is 20 mM Citrate + 10 mM Glycine, 8% Sucrose, and 0.01% Polysorbate 80, with a target pH of 5.5. The placebo will be filled into glass vials at 10 mL per vial. The formulation buffer is a clear colorless liquid. It will be produced by KBio in a GMP lot.

Formulation Buffer: Diluent and IV flush

When the volume of study product is less than 20 mL, the formulation buffer will be added to the study product to attain a volume of 20 mL for the purpose of having sufficient volume for the infusion.

Following all infusions, a flush of the IV will be done with formulation buffer to ensure that all study product is delivered to the participant.

4.1.2 Product Storage and Stability

EV68-228-N: Study Product

Vials of EV68-228-N study product must be stored at -20 ± 5ºC until use. Vials will be thawed for 15 minutes to reach room temperature prior to compounding. Thawing may be done via hand-rolling, incubator, or other appropriate method. All infusions must be completed within 4 hours of compounding.

Stability testing of the EV68-228-N study product has been performed to evaluate the effects of storage at real-time (-20 ± 5ºC) and accelerated (5 ± 3ºC) conditions on quality attributes such as antibody concentration, potency, and purity. Results from ongoing stability studies support a shelf life of at least six months of storage at -20 ± 5ºC for the EV68-228-N study product. Unopened vials thawed for no more than 8 hours at room temperature can be re-frozen and stored at -20 ± 5ºC one time and used within six months.

Formulation Buffer: Placebo and Diluent

The formulation buffer should be stored at 2-8 ºC until use. Vials must be brought to room temperature prior to compounding. Stability testing of the formulation buffer has shown stability for 12 months at 2-8 ºC.

4.2 Acquisition/Distribution

Product 1: EV68-228-N

Will be provided by KBio via the DMID Clinical Material Services (CMS).

Upon request by DMID, EV68-228-N will be transferred to the following address:

DMID Clinical Materials Services Contract
Fisher BioServices
20439 Seneca Meadows Parkway
Germantown, MD 20876
Phone: 240-477-1350
Fax: 240-477-1360
Email: DMID.CMS@thermofisher.com

Product 2: Placebo/Diluent

Will be provided by KBio via the DMID CMS.

Upon request by DMID, EV68-228-N formulation buffer/placebo will be transferred to the following address:

DMID Clinical Materials Services Contract

Fisher BioServices
20439 Seneca Meadows Parkway
Germantown, MD 20876
Phone: 240-477-1350
Fax: 240-477-1360
Email: DMID.CMS@thermofisher.com

All study products will be shipped to the clinical research site upon request and approval from DMID.

4.3 Dosage/Regimen, Preparation, Dispensing and Administration of Study Product

Product 1 EV68-228-N: Study Product

Vials of EV68-228-N study product must be thawed at room temperature prior to use. Once thawed, product must be used within 4 hours, including infusion time.

If the calculated dose of study product is less than 20mL, enough formulation buffer will be added to obtain a volume of 20mL. If the volume of calculated dose of study product is greater than 20mL, no diluent will be used. If the volume of the calculated dose of study product is 50mL or less, study product will be delivered using a syringe pump. If the volume of the calculated dose of study product is more than 50mL, study product will be delivered in an infusion bag. Dose and volume preparation for study product will be further described in the Manual of Procedures (MOP). Prepared study product in infusion bags/syringes may be stored at room temperature for up to 4 hours including the time required for infusion.

On Day 1, IV access for the infusion will be established using aseptic technique. A different IV site or repeated phlebotomy should be used for collection of PK blood samples. When volume of infusion is 20mL, the study product will be infused at a rate of 80mL/hr to infuse over approximately 15 minutes. When volume of infusion is greater than 20mL, study product will be infused over approximately 30 minutes. Total infusion time will include the time of flush, which will take approximately 10 minutes. Longer infusion times will be permitted if the participant experiences side effects during the infusion and the rate of infusion must be slowed. The rate of infusion may be slowed or stopped to alleviate the symptoms as described in the MOP.

Product 2 Formulation Buffer: Placebo

The volume of formulation buffer used will be equivalent to the volume of study product that the participant would receive if they were randomized to the study product arm. Preparation of matching placebo is further described in the MOP.

On Day 1, IV access for the infusion will be established using aseptic technique. A different line, other than the infusion line, should be used for collection of PK blood samples. The placebo will be infused over a period of approximately 15 minutes if the volume equals 20mL and over a period of approximately 30 minutes if the volume is greater than 20 mL. Total infusion time will include the time of flush, which will take approximately 10 minutes. Longer infusion times will be permitted if the participant experiences side effects during the infusion and the rate of infusion must be slowed. The rate of infusion may be slowed or stopped to alleviate the symptoms as described in the MOP.

4.4 Accountability Procedures for the Study Product(s)

EV68-228-N will be stored and shipped from the DMID CMS to the Clinical Sites. Once received, EV68-228-N will be stored in and dispensed by the Investigational Pharmacy.

The FDA requires accounting for the disposition of all investigational products. The Investigator is responsible for ensuring that a current record of product disposition is maintained, and product is dispensed only at an official study site by authorized personnel as required by applicable regulations and guidelines. Records of product disposition, as required by federal law, consist of the date received, date administered, quantity administered, and the participant number to whom the drug was administered.

The Investigational Pharmacist will be responsible for maintaining accurate records of the shipment and dispensing of the study product. The pharmacy records must be available for inspection by the DMID monitoring contractors and are subject to inspection by a regulatory agency (e.g., FDA) at any time. An assigned Study Monitor will review the pharmacy records.

Unused study product vials will be stored at -20ºC ± 5ºC in the Investigational Pharmacy until clinical trial accountability is completed. At study termination, all unused study product will be disposed of in accordance with the MOP following complete drug accountability and monitoring.

5 SELECTION OF PARTICIPANTS AND STUDY ENROLLMENT AND WITHDRAWAL

Approximately 36 individuals, including males and non-pregnant, non-lactating females 18 to 49 years old, inclusive, at the time of enrollment, who are in good health by history and screening values and meet the inclusion and exclusion criteria will be enrolled in the study. The eligibility criteria apply only to enrollment of participants into the study. The focus population should reflect the community at large at the site.

Participant Inclusion and Exclusion Criteria must be confirmed by a study clinician licensed to make medical diagnoses.

No exemptions are granted on Subject Inclusion/Exclusion Criteria in DMID-sponsored studies. Questions about eligibility will be directed toward the DMID Medical Officer.

5.1 Eligibility Criteria

5.1.1 Participant Inclusion Criteria

1. Provides written informed consent prior to initiation of any study procedures.
2. Is able to understand and agrees to adhere to planned study procedures and is available for all study visits.
3. Adult volunteers 18 to 49 years of age, inclusive.
4. Females who are of childbearing potential[1] must agree not to become pregnant.

   [1] Not of childbearing potential includes post-menopausal females (defined as no menses for at least 12 months without an alternative medical cause for amenorrhea) or surgically sterile females with documented history of hysterectomy, bilateral oophorectomy, tubal ligation/salpingectomy, or Essure® placement.

5. Females who have sexual intercourse with male partners must agree to use at least one acceptable form of contraception for the duration of the study[2,3].

   [2] Acceptable methods of birth control include long-acting reversible contraception (LARC), combined pill, progestin-only pill, hormone-releasing transdermal patch or vaginal ring, and depot medroxyprogesterone acetate (DMPA) injection. Participants who choose to use a licensed hormonal product should use them for a minimum of 28 days prior to study infusion. True sexual abstinence or a monogamous relationship with a vasectomized partner who has

been vasectomized for 180 days or more prior to the participant's first infusion are also acceptable contraceptive methods.

[3] Participants who report practicing true abstinence, defined as no heterosexual vaginal-penile intercourse, need to practice true abstinence at all times during the study. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and the withdrawal method are not acceptable methods of contraception.

6. Females of childbearing potential must agree to not donate ova or oocytes during the study.
7. Participant is in good health[4].

[4] Good health is defined by the absence of a medical condition described in the exclusion criteria. If the participant has another current, ongoing medical condition, the condition cannot meet any of the following criteria: (1) was first diagnosed within 3 months of enrollment with a clinically significant condition, in the opinion of investigator that has worsened within 3 months of enrollment; (2) had non-elective surgery, clinically significant medical procedure, or hospitalization within 3 months of enrollment; (3) received new prescription for systemic medication within 30 days of enrollment, unless the new prescription is in the same class of agent or a transition from generic to/from brand name equivalent; or (4) takes medication that may pose a risk to participant's safety or impede assessment of adverse events or study endpoints if they participate in the study.

8. Must agree to refrain from donating blood or blood products[5] during the study.

[5] This includes whole blood cells, red blood cells, platelets, plasma, and plasma derivatives collected and donated outside of the study blood draws.

9. Body mass index (BMI) 18 kg/m^2 to 32 kg/m^2, inclusive, and a weight of 125 kg or less at time of screening.
10. Must have adequate venous access for intravenous (IV) infusion and blood sampling.

5.1.2 Participant Exclusion Criteria

All participants meeting any of the exclusion criteria at baseline will be excluded from study participation.

1. Positive pregnancy test at screening or prior to infusion.
2. Female participant who is lactating.

3. Presence of significant psychiatric condition, that in the opinion of the site PI or appropriate sub-investigator, precludes study participation.

4. History of drug abuse or alcohol abuse within 6 months of enrollment that, in the opinion of the site PI or appropriate sub-investigator, precludes study participation.

5. Has a significant acute illness (with or without fever), as determined by the site PI or appropriate sub-investigator, within 72 hours prior to infusion[1].

   [1]If the participant meets all other eligibility criteria, they may be enrolled and dosed once they meet this eligibility criterion. If the illness resolves within the 28-day screening window, they do not need to be rescreened, otherwise they will need to be rescreened.

6. Currently enrolled in or plans to participate in another clinical trial with an investigational agent that will be received during the study-reporting period.

7. Has a history of significant hypersensitivity, intolerance, or allergy to any drug compound, vaccine, food, or other substance, unless approved by the Investigator (or designee)[2].

   [2]Sensitivity to glycine, citric acid, trisodium citrate, sorbitol, or polysorbate 80 (components of the study product) is exclusionary.

8. Any history of an infusion reaction to any biologic product.

9. Receipt of a monoclonal antibody in the 180 days prior to infusion.

10. Receipt of a blood product within 120 days prior to infusion.

11. Received any live-attenuated vaccine in the 28 days prior or any other vaccine in the 14 days prior to infusion.

12. Has used any prohibited medication within 30 days prior to Day 1 or plans to use prohibited medication[3] during the study.

    [3]Prohibited medications include systemic immunosuppressive drugs, immune modulators (except acetaminophen or non-steroidal anti-inflammatory drugs), oral corticosteroids, and systemic anti-neoplastic agents. Topical, inhaled, and intranasal steroids, as well as topical anti-neoplastic agents are acceptable.

13. Has clinically significant findings[4] on 12-lead electrocardiogram.
    [4]Clinical significance will be determined by a cardiologist. Examples of findings that will lead to exclusion are significant left ventricular hypertrophy, right or left bundle branch

block, advanced A-V heart block, non-sinus rhythm (excluding isolated premature atrial contractions), pathologic Q wave abnormalities, significant ST-T wave changes, and prolonged QTc interval. Long QT interval is defined in males as a median QTcB greater than 450 msec or in females as a median QTcB greater than 460 msec (Bazett's correction) at screening.

14. Abnormal vital signs (Grade 1 or higher)[5] at screening or on Day 1.

[5] *Grade 1 or higher is equivalent to:*

Systolic blood pressure (SBP) > 140 mmHg or < 85 mmHg

Diastolic blood pressure (DBP) > 90 mmHg

Oral temperature ≥ 38.0°C (100.4°F)

15. Abnormal laboratory results[6] that are Grade 1 or worse at screening based on the Toxicity Tables in Appendix B[7].

[6]*Creatinine, alanine transaminase (ALT), hemoglobin (Hgb), platelets (PLT), white blood cell count (WBC), and total bilirubin (T bili).*

[7]*Laboratory studies can be repeated once if an alternative, transient etiology for abnormal laboratory values is identified.*

16. Known, current human immunodeficiency virus (HIV), hepatitis B Virus (HBV), or hepatitis C virus (HCV) infection

17. Has any medical disease or condition[8] that, in the opinion of the site PI or appropriate sub-investigator, precludes study participation[9].

[8]*Medical conditions include, but are not limited to, kidney disease with creatinine clearance < 90 mL/min/1.73 cm^2 (CKD-EPI method); known active liver disease including steatosis; ischemic heart disease, clinically significant cardiac conduction disorder, arrythmia requiring treatment, congenital long QT syndrome, uncompensated heart failure; diabetes requiring insulin; neuropathy or myopathy; and malignancy (not including squamous cell skin cancer, basal cell skin cancer, or cervical low-grade squamous intraepithelial lesions).*

[9]*Participation may be precluded due to safety concerns or inability to adequately evaluate clinical trial endpoints.*

5.1.3 Withdrawal from the Study or Discontinuation of the Study Product

Participants may voluntarily withdraw their consent for study participation at any time without penalty or loss of benefits to which they are otherwise entitled.

An investigator may also withdraw a participant prior to receiving the study product or after receiving the study product for any reason. Follow-up safety evaluations will be conducted, if the participant agrees. If a participant withdraws or is withdrawn prior to completion of the study, the reason for this decision must be recorded in the case report forms (CRFs).

The reasons might include, but are not limited to the following:

- Participant becomes pregnant prior to infusion
- Participant no longer meets eligibility criteria (e.g., develops acute febrile illness on Day 1 prior to infusion)
- Participant meets individual halting criteria (reference to section 8.6.4)
- Participant becomes non-adherent
- Participant develops a medical disease or condition, or new clinical finding(s) for which continued participation, in the opinion of the investigator might compromise the safety of the participant, interfere with the participant's successful completion of this study, or interfere with the evaluation of responses
- Participant is lost to follow-up
- Determined by a physician’s discretion to require additional therapy not indicated in the protocol to ensure participant’s health and well-being

If the participant consents, every attempt will be made to follow all AEs and pregnancies through resolution. The investigator should be explicit regarding study follow-up (e.g., safety follow-up) that might be carried out. The procedures that collect safety data for the purposes of research must be inclusive in the original informed consent or the investigator may seek subsequent informed consent using an IRB/IEC-approved consent form with the revised procedures.

The investigator will inform the participant that already collected data will be retained and analyzed even if the participant withdraws from this study.

5.1.4 Participant Replacement

Participants who withdraw, or are withdrawn from this study, or are lost to follow-up after signing the informed consent form (ICF) and receiving an infusion will not be replaced. Participants who withdraw, or are withdrawn from this study, or are lost to follow-up after signing the ICF but before infusion may be replaced.

5.1.5 Study Termination

If the study is prematurely terminated by the sponsor, any regulatory authority, or the investigator for any reason, the investigator will promptly inform the study participants and assure appropriate therapy or follow-up for the participants, as necessary. The investigator will provide a detailed written explanation of the termination to the IRB.

6 STUDY PROCEDURES

Study Duration

It is projected that the length of the overall study, from initiating screening to the completion of the last safety communication follow-up visit, will be approximately 10 months.

Duration of Individual Participant Participation

Participation for an individual participant will be approximately 6 months, which includes a 1 to 28-day screening period, infusion on Day 1, and follow-up visits on Day 2, 3, 8, 15, 29, 61, 91, and 121.

Study Schedule

Complete study schedule details listed by type of visit are described below. Refer also to the Schedule of Activities in Appendix A.

6.1 Screening/Visit 0

Day -28 to -1

The following activities will be performed at screening and may be done all at one visit or split into separate visits.

- Participants will be provided with a description of this trial (purpose and study procedures) and asked to read and sign the ICF. The ICF will be signed prior to performing any study procedures, including any screening procedures.
- Demographic information will be obtained by interviewing participants.
- Eligibility criteria will be reviewed with participants.

The following screening procedures will be completed to ensure that the potential participant is eligible for the study:

- Complete medical history will be obtained by interview of participants, including current contraceptive history with females of childbearing potential. History of all concomitant medications and vaccines taken within 28 days prior to signing the ICF will be obtained by interview of participants or abstraction from the electronic case report form (eCRF).
- Vital signs, including oral temperature, pulse, and blood pressure (BP), will be obtained.
- Height and weight will be collected for BMI calculation.
- A physical examination will be performed to include the following organs and organ systems: general appearance, skin, head and neck, lungs, heart, liver, spleen, extremities,

musculoskeletal and lymph nodes by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site PI or sub-investigator. Breast, genitourinary, and rectal exams are not considered a routine part of the full physical exam done at the screening visit.

- A 12-lead electrocardiogram (ECG) will be conducted.
- A urine pregnancy test will be performed on all females of childbearing potential and must be negative.
- Counsel on avoidance of pregnancy for females of childbearing potential.
- Venous blood will be collected for **safety laboratory tests**. These include:
    - Hematology: WBC, Hgb, and PLTs
    - Chemistry: Total bilirubin, ALT, Creatinine (Cr)

Testing results must be confirmed to meet the eligibility criteria as outlined in the Inclusion / Exclusion Criteria prior to randomization. Unacceptable screening laboratory values are those that are Grade 1 or worse at screening based on the toxicity scale (13). A creatinine value or an ALT value below the lower limits are acceptable for study inclusion as they are not considered to be clinically significant (CS). Participants with a Grade 1 abnormality may be re-screened once if there is a suspected inter-current, short-term medical illness or a suspected laboratory error. If screening labs are collected more than 28 days before infusion, the screening labs may be re-collected to determine if the participant remains eligible for study participation.

Participants who have a significant acute illness (with or without fever) in the 72 hours prior to infusion are not eligible for enrollment (see exclusion criteria). However, if the participant meets all other eligibility criteria, they may be enrolled after they have recovered, as determined by the site PI or sub-PI. Participants must be afebrile and symptom-free for at least 72 hours prior to infusion. If enrollment and infusion can be accomplished within the 28-day screening window, participants do not require rescreening, unless indicated at the investigator's discretion; if this cannot be accomplished, rescreening is required.

No participant may be screened **more than twice due to a screening failure result as defined above.** The Study PI will be contacted for clarification or questions regarding screening failures. All eligibility criteria must be satisfied before a participant is enrolled.

A participant may also be re-screened if the participant was screened eligible for a previous cohort but was not enrolled (e.g., scheduling conflict, vacation). Participants will be informed of any abnormal/clinically significant screening test results by a member of the study team. Participants will be encouraged to follow up with their primary care providers if results are of clinical significance or are sensitive in nature.

6.2 Enrollment

6.2.1 Visit 1, Day 1

Participants who pass screening and meet all the inclusion criteria and none of the exclusion criteria may be considered for study enrollment.

- Screening blood testing and study entry requirements will be reviewed with participants prior to the study treatment to ensure participants continue to be eligible. Laboratory results must be within acceptable parameters as outlined in the inclusion / exclusion criteria.
- Interim medical history, including an assessment for new medical conditions, stability of chronic diseases, will be obtained by interview of participants prior to the infusion. Any changes in medical history since the screening visit will be reviewed to ensure participants continue to remain eligible.
- A focused physical examination will be performed.
- All concomitant medications will be reviewed with participants prior to the infusion. Any new concomitant medications will be reviewed to ensure participants continue to qualify for participation.
- Vital signs, including oral temperature, pulse, and BP, will be obtained prior to the infusion at two timepoints: once to confirm eligibility and again at approximately 15 minutes prior to the infusion. Vital signs assessed 15 minutes prior to infusion will be considered as baseline.
- Oximetry measurements will be collected 15 minutes prior to infusion, at the end of infusion, and at approximately 1-hour post infusion. For infusions lasting at least 30 minutes, oximetry measurements will be collected at approximately 15 minutes after the beginning of the infusion.
- During infusion and up to one-hour post-infusion, continuous cardio-pulmonary monitoring will be conducted to assess for acute changes.
- Weight will be assessed to determine study product dose.
- An ECG will be performed approximately 15 minutes prior to the infusion and repeated one hour post infusion. The Day 1 pre-infusion ECG will be considered as baseline and is not utilized for eligibility purposes.
- For all participants of childbearing potential, a urine pregnancy test will be performed within 24 hours of the infusion. Results must be negative and known prior to the infusion.
- Venous blood will be collected approximately 15 minutes prior to the infusion for:
    - WBC, Hgb, PLT, Cr, ALT, T. Bilirubin
    - PK samples

- Serum samples for ADA
- Serum for baseline hypersensitivity panel

The results from the Day 1 safety labs, drawn pre-infusion, will be considered the new baseline for the participant.

Participants will be enrolled in Advantage eClinical® and randomly assigned to a treatment arm prior to the infusion.

Participants will receive a single intravenous dose of either study product or placebo. The time of administration to the participant will be recorded on the appropriate eCRF. Infusions will be administered over approximately 15 minutes or 30 minutes, depending upon the volume of the infusion, in the absence of infusion reactions or non-drug, infusion related safety issues; longer infusion times will be permitted. All infusions must be completed within 4 hours of compounding.

Once the IV bag/syringe containing the infusion runs dry, a flush of the tubing using formulation buffer will be conducted, allowing for any remaining product in the tubing to be infused to the patient. Refer to MOP for full procedures. The end of infusion time will be considered the time of completion of the flush and recorded on the appropriate eCRF.

Participants will be monitored during the infusion for safety. ECG, vital signs and oximetry measurements will be collected approximately 15 minutes prior to infusion. During infusion and up to one-hour post-infusion, continuous cardio-pulmonary monitoring will be conducted to assess for acute changes. Vital signs and oximetry will be collected at end of infusion, and at 1-hour post-infusion. For infusions that last at least 30 minutes, vital signs and oximetry will be collected at approximately 15 minutes after the infusion begins. An ECG will be conducted at 1-hour post-infusion.

For drug-related infusion reactions, appropriate interventions should be available to be administered as clinically indicated. Should a hypersensitivity or anaphylaxis symptom occur, refer to the site SOP for standard anaphylaxis protocol. If a participant experiences anaphylaxis or an anaphylactoid event related to the infusion, three additional hypersensitivity panel laboratory samples will be collected: 1) at onset, 2) 2 or more hours after onset and 3) after resolution of symptoms.

If the infusion is interrupted due to drug-related reactions such as anaphylaxis or hypersensitivity, stop the infusion immediately. If there are clinically significant changes in blood pressure, pause the infusion and monitor the participant. Refer to the MOP for further guidance on infusion interruption and restarting.

Participants will be observed in the unit until completion of the 5-hour PK timepoint and until the study clinician assesses it is safe for the participant to leave. Any AE/SAEs will be recorded on the appropriate CRF prior to discharge from the clinic.

Pharmacokinetic samples will be collected before infusion (approximately 15 minutes before), end of infusion (+ 5 minutes window), and at 1-hour (±15 minutes), 3-hours (±15 minutes), and 5-hours (±15 minutes) after end of infusion. If an infusion is terminated prior to completion, consult the MOP to determine updated PK sample collection directions.

Research staff will counsel participants on avoidance of pregnancy for participants of childbearing potential.

Research staff will review the use of the electronic Memory Aid (e-Memory Aid) with participants for the collection of solicited and unsolicited AEs.

6.3 Planned Study Visits

6.3.1 Visit 2: Day 2

The participant will return to the clinic approximately 24 hours after the end of the infusion, within a ± 3-hour window, which is required for the PK sample. The following procedures will occur:

- Review e-Memory Aid for solicited and unsolicited AEs
- Interim medical history, including an assessment for new medical conditions, stability of chronic diseases, and MAAEs, will be obtained by interview of participants and any changes since the previous visit will be noted.
- All concomitant medications will be reviewed with participants and recorded on the appropriate eCRF.
- Vital signs, including oral temperature, pulse, and BP, will be obtained.
- A focused physical examination will be performed.
- An ECG will be performed.
- All AE/SAEs will be recorded on the appropriate eCRF.
- Any previous AE/SAEs will be updated as required
- Venous blood will be collected for the following tests:
  - WBC, Hgb, PLT, Cr, ALT, T. Bilirubin
  - PK samples

6.3.2 Visit 3: Day 3

The participant will return to the clinic approximately 48 hours after the end of the infusion within a ± 3-hour window, which is required for the PK sample. The following procedures will occur:

- Review e-Memory Aid for solicited and unsolicited AEs
- Interim medical history, including an assessment for new medical conditions, stability of chronic diseases, and MAAEs will be obtained by interview of participants and any changes since the previous visit will be noted.
- All concomitant medications will be reviewed with participants and recorded on the appropriate eCRF.
- Vital signs, including oral temperature, pulse, and BP, will be obtained.
- A focused physical examination will be performed.
- All AE/SAEs will be recorded on the appropriate eCRF.
- Any previous AE/SAEs will be updated as required
- Venous blood will be collected for the following tests:
  - WBC, Hgb, PLT, Cr, ALT, Total Bilirubin
  - PK samples

6.3.3 Visit 4: Day 8 (± 1 day)

The following procedures will occur:

- Review e-Memory Aid for solicited and unsolicited AEs
- Interim medical history, including an assessment for new medical conditions, stability of chronic diseases, and MAAEs will be obtained by interview of participants and any changes since the previous visit will be noted.
- All concomitant medications will be reviewed with participants and recorded on the appropriate eCRF.
- Vital signs, including oral temperature, pulse, and BP, will be obtained.
- A focused physical examination will be performed.
- All AE/SAEs will be recorded on the appropriate eCRF.
- Any previous AE/SAEs will be updated as required
- Venous blood will be collected for the following tests:
  - WBC, Hgb, PLT, Cr, ALT, Total Bilirubin
  - PK samples
  - Serum samples for ADA

6.3.4 Visit 5: Day 15 (± 2 days)

The following procedures will occur:

- Interim medical history, including an assessment for new medical conditions, stability of chronic diseases, MAAEs, and NOCMCs, will be obtained by interview of participants and any changes since the previous visit will be noted.
- All concomitant medications will be reviewed with participants and recorded on the appropriate eCRF.
- Vital signs, including oral temperature, pulse, and BP, will be obtained.
- A symptom-driven physical examination may be performed if indicated based on review of complete medical history and any updates obtained by interview of participants since the previous visit.
- All AE/SAEs will be recorded on the appropriate eCRF.
- Any previous AE/SAEs will be updated as required
- Venous blood will be collected for the following tests:
  - PK samples
  - Serum samples for ADA

6.3.5 Visit 6: Day 29 (± 3 days)

The following procedures will occur:

- Interim medical history, including an assessment for new medical conditions, stability of chronic diseases, MAAEs, and NOCMCs, will be obtained by interview of participants and any changes since the previous visit will be noted.
- All concomitant medications will be reviewed with participants and recorded on the appropriate eCRF.
- Vital signs, including oral temperature, pulse, and BP, will be obtained.
- A symptom-driven physical examination may be performed if indicated based on review of complete medical history and any updates obtained by interview of participants since the previous visit.
- All AE/SAEs will be recorded on the appropriate eCRF.
- Any previous AE/SAEs will be updated as required
- Venous blood will be collected for the following tests:
  - WBC, PLT, Hgb, ALT, Creatinine, Total Bilirubin
  - PK samples
  - Serum samples for ADA

6.3.6 Visit 7: Day 61 (± 7 days)

The following procedures will occur:

- Interim medical history, including an assessment for new medical conditions, stability of chronic diseases, MAAEs, and NOCMCs, will be obtained by interview of participants and any changes since the previous visit will be noted.

- A symptom-driven physical examination may be performed if indicated based on review of complete medical history and any updates obtained by interview of participants since the previous visit.
- All SAEs will be recorded on the appropriate eCRF.
- Any previous AE/SAEs will be updated as required
- Venous blood will be collected for the following tests:
  - PK samples
  - Serum samples for ADA

6.3.7 Visit 8: Day 91 (± 14 days)

The following procedures will occur:

- Interim medical history, including an assessment for new medical conditions, stability of chronic diseases, MAAEs, and NOCMCs, will be obtained by interview of participants and any changes since the previous visit will be noted.
- A symptom-driven physical examination may be performed if indicated based on review of complete medical history and any updates obtained by interview of participants since the previous visit.
- All SAEs will be recorded on the appropriate eCRF.
- Any previous AE/SAEs will be updated as required
- Venous blood will be collected for the following tests:
  - PK samples
  - Serum samples for ADA

6.3.8 Final Study Visit: Day 121 (± 14 days)

At the final study visit, the following study activities should be completed:

- Interim medical history, including an assessment for new medical conditions, stability of chronic diseases, MAAEs, and NOCMCs, will be obtained by interview of participants and any changes since the previous visit will be noted.
- A symptom-driven physical examination may be performed if indicated based on review of complete medical history and any updates obtained by interview of participants since the previous visit.
- All SAEs will be recorded on the appropriate eCRF.
- Any previous AE/SAEs will be updated as required
- Venous blood will be collected for the following tests:
  - PK samples
  - Serum samples for ADA

6.3.9 Early Termination Visit

If the participant decides to withdraw consent after receiving the infusion and declines study duration follow up, it will be requested that the participant return for a final visit where follow-up safety evaluations will be conducted. If the participant refuses or is unable to come to the study clinic, the team will attempt to collect as much data as possible by phone or a HIPPA-compliant telehealth platform.

If the early termination visit occurs **within 7 days** of the infusion, the following procedures will occur:

- Interim medical history, including an assessment for new medical conditions, stability of chronic diseases, MAAEs, and NOCMCs, will be obtained by interview of participants and any changes since the previous visit will be noted.
- All concomitant medications will be reviewed with participants and recorded on the appropriate eCRF.
- A focused physical examination will be performed, if possible
- Vital signs, including oral temperature, pulse, and BP, will be obtained if possible.
- All AEs/SAEs will be recorded on the appropriate eCRF.
- Any previous AE/SAEs will be updated as required
- Venous blood will be collected if possible for the following tests:
  - WBC, PLT, Hgb, ALT, Cr, Total Bilirubin
  - PK samples
  - Serum samples for ADA

If the early termination visit occurs **later than 7 days** from infusion, the following procedures will occur:

- Interim medical history, including an assessment for new medical conditions, stability of chronic diseases, MAAEs, and NOCMCs, will be obtained by interview of participants and any changes since the previous visit will be noted.
- A symptom-driven physical examination may be performed if indicated based on review of complete medical history and any updates obtained by interview of participants since the previous visit.
- All SAEs will be recorded on the appropriate eCRF.
- Any previous AE/SAEs will be updated as required
- Venous blood will be collected if possible for the following tests:
  - PK samples
  - Serum samples for ADA

6.4 Unscheduled Study Visits

Participants will be asked to notify the study staff promptly if they develop any illness or new medical condition, or if they sought medical care. If the study staff determines the symptoms are potentially significant, the participant will be asked to come to the clinic for an evaluation. Participants will be asked to complete an unscheduled visit for any event that warrants follow-up. All adverse events will be followed to resolution, until determined to be stable, or until study completion. All serious adverse events will be followed until resolution or until determined to be stable.

At an unscheduled visit, the following study activities should be completed:

- Interim medical history, including an assessment for new medical conditions, stability of chronic diseases, MAAEs, and NOCMCs, will be obtained by interview of participants and any changes since the previous visit will be noted.
- All AEs/SAEs will be recorded on the appropriate eCRF.
- Any previous AE/SAEs will be updated as required
- A symptom-driven physical examination may be performed if indicated based on review of complete medical history and any updates obtained by interview of participants since the previous visit.

If the unscheduled visit occurs within 7 days of the infusion, the following procedures will also occur:

- A focused physical examination will be performed
- Vital signs, including oral temperature, pulse, and BP, will be obtained.
- All concomitant medications will be reviewed with participants and recorded on the appropriate eCRF.

6.5 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or protocol-specific MOP requirements. The noncompliance may be due to various reasons, including on the part of the participant, the site principal investigator, or the site personnel. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with International Conference on Harmonisation (ICH) E6:

4.5 Compliance with Protocol, Sections 4.5.1, 4.5.2, and 4.5.3

5.1 Quality Assurance and Quality Control, Section 5.1.1

5.20 Noncompliance, Sections 5.20.1, and 5.20.2.

It is the responsibility of the site PI and personnel to use continuous vigilance to identify and report deviations. All deviations must be promptly reported to DMID per the Statistical and Data Coordinating Center (SDCC) protocol deviation reporting procedures.

All protocol deviations, as defined above, must be addressed in study participant eCRFs. A contemporaneous log of protocol deviations should be maintained in the Regulatory File.. Protocol deviations must be sent to the IRB/IEC of record per their guidelines. The site principal investigator and personnel are responsible for knowing and adhering to their local IRB requirements, if applicable.

7 DESCRIPTION OF CLINICAL AND LABORATORY EVALUATIONS

7.1 Clinical Evaluations

Clinical evaluations will be done to evaluate safety endpoints. These evaluations will include monitoring and recording of AEs and SAEs, clinical safety laboratory test results, vital sign measurements, 12-lead ECG results, and physical examination findings. The timing and frequency of all safety assessments are listed in the Schedule of Activities (Appendix A).

Medical History

Medical history will be obtained by direct interview and include a history of significant medical disorders of the head, eyes, ears, nose, throat, cardiovascular system, lungs, gastrointestinal tract, liver, kidney, urologic, nervous system, blood, lymph glands, endocrine system, musculoskeletal system, skin, and genital/reproductive tract. A history of any allergies, cancer, immunodeficiency, psychiatric illness, substance abuse, and autoimmune disease will be solicited. The medical history will include current and past medical diagnoses, hospitalizations, and major surgical procedures. Females will be asked about lactation, current use of contraceptive methods, the date of last menses, and history of sterilization. At each visit, participants will be asked about any changes in health, new or worsening symptoms, or medications taken since their last visit. Any new adverse health changes will be recorded as an AE.

Vital Signs

Vital sign measurements will include pulse, systolic and diastolic blood pressure and oral body temperature. The participant will have rested comfortably in a seated or supine position for at least 5 minutes before all measurements are taken. Participants must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature. For time points when ECGs and vital signs occur at the same time point, it is acceptable to perform the vital sign measurements approximately 2 minutes after the ECG if the participant remains resting in the supine position. Additional vital signs may be measured for the safety of the participants at the discretion of the Investigator. All measurements will be recorded in the participant's eCRF.

If a physiologic parameter (e.g., a vital sign) is outside the protocol-specified range, then the measurement may be repeated once if, in the judgment of the site PI, the abnormality is the result of an acute, short-term, rapidly reversible condition (e.g., stress, anxiety, or "white coat syndrome"). A physiologic parameter may also be repeated if there is a technical problem with

the measurement caused by a malfunctioning or inappropriate measuring device (e.g., inappropriately sized BP cuff).

Cardiopulmonary Monitoring and Oximetry Measurements

Oximetry measurements will be collected approximately 15 minutes prior to infusion, at the end of the infusion, and at 1-hour post-infusion. For infusions lasting at least 30 minutes, oximetry measurements will be collected at approximately 15 minutes after the beginning of the infusion.

During infusion and up to one-hour post-infusion, continuous cardio-pulmonary monitoring will be conducted to assess for acute changes. If a CS change occurs, the monitoring will be continued until it has resolved and it is deemed safe to discontinue monitoring by the site PI or designee.

Physical Examination

A full physical examination will be performed at Screening (skin, head, ears, eyes, nose, throat, neck, thyroid, lungs, heart, cardiovascular, abdomen, lymph nodes, and musculoskeletal system/extremities). A focused examination (lungs, cardiovascular, abdomen, and skin) will be performed on Days 1, 2, 3 and 8. Targeted, symptom-directed physical examinations will be performed at all other follow-up visits if indicated. All physical examinations will be performed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site PI or sub-investigator.

Height and weight measurement will be done at screening to calculate BMI. Weight collected at enrollment will be used to calculate the dose for the individual.

Electrocardiogram (ECG)

A single, standard 12-lead ECG will be done at screening to determine eligibility. Additional standard 12-lead ECG recordings will be obtained at 15 minutes pre-infusion (baseline), one hour after the infusion, and Day 2. The 12-lead ECG will be obtained after the participant has rested comfortably in a supine position for at least 5 minutes. A study clinician should review the ECG for any immediate issues. Electrocardiogram assessments will include comments on whether the tracings are normal or abnormal, rhythm, presence of arrhythmia or conduction defects, morphology, any evidence of myocardial infarction, or ST-segment, T-wave, and U-wave abnormalities.

- All ECG recordings will be taken before obtaining any blood sample. Significant changes are defined as the following:
    - Any significant change in rate or rhythm as determined by the site PI
    - QTcB interval of greater than 450 ms (male) or greater than 460 ms (female)

- Increase from the QTcB baseline (defined as median of pre-dose measurements) greater than 50 ms until the change resolves.

If any of these changes are observed, triplicate ECG tracings, approximately 2 minutes apart, should be recorded and repeated hourly until cessation of the abnormality.

A cardiologist at the site will provide an interpretation within 3 business days using the following categories:

- Normal
- Abnormal—not clinically significant (NCS)
- Abnormal—clinically significant (CS)
  - Clinically significant ECG findings with new onset (or change in severity or frequency) any time after the infusion will be recorded as AEs. Clinical significance of ECG findings will be determined by the site PI based on their overall interpretation of data. Original ECG tracings will be retained as source documentation in the participant's records.

Memory Aid

All participants will complete an e-Memory Aid from the time of infusion through 48 hours post infusion. Participants will only require access to internet access. e-Memory Aids will be reviewed with the participants for any AEs (solicited and unsolicited AEs), SAEs, NOCMCs, MAAES and concomitant medications at clinic study visits on Days 2, 3 and 8. Solicited systemic parameters occurring during the 48 hours after receipt of infusion will include: headache, nausea, vomiting, diarrhea, rash, fatigue, and myalgia. Participant e-Memory Aids are reviewed for accuracy and completeness at follow-up visits. The research team will follow and collect resolution information for any symptoms that are not resolved within 3 days.

7.1.1 Assessment of Concomitant Medications/Treatments other than Study Product

Restrictions for prior medications and therapies are provided in Section 5.1.2. In this study, individuals who take immunosuppressive drugs, immune modulators (except acetaminophen), oral corticosteroids, inhaled or intranasal steroids (<800 µg/day beclomethasone or equivalent is acceptable), and anti-neoplastic agents within 28 days of Day 1 are ineligible for the study. Topical steroids are acceptable. Information regarding concomitant medications taken by the participant within the 28 days of Day 1 and those taken during the study will be recorded in the participant's eCRF. Concomitant medications include prescription medications and treatments; over the counter drugs, vitamins, herbals, and supplements; illicit substances; and vaccines.

The Investigator is responsible for ensuring that details regarding the medication are adequately recorded in the participant's source documents and in the eCRF. Concomitant medication use including receipt of treatments and vaccines in the 28 days prior to Day 1 will be obtained and

recorded at the Screening visit and at Day 1. At each follow-up study visit through Day 29, new concomitant medication and changes to existing medications will be recorded. Any concomitant medication deemed necessary for the welfare of the participant during the study should be given.

7.1.2 Clinical Laboratory Evaluations

- Hematology: Hgb, WBC, PLT
- Chemistry: Creatinine, Total Bilirubin, ALT
- Urine pregnancy testing will be performed in the clinic by a qualified study team member using a commercially available test at screening and at Day 1 prior to study intervention to reconfirm negative pregnancy status. Pregnancy test results must be negative for participants to be eligible for enrollment and to be qualified to receive an infusion.
- Hypersensitivity testing: hypersensitivity testing will be conducted if a participant experiences anaphylaxis or an anaphylactoid event related to the infusion. One sample will be obtained at approximately 15 minutes prior to the infusion; if the participant experiences a reaction, three additional samples will be drawn: 1) at the onset of symptoms, 2) 2 or more hours after onset of symptoms, and 3) after resolution of symptoms. The hypersensitivity panel will measure complement levels and activity (C3, C4, and CH50), Immunoglobulin E (IgE) levels, and tryptase on serum.

7.1.3 Research Assays

Serum EV68-228-N concentrations and ADA will be measured with validated assays developed by PPD Laboratories- Bioanalytical Lab. Detailed information on the endpoint lab as well as timeline for assay completion and data analyses can be found in the Central Assay Plan (CAP).

- Serum EV68-228-N concentrations: A quantitative anti-idiotype ELISA will be performed on samples that collected approximately 15 minutes prior to infusion; at end of infusion (+5 minute window); 1-, 3-, and 5-hours after end of infusion, within a ±15 minute window; 24- and 48-hours after the end of the infusion (within a ±3-hour window) and on Days 8, 15, 29, 61, 91, and 121 (the visit window will be the window for the PK blood draw). An EV68-228-N anti-idiotype antibody will be used in the assay to capture EV68-228-N and this will be detected with an anti-Human IgG (Fc)-biotin antibody, secondary detection is Streptavidin SULFO-TAG. The PK parameters will be estimated based on the time-concentration data and listed in section 10.5.
- Anti-Drug Response: A bridging ELISA will be performed on samples collected approximately within 15 minutes prior to the infusion on Day 1 and at Days 8, 15, 29, 61, 91 and 121 (with corresponding windows) for serum levels of anti-EV68-228-N antibodies. To measure ADA, anti-drug antibodies will be captured by EV68-228-N and detected with labeled EV68-228-N (e.g., biotin) followed by standard detection (e.g., streptavidin - SULFO-TAG).

7.1.3.1 Laboratory Specimen Preparation, Handling, and Storage

Blood samples are obtained at the research clinic for hematology and chemistry testing. Instructions for specimen preparation, handling and storage are included in the protocol-specific MOP.

Blood samples are obtained at the research clinic and processed in the sample processing laboratory according to SOPs to obtain aliquots of serum for PK and ADA analysis. These specimens will be frozen and sent to DMID CMS where they will be stored per SOP until the timepoint that the IDCRC central processing lab has determined is appropriate for testing based on stability testing. Instructions for specimen preparation, handling and storage are included in the protocol-specific MOP.

7.1.3.2 Laboratory Specimen Shipping

Instructions for specimen preparation, handling, storage, and shipment as applicable are included in the protocol-specific MOP. International Air Transport Association (IATA) guidelines will be followed for specimen handling, transport and shipping.

8 ASSESSMENT OF SAFETY

8.1 Assessing and Recording Safety Parameters

Safety will be assessed through the evaluation of AEs, vital signs, ECGs, and conventional clinical laboratory data as outlined in the Schedule of Activities.

8.1.1 Adverse Events (AEs)

ICH E6 defines an AE as any untoward medical occurrence in a patient or clinical investigation participant administered a pharmaceutical product regardless of its causal relationship to the study treatment. The FDA defines an AE as any untoward medical occurrence associated with the use of a drug in humans, whether or not it is considered drug related.

An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product. The occurrence of an AE may come to the attention of study personnel during study visits and interviews of a study recipient presenting for medical care, or upon review by a study monitor.

A treatment-emergent AE (TEAE) is defined as an AE that occurs during or after the study product infusion and up through the final visit. Only treatment-emergent adverse events will be documented as adverse events in this study. Any medical condition, vital signs, ECG results, or clinical lab test values that are collected prior to infusion will be considered a baseline. Any medical condition that is reported after screening but before the infusion will be evaluated and reported as Medical History update.

All AEs will be captured on the appropriate eCRF. Information to be collected for AEs includes event 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/sub-investigator), date of resolution, seriousness, and outcome. AEs occurring during the study collection and reporting period will be documented appropriately regardless of relationship. Adverse events will be followed through resolution or stabilization, as indicated by an investigator.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the severity of any preexisting medical condition increases, it should be recorded as an AE.

If laboratory tests (emergency or unscheduled) are indicated for an AE or SAE, they should be conducted through the central laboratory if they are tests within the central laboratory’s scope of

work for this protocol (WBC, Hgb, PLT, Cr, ALT, T Bili). If the laboratory tests are outside the central laboratory's scope of work, they should be conducted at a local laboratory.

The investigator should employ their best medical judgment in determining how to manage AEs and SAEs.

Solicited adverse events will be collected during days 1 through 3. Participants will be given an e-Memory Aid to assist with recording solicited adverse events. Unsolicited AEs will be collected through Day 29. SAEs, NOCMCs, and MAAEs will be collected Days 1-121.

8.1.1.1 Adverse Events Grading

All AEs (laboratory and clinical symptoms) will be graded by the licensed study physicians on Form FDA 1572 for severity according to the Toxicity Tables in Appendix B, based upon the Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0, November 2017, and assessed for relationship to study product (13). The start and stop date of each reported AE will be recorded on the appropriate eCRF. Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of intensity.

Severity of Event

All AEs (laboratory and clinical symptoms) will be graded for severity according to the Toxicity Tables in Appendix B, based upon the CTCAE Version 5.0, November 2017, and assessed for relationship to study product (13). AEs characterized as intermittent require documentation of onset and duration of each episode. The start and stop date of each reported AE will be recorded on the appropriate data collection form and eCRF.

- 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 affect clinical status, may require intensive therapeutic intervention, or death related to an AE. Severe events are usually incapacitating.
- Life Threatening (Grade 4): Life threatening consequences; urgent intervention indicated.
- Death (Grade 5): Related to adverse event.

Relationship to Study Product

The assessment of the AE's relationship to study product will be done by the licensed study physician indicated on the Form FDA 1572 and the assessment will be part of the documentation

process. Whether the AE is related or not is not a factor in determining what is or is not reported in this study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported.

In a clinical study, the study product must always be suspect. The relationship to study product will be assessed for AEs using the terms "Related" and "Not Related":

- Related – There is a reasonable possibility that the study product caused the AE. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the AE.
- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

An investigator should use good clinical judgement. If the event is believed to be unrelated to the study product, an alternative plausible explanation must be provided. Otherwise, adverse events will be considered related to the study product.

8.1.2 Serious Adverse Events

An AE or suspected adverse reaction is considered an SAE if it occurs between the time of receiving study medication and the final study visit, and in the view of either the site PI or sponsor; it results in any of the following outcomes:

- Death,
- A life-threatening* AE,
- 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 hospitalizations 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, blood dyscrasias, or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

*Life-threatening AE: An AE is considered "life-threatening" if, in the view of either the site PI or sponsor, its occurrence places the patient or participant at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death.

Refer to section 8.3.3 to determine if a pregnancy-related event should be considered an AE or SAE.

SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed study physician listed on the FDA Form 1572 or by the Institution as the site PI or sub-investigator.
- Recorded on the appropriate SAE data collection form and eCRF.
- Followed through resolution by a licensed study physician (for IND studies, a physician listed on the Form FDA 1572 as the site PI or sub-investigator).
- Reviewed and evaluated by DMID, the SMC (periodic review unless related), and the IRB/IEC.

8.1.2.1 Suspected Unexpected Serious Adverse Reactions (SUSAR)

A SUSAR is any SAE where a causal relationship with the study product is at least reasonably possible but is not listed in the IB, Package Insert, and/or Summary of Product Characteristics.

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

- *Suspected Adverse Reaction* means any AE for which there is a reasonable possibility that the drug caused the AE.
- *Unexpected Adverse Event* means an AE that is not listed at the specificity or severity that has been observed.

All SUSARs (as determined by the IND Sponsor) will be reported to the FDA as IND Safety Reports per 21 CFR 312.32 as soon as possible but not exceeding 7 calendar days for unexpected fatal or life-threatening events, and not exceeding 15 calendar days for other qualifying events.

IND Safety Reports will also be provided to the IRB.

8.1.3 Unanticipated Problem

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

- Unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the 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 (which may include research staff, family members or other individuals not directly participating in the research) at a

greater risk of harm (including physical, psychological, economic, or social harm) related to the research than was previously known or expected.
- A UP must be reported within 7 calendar days of an investigator becoming aware of the actual or suspected UP.

8.2 Specification of Safety Parameters

Safety will be assessed by the frequency and severity of AEs and SAEs; the incidence and maximum intensity of AEs and SAEs; clinically important changes in vital sign measurements, ECG findings, and clinical laboratory values, defined as a change to a higher grade relative to baseline (13).

AEs and SAEs will be addressed as they are reported or identified by a staff member. Safety laboratory reports are reviewed as available, generally within 24 hours of sample collection.

8.2.1 Solicited Events

Solicited events are AEs that may be anticipated based on the class of study product (i.e., monoclonal antibody). As the study product is a monoclonal antibody, it is reasonable to expect that events commonly seen after other mAb infusions may occur in this first-in-human study. Solicited events for this study are:

- Headache
- Rash
- Nausea
- Vomiting
- Diarrhea
- Fatigue
- Myalgia

The investigator will assess if any reported solicited AEs are related to the infusion.

8.2.2 Unsolicited Events

Unsolicited events are any other AEs that occur following infusion.

8.2.3 New-Onset Chronic Medical Conditions (NOCMCs)

NOCMCs are defined as any new ICD-10 diagnosis that is applied to the participant during the duration of the study, after receipt of the study agent, that is expected to continue for at least 3 months and requires continued health care intervention.

8.2.4 Medically Attended Adverse Events (MAAEs)

For each unsolicited AE experienced, the participant will be asked if they have received medical attention, defined as hospitalization, an ER visit, or an otherwise unscheduled visit with medical personnel for any reason. AEs characterized by such unscheduled medical care will be designated as MAAEs.

8.2.5 Dose Escalation Process

8.2.5.1 Dose Escalation to Next Cohort

Before proceeding to the next cohort, blinded safety and clinical laboratory data up to the Day 8 visit will be evaluated by the PSRT. The safety review will include data from SAEs, AEs, safety clinical laboratory results, ECGs and vital signs. The PSRT will confirm if any halting criteria have been met or occurrence of any safety concerns. If none of the pre-defined halting criteria are met, the DMID Medical Monitor will recommend to proceed to the next cohort, or alternatively recommend convening of the SMC.

After PSRT safety data review, advancing to the next cohort will be discussed via email communication with the SMC members for their concurrence before the dose escalation. Electronic review of the safety data by the SMC is required prior to the cohort dose escalation when halting rules are met or there are any safety concerns.

8.2.5.2 Sentinel Participants

Dosing will begin with two sentinel participants in each cohort who will be monitored for AEs and safety laboratory data after infusion through the Day 3 visit. The enrolling site PI(s) will review sentinel safety data to confirm that no halting criteria (see Section 8.6.3) have been met. The site will notify the DMID Medical Monitor of any safety concerns.

Sentinel safety data will be reviewed by the study PI, DMID Medical Monitor and DMID Medical Officer. Data to be reviewed will include clinical data collected from the day of infusion through in-clinic study visits on Day 2 and Day 3, results of laboratory testing conducted at these visits, solicited AEs (reported based on Memory-aid), and any adverse events that occur through the study visit 3 time point. During that safety review period no new participants will be enrolled and given the study product or placebo, but screening may continue.

If no specific safety concerns are identified by the blinded data review of sentinel participants, and after approval from the DMID Medical Monitor, the remaining participants in the cohort will be enrolled and dosed.

If sentinel halting rules are met, the study will be suspended by the DMID Medical Monitor, and the PSRT will determine if an SMC ad-hoc meeting should convene to decide how to proceed.

8.3 Reporting Procedures

8.3.1 Reporting Serious Adverse Events

Serious AEs will be followed until resolution even if this extends beyond the study reporting period. Resolution of an AE is defined as the return to pretreatment status or stabilization of the condition with the expectation that it will remain chronic.

Any AE that meets a protocol-defined serious criterion must be submitted immediately (within 24 hours of site awareness) on a DMID 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 data entry 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 and DMID Clinical Project Manager will be notified of the SAE by the DMID Pharmacovigilance Group. 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 PI 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.

8.3.2 Regulatory Reporting for Studies Conducted Under DMID-Sponsored IND

Following notification from the site Principal Investigator or appropriate sub-investigator, DMID, as the IND sponsor, will report any suspected unexpected serious adverse event. 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. 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. 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.

All SAEs designated as "not related" to study product(s), will be reported to the FDA at least annually in a summary format.

The Suspected Unexpected Serious Adverse Reaction (SUSAR) report is equal to an IND Safety Report. The difference is the regulatory agency and the country(ies) the study is conducted in.

For studies conducted in Europe or the United Kingdom, the IND safety report called SUSAR will be submitted on a CIOMS form. The FDA will accept the CIOMS format as well.

If the US IND holder conducts studies internationally, the sponsor is required to submit both SUSAR and IND safety reports (CIOMS form, 3500A).

8.3.3 Reporting of Pregnancy

Females of childbearing potential are excluded from this study unless they consent to adequate contraception.

Female participants must be instructed to inform the investigator immediately if they become pregnant during the study. In the event of a confirmed pregnancy or positive pregnancy test, the following actions should be taken:

- Pregnancy should be reported to Emmes within 24 hours of notification, using the applicable pregnancy report form.
- Investigator should counsel the participant regarding the need to inform the study site of the outcome of the pregnancy.

- Participant should be monitored, if possible, until the immediate postnatal period (6 weeks) or until termination of the pregnancy. The outcome should be reported to the medical monitor using the Pregnancy Outcome or Abnormal Pregnancy Outcome form.

Pregnancy is not an AE itself. However, any pregnancy complication or elective termination of a pregnancy for medical reasons will be recorded as an AE or SAE. A spontaneous abortion is always considered an SAE and will be reported as described in the AE and SAE sections.

Furthermore, any SAE occurring as an adverse pregnancy outcome post-study must be reported to the sponsor/DMID.

8.4 Type and Duration of Follow-up of Participants after Adverse Events

The site PI or sub-investigator is responsible for ensuring the recording of all AE/SAEs that are observed or reported during this trial, regardless of the relationship to study product.

AE/SAEs, abnormal laboratory test values, or abnormal clinical findings will be collected, assessed, documented, and followed appropriately. Grading of the ECG, laboratory and vital signs AEs will be according to the Toxicity Tables in Appendix B, based on the CTCAE (13).

8.5 Procedures to be Followed in the Event of Abnormal Laboratory Test Values or Abnormal Clinical Findings

Isolated laboratory test abnormalities should not be recorded as AEs or SAEs; for example, if the laboratory abnormality is part of a clinical condition or syndrome, it should be recorded as the syndrome or diagnosis rather than as the individual laboratory abnormality. In addition, laboratory abnormalities or other abnormal test assessments (e.g., ECGs) that are associated with signs or symptoms must be recorded as AEs or SAEs if they meet the definition of an AE (or SAE) as described in Section 8.1. Laboratory test abnormalities are documented on the laboratory form and not recorded as a separate Adverse Event eCRF but the relationship, clinical significance, and grade are noted on the laboratory screen.

The site is utilizing standard panels for this protocol; therefore, if there are laboratory parameters not solicited in this protocol but part of a standard panel that are abnormal AND clinically significant as determined by the PI, then the abnormal parameter will be reported on the Adverse Event eCRF.

8.6 Halting Rules for the Study

The halting rules outlined below will be used to evaluate whether it is safe to proceed with dosing the remaining participants in a cohort, dose escalation to the next cohort, or whether the study should be suspended for further safety evaluation.

If a dose-escalation halting rule is met, the SMC will convene. Dose-escalation decisions will be documented by the DMID Medical Monitor. This documentation will include whether halting criteria were met and any review and recommendations of the SMC.

8.6.1 Study Halting Criteria

Additional enrollment and administration of study products will be halted for SMC review and recommendation if any of the following are reported after infusion:

- One participant experiences an SAE that is determined to be related to study product.
- Two or more participants (cumulative among all cohorts) experience generalized urticaria (defined as occurring at more than two body parts) within 48 hours after administration of study product that is considered related to study product.
- Three or more participants in the study (cumulative among all cohorts) experience a Grade 3 or higher related AE (laboratory or clinical) that is coded in the same High Level Group Term (HLGT) per MedDRA classification.

8.6.2 Dose Escalation Halting Criteria

Study product administration in the cohort will be halted for SMC ad-hoc review if any of the following events are reported after infusion:

- One participant experiences anaphylaxis, regardless of seriousness or severity of the event.
- Two or more participants experience Grade 2 or higher hypersensitivity reactions that are not considered anaphylaxis.
- One participant has an SAE that is determined to be related to the study product.
- Two or more participants in a cohort experience Grade 3 or higher related AE (laboratory or clinical) that is coded in the same HLGT per MedDRA classification.

If the pre-defined dose escalation halting criteria are met, the SMC will convene for an ad-hoc meeting to review the unblinded safety data and provide guidance on how to proceed.

Any event of anaphylaxis will be reported to the FDA within 24 hours of occurrence. The FDA will also be alerted if the study or dosing is stopped due to hypersensitivity reactions.

8.6.3 Sentinel Participants Halting Rules

Progression to the remaining participants in the cohort will be halted if any of the sentinel participants experience the following:

- A participant experiences any SAE, regardless of the relationship to the study product (with exception of death or hospitalization that was a result of trauma or accident).
- A participant experiences a related Grade 3 or higher AE (laboratory or clinical) not resolved within 48 hours of onset (through day 8); resolution is defined as a return to Baseline or Grade 1.

8.6.4 Halting Rules for an Individual Participant

Infusion of the Investigational Product will be halted if any of the following manifestations of anaphylaxis or generalized urticaria develop and will not be restarted (symptoms of anaphylaxis may begin in seconds or minutes of infusion). Symptoms of anaphylaxis or hypersensitivity may include the following:

- Acute onset of an illness (minutes to hours)
- Skin or mucous membrane manifestations: hives, moderate or severe pruritus, or angioedema (usually of face, eyelids, or lips, tongue, or uvula)
- Respiratory compromise: sensation of throat closure or choking, chest tightness, wheezing, stridor
- A decrease in systolic BP to < 90 mmHg or > 30% decrease from baseline in systolic or diastolic BP
- Tachycardia with an increase in resting heart rate to ≥ 130 beats per minute (bpm); or development of a ventricular dysrhythmia; or bradycardia < 45 bpm (or < 40 bpm in subjects with a baseline of < 60 bpm) that is associated with complaints of dizziness, nausea, or feeling faint
- Syncope
- Slurred speech and/or confusion
- Any other condition that the site PI judges to increase the risk to the subject unduly

Infusion related reactions to monoclonal antibodies typically develop within 30 minutes to two hours after the initiation of drug infusion although symptoms may be delayed for up to 24 hours. The most common signs and symptoms of infusion related reactions are:

- Fever and/or shaking chills
- Flushing and/or itching
- Alterations in heart rate and BP
- Shortness of breath, chest discomfort

- Nausea, vomiting, and/or diarrhea
- Various types of skin rashes

The study may also be suspended (participant enrollment and/or study interventions suspended) because of safety findings, such as an SAE or an overall pattern of symptomatic, clinical, or laboratory events that DMID or the PSRT consider to be related to the study product. These may appear minor in terms of individual events but might collectively represent potential concern for safety.

The DMID Medical Monitor may stop enrollment and/or administration of study product for any specific safety concerns or if AEs that meet the halting criteria are reported.

If any of the halting rules are met following any participant receipt of study product, the trial will not continue with the remaining enrollments or study infusions without a review by and recommendation from the SMC to proceed.

8.7 Safety Oversight

8.7.1 Safety Monitoring Committee (SMC)

This clinical study will utilize an SMC, which is an independent group of experts that advises DMID. The primary responsibility of the SMC is to monitor participant safety. The SMC is external to DMID and comprises at least 3 voting members. The SMC will consist of members with appropriate phase 1 study expertise to contribute to the interpretation of the data from this trial. Its activities will be delineated in an SMC Charter that will describe the membership, responsibilities, and the scope and frequency of data reviews. The SMC will operate on a conflict-free basis independently of the study team. DMID or the SMC may convene ad hoc meetings of the SMC according to protocol criteria or if there are safety concerns that arise during the study.

As defined in the charter, the SMC will review data at specified times during the course of the study for participant and overall study progress and will conduct ad hoc reviews as appropriate when a halting rule is met or for immediate concerns regarding observations during this study. The SMC will receive email notifications about SAEs and related AEs that are Grade 2 or higher.

If any of the predefined halting criteria are met, study enrollment and dosing will be stopped until the SMC members e-review unblinded safety data report and provide recommendations regarding continuation of the study during an ad hoc SMC meeting or by electronic communication.

The SMC will electronically review the safety data between cohorts and in case of safety concerns, there will be an SMC ad hoc meeting scheduled for SMC unblinded safety review.

9 HUMAN SUBJECTS PROTECTION

9.1 Institutional Review Board/Independent Ethics Committee

Each site principal investigator will obtain IRB approval for this protocol to be conducted at their research site(s) and send supporting documentation to the DMID before initiating recruitment of participants. The investigator will submit applicable information to the IRB/IEC on which it relies for the review, to conduct the review in accordance with 45 CFR 46, ICH E6 GCP, and as applicable, 21 CFR 56 (Institutional Review Boards) and 21 CFR 50 (Protection of Human Subjects), other federal, state, and local regulations. The IRB/IEC must be registered with OHRP as applicable to the research. DMID must receive the documentation that verifies IRB/IEC-approval for this protocol, associated informed consent documents, and upon request any recruitment material and handouts or surveys intended for the participants, prior to the recruitment and enrollment of participants.

Any amendments to the protocol or consent materials will be approved by the IRB/IEC before they are implemented. IRB/IEC review and approval will occur at least annually throughout the enrollment and follow-up of participants and may cease if annual review is no longer required by applicable regulations and the IRB/IEC. The investigator will notify the IRB/IEC of deviations from the protocol and reportable SAEs, as applicable to the IRB/IEC policy.

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.

A single IRB of record, Vanderbilt IRB, will be accountable for compliance with regulatory requirements for this multi-centered study, at participating sites. Written reliance agreements between the single IRB and participating sites will be required. The agreements will set forth the specific responsibilities of the IRB and each participating site. Participating sites will then rely on the IRB of record to satisfy the regulatory requirements relevant to the IRB review. The participating sites will maintain essential required documentation of IRB reviews, approvals, and correspondence, and must provide copies of any agreements and essential documentation to the DMID or regulatory authorities upon request.

9.2 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 explanation will be organized and presented in lay terminology and language that facilitates understanding why one might or might not want to participate.

An investigator or designee will describe the protocol to potential participants face-to-face. The key information about the purpose of the study, the procedures and experimental aspects of the study, risks and discomforts, any expected benefits to the participant, and alternative treatment will be presented first to the participant.

Participants will also receive an explanation that the trial involves research, and a detailed summary of the proposed study procedures and study interventions/products. This will include aspects of the trial that are experimental, the probability for random assignment to treatment groups, any expected benefits, all possible risks (including a statement that the particular treatment or procedure may involve risks to the participant or to the embryo or fetus, if the participant is or may become pregnant, that are currently unforeseeable), the expected duration of the participant's participation in the trial, alternative procedures that may be available and the important potential benefits and risks of these available alternative procedures.

Participants will be informed that they will be notified in a timely manner if information becomes available that may be relevant to their willingness to continue participation in the trial. 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.

The extent of the confidentiality of the participants' records will be defined, and participants will be informed that applicable data protection legislation will be followed. 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 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 allowed sufficient time to consider participation in this research trial and have the opportunity to discuss this trial with their family and friends 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. The participant(s) may withdraw consent at any time throughout the course of the trial. The rights and welfare of the participant(s) will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

Study personnel may employ recruitment efforts prior to obtaining study consent if a patient-specific screening consent is on record or if the IRB has agreed that chart review is allowed without a fully executed screening consent. Research staff will obtain written consent per the standard informed consent process before conducting protocol-specific screening activities.

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. Participants will be given a copy of all informed consent forms that they sign.

9.3 Consent for Secondary Use of Stored Specimens and Data

Residual samples/specimens are those that are left over after protocol-specified testing and this study has been completed. Participants will be asked for permission to keep any remaining (residual) specimens (serum) derived from venous blood samples for possible use in secondary research studies, such as examining additional immunological assessments or testing for antibodies against other viruses or bacteria. These residual specimens will be stored coded indefinitely at Fisher, a central clinical storage facility with IRB oversight. Specimens may be shared with investigators at the participating site and with other investigators at U.S. institutions. The recipients of specimens will be informed that these specimens have a NIH certificate of confidentiality. The information provided to a recipient will not contain direct identifiable information. Use of the specimens will require review by an IRB.

Residual specimens will be available upon the completion of this trial. Exceptions for early release of residual specimens must be approved by the study team and IDCRC leadership.

There are no benefits to participants in the collection, storage and subsequent secondary use of their samples/specimens. Secondary use samples/specimens will not be sold or used directly for production of any commercial product. No genetic tests will be performed on samples/specimens. Each sample/specimen will be encoded (labeled) only with a barcode and a unique tracking number that connects to a code key at the study site. Restricted access to the code key is maintained by the principal investigator to protect participant confidentiality. Reports from secondary research studies performed using participants' samples/specimens will NOT be kept in their health records.

Participants may be given the option to decide if they want their **residual** specimens to be used for secondary research or have these specimens destroyed at the end of this trial. The participant's decision can be changed at any time by notifying the study doctors or nurses in writing. However, if the participant originally consents to the secondary use of **residual** specimens and subsequently changes their decision, any data from a previously collected specimen may still be used for secondary research.

9.4 Exclusion of Women, Minorities, and Children (Special Populations)

Special populations (e.g., pregnant or lactating women, non-English speakers, children, illiterate or non-writing individuals and vulnerable populations) will not be enrolled in this study as this is a Phase 1 first-in-humans study. A successful outcome of the current trial may lead to future evaluation of the study product which then may incorporate a broader participant population.

9.5 Participant Confidentiality

Participant confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality includes documentation, investigation data, participant's clinical information, and all other information generated during participation in the study. No information concerning the study nor the data generated from the study will be released to any unauthorized third party without prior written approval of the DMID and the participant. Participant confidentiality will be maintained when study results are published or discussed in conferences. The study monitors or other authorized representatives of the sponsor or governmental 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 records will be kept locked and all computer entry and networking programs will be carried out with coded numbers only and with password protected systems. All non-clinical specimens,

evaluation forms, reports, and other records that leave the site will be identified only by a coded number.

9.6 Certificate of Confidentiality

To protect privacy, this study is covered by a Certificate of Confidentiality. With this Certificate, the researchers cannot be forced to release information that may identify the research participant, even by a court subpoena, in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings. The researchers will use the Certificate to resist any demands for information that would identify the participant, except as explained below.

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 individual 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 is in compliance with applicable Federal regulations governing the protection of human subjects in research.

9.7 Costs, Participant Compensation, and Research Related Injuries

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. Participants may be compensated for their participation in this trial. Compensation will be in accordance with the local IRB's policies and procedures, and subject to IRB approval.

If it is determined by the site principal investigator that an injury occurred to a participant as a direct result of the tests or treatments that are done for this trial, then referrals to appropriate health care facilities will be provided to the participant. Study personnel will try to reduce,

control, and treat any complications from this trial. Immediate medical treatment may be provided by the participating site. No financial compensation will be provided to the participant by the NIH, NIAID, or the participating site for any injury suffered due to participation in this trial.

10 STATISTICAL CONSIDERATIONS

10.1 Study Hypotheses

This is a Phase I, dose-ranging clinical trial and is not designed to test a specific hypothesis. Rather, it is intended to obtain preliminary estimates in healthy adults of the safety and pharmacokinetics of single IV administrations of EV68-228-N.

10.2 Sample Size Considerations

As a Phase 1, first-in-human study, the number of participants was chosen principally on feasibility and using a 5:1 randomization scheme. Thus, three cohorts of 10 participants each (n=30) and 6 placebo recipients (total N=36) are proposed. With 10 participants in each active dose group, the chance of observing at least one AE of probability 20% or more is approximately 89.3% (see Table 3). Therefore, if no AEs of a given type occur in an active dose group, we can be relatively confident that they will occur in fewer than 20% of people once the treatment is implemented.

Table 3: Probability to observe at least n AEs within study population

Adverse Event Probability	Probability to Observe At Least n AEs									
	n=1	n=2	n=3	n=4	n=5	n=6	n=7	n=8	n=9	n=10
5%	40.1%	8.6%	1.2%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
10%	65.1%	26.4%	7.0%	1.3%	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%
15%	80.3%	45.6%	18.0%	5.0%	1.0%	0.1%	0.0%	0.0%	0.0%	0.0%
20%	89.3%	62.4%	32.2%	12.1%	3.3%	0.6%	0.1%	0.0%	0.0%	0.0%
25%	94.4%	75.6%	47.4%	22.4%	7.8%	2.0%	0.4%	0.0%	0.0%	0.0%
30%	97.2%	85.1%	61.7%	35.0%	15.0%	4.7%	1.1%	0.2%	0.0%	0.0%

PK parameters are not a primary outcome of the study, so it has not been powered for this, however with a sample size of 10 participants per dose level, assuming the pharmacokinetics are not dose proportional, a parameter with a coefficient of variation of 120% or less has a 90% chance to be observed within 80% and 120% of the geometric mean.

10.3 Treatment Assignment Procedures

Participants will be randomized to study intervention in a 5:1 ratio. The study will be double blind and study sites will administer product to a participant according to which study arm the participant has been assigned.

10.3.1 Randomization Procedures

The trial will be randomized. The randomization scheme will be generated by the SDCC and provided to unblinded study personnel (i.e., research pharmacists or other trained/delegated personnel performing study product preparations) at each participating site or subcontracted site.

The list of randomized treatment assignments will be prepared by statisticians at the Emmes Corporation and included in the enrollment module Internet Data Entry System (IDES) for the trial. IDES will assign each participant a treatment code after demographic and eligibility data have been entered into the system. A designated individual at the site will be provided with a treatment key, which links the treatment code to the actual treatment assigned, which will be kept in a secure place.

Instructions for use of the enrollment module will be included in the IDES User's Guide. Manual back-up randomization procedures and instructions are provided for use if the site temporarily loses network access or the online enrollment system is unavailable.

Per GCP, screening records will be kept at the study site to document the reason why an individual was screened but failed trial entry criteria. The reasons why individuals failed screening will be recorded in IDES.

10.3.2 Masking Procedures

This is a double-blind study. Participants, investigators, study personnel performing any study-related assessments following study product administration, and laboratory personnel will be blinded to treatment assignments.

The unblinded study product preparer is a study personnel member credentialed to prepare study product and will not be involved in study-related assessments or have participant contact for data collection.

The SMC may receive data in aggregate and presented by treatment arm and cohort. The SMC may request the treatment assignment be unblinded for an individual participant if required for safety assessment. In the open session the SMC may review data by participant group with all summaries presented in aggregate over the dose levels. In the closed session the SMC may review unblinded data.

10.4 Planned Interim Analyses

10.4.1 Interim Safety Review

Not applicable.

10.4.2 Interim Immunogenicity or Efficacy Review

There are no planned interim analyses for immunogenicity or pharmacokinetics.

10.5 Final Analysis Plan

The final analysis will be performed after the final data lock, and clinical study report (CSR) completed when all primary safety endpoint data and all secondary immunogenicity and pharmacokinetics endpoint data are available and received by the SDCC. A full statistical analysis plan (SAP) will be developed by the SDCC and finalized prior to the primary data lock.

Unless otherwise noted in the SAP, continuous variables will be summarized using the following descriptive statistics: n (non-missing sample size), mean, standard deviation, median, maximum and minimum. The frequency and percentages (based on the non-missing sample size) of observed levels will be reported for all categorical measures.

Summaries and analysis of safety data will be presented for the Safety Analysis Population defined as any participant who received all or part of the study product.

Solicited AEs will be collected from the end of each infusion through Day 3. Solicited AEs will be summarized by severity for each day post administration (Days 1-3) and as the maximum severity over all 3 days. Additionally, solicited AEs will be analyzed using standard techniques, such as exact confidence intervals (CI), to summarize the proportion of participants reporting each symptom.

Unsolicited AEs will be collected from the time of infusion through Day 29. Unsolicited AEs will be coded by MedDRA for preferred term and system organ class (SOC). SAEs, MAAEs and NOCMCs will be collected from the time of infusion through end of study. The numbers of SAEs, MAAEs, and NOCMCS will be reported by detailed listings showing the event description, MedDRA preferred term and SOC, relevant dates (product administration and AEs), severity, relatedness, and outcome for each event. Non-serious unsolicited AEs will be summarized as number and percentage of participants reporting at least one event in each MedDRA preferred term and SOC, cross tabulated by severity and relationship to study product. Additionally, the proportion of participants and exact 95% CIs of AEs in aggregate and by MedDRA categories will be computed.

Descriptive summaries of immunogenicity data will be presented for the modified intent-to-treat (mITT) population. The mITT population includes all participants who received at least a partial dose of study product and contributed both pre- and at least one post-dose venous blood sample for immunogenicity testing for which valid results were reported. If there are protocol deviations which may affect the analysis, a per-protocol analysis may also be performed.

In the final analysis, protocol deviations will be reviewed to determine which protocol deviations may affect the analysis. The per protocol 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 subsequent to the protocol deviations that are considered to affect the science.
- Data from any visit that occurs substantially out of window.

Geometric Mean Titers (GMT) of ADAs will be calculated, along with 95% CIs, for all groups, and dose levels at each timepoint. Summaries will also be displayed graphically. The proportion of participants with detectable ADAs and exact 95% CIs will also be computed. The proportion of participants developing ADAs will be evaluated over time to assess persistence of ADAs.

The PK analysis set will consist of participants in the safety population who have evaluable serum PK samples for the estimation of Cmax or AUC (0-∞) and received a full dose. This set will be used to assess the PK endpoints. Any participants or data values excluded from this analysis set will be identified, along with their reason for exclusion, in the CSR.

All PK parameters will be estimated through a non-compartmental analysis. The recorded true time points will be used for PK calculation. Estimated total serum PK parameters will include the following:

- $AUC_{(0-\infty)}$: Area under the concentration time-curve from time 0 extrapolated to infinity
- AUC(0-t): Area under the concentration time curve from time 0 to time t
- $AUC_{(0\text{-last})}$: Area under the concentration time-curve from time 0 to the last concentration above the lower limit of quantitation
- $AUC_{(0-48)}$: Area under the concentration time-curve from time 0 to 48hrs
- C_{max}: Maximum concentration
- T_{max}: Time of maximum concentration
- $t_{1/2}$: Apparent terminal elimination half-life
- CL: Total serum clearance
- V_z: Volume of Distribution during terminal phase

Calculated PK parameters will be summarized using standard summary statistics (such as mean, median, standard deviation, coefficient of variation, or range).

11 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

Each participating site will maintain appropriate medical and research records in compliance with ICH E6, Section 4.9 and regulatory and institutional requirements for the protection of confidentiality of participants. Each site will permit authorized representatives of DMID, its designees, and appropriate regulatory agencies to examine (and when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study safety and progress. These representatives will be permitted access to all source data and source documents, which include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, and participant files and records kept at the pharmacy, at the laboratories, and medico-technical departments involved in the clinical trial.

This study uses direct data entry for the participating clinic site and the eCRFs serve as the source documents for data collected.

Participants will use a web-based e-Memory Aid. Data entered by participants into the e-Memory Aid are stored in the data system for clinic staff review during scheduled visits. The e-Memory Aid is not considered source data. After clinic staff review and save the data, the data will be entered into Advantage eClinical as source.

12 QUALITY CONTROL AND QUALITY ASSURANCE

Following a written DMID-accepted site quality management plan, each participating site(s) and its subcontractors are responsible for conducting routine quality assurance (QA) and quality control (QC) activities to internally monitor study progress and protocol compliance. The site principal investigator will provide direct access to all study-related sites, source data/data collection forms, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities. The site principal investigator will ensure all study personnel are appropriately trained and applicable documentation is maintained on site.

The SDCC will implement quality control procedures beginning with the data entry system and generate data quality control checks that will be run on the database. Any missing data or data anomalies will be communicated to the participating site(s) for clarification and resolution.

13 DATA HANDLING AND RECORD KEEPING

13.1 Data Management Responsibilities

The site principal investigator is responsible for ensuring the accuracy, completeness, and timeliness of the data reported.

Data will be entered electronically over the Internet by site study staff into Advantage eClinical, developed and maintained by the SDCC. The eCRFs serve as the source documents for data collected. Paper case report forms derived from the eCRF are provided by the SDCC and are to be used only when Advantage eClinical is unavailable. Details on data handling procedures, procedures for data monitoring, and instructions for use of the system and completion of the eCRFs are provided in the study MOP, eCRF Instructions, and Advantage eClinical User's Guide.

The sponsor and/or its designee will provide guidance to the site principal investigators and other study personnel on making corrections to the eCRF.

13.2 Data Coordinating Center/Biostatistician Responsibilities

Data collection is the responsibility of the study personnel at the participating clinical study site under the supervision of the site principal investigator. During the study, the site principal investigator must maintain complete and accurate documentation for the study.

The data coordinating center for this study will be responsible for data management, quality review, analysis, and reporting of the study data.

13.3 Data Capture Methods

Clinical (including, but not limited to, AE/SAEs, concomitant medications, medical history, vital signs, ECG data, physical assessments, and clinical laboratory values) will be entered into eCRFs via a 21 CFR Part 11-compliant internet data entry system provided by the SDCC. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate.

Site staff who are delegated the responsibility by the site PI will be the data originators for clinical data entered directly into the eCRF. A list of all authorized data originators, including site staff, will be included on the Study Personnel/Signature Responsibility List.

13.4 Types of Data

Data for this trial will include clinical, safety, and outcome measures (e.g., clinical laboratory values, pharmacokinetic, and immunogenicity data).

13.5 Study Records Retention

Study records and reports including, but not limited to, eCRFs, source documents, ICFs, laboratory test results, and study drug disposition records will be retained for 2 years after a marketing application is approved for the study 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 the study product, until 2 years after the investigation is discontinued and the FDA has been notified. These documents will be retained for a longer period, however, if required by local regulations. ICFs for secondary use will be maintained as long as the sample/specimen exists.

No records will be destroyed without the written consent of the sponsor. It is the responsibility of the sponsor to inform the site principal investigators when these documents no longer need to be retained. The participating VTEU sites must contact DMID for authorization prior to the destruction of any study records.

14 CLINICAL MONITORING

Site monitoring is conducted to ensure that the human participants' protections, study and laboratory procedures, study intervention administration, and data collection processes are of high quality and meet sponsor, ICH/GCP guidelines and applicable regulations, and that this trial is conducted in accordance with the protocol, protocol-specific MOP and applicable sponsor standard operating procedures. DMID, the sponsoring agency, or its designee will conduct site-monitoring visits as detailed in the clinical monitoring plan.

Site visits will be made at standard intervals as defined by DMID and may be made more frequently as directed by DMID. Monitoring visits will include, but are not limited to, review of regulatory files, accountability records, eCRFs, informed consent forms, medical and laboratory reports, and protocol and GCP compliance. Site monitors will have access to each participating site, study personnel, and all study documentation according to the DMID-approved site monitoring plan. Study monitors will meet with site principal investigators to discuss any problems and actions to be taken and will document site visit findings and discussions.

15 PUBLICATION POLICY

Following completion of the study, the lead Principal Investigator is expected to publish the results of this research in a scientific journal. The results of all completed Infectious Diseases Clinical Research Consortium (IDCRC) trials should be published in peer-reviewed journals. The results of primary and secondary objectives should be made public. The publication section of the IDCRC MOP details the process and requirements for preparation and review of abstracts, manuscripts, executive summaries, and other documents through which study-related results are disseminated.

All investigators funded by the NIH must submit or have submitted for them to the National Library of Medicine's PubMed Central (http://www.ncbi.nlm.nih.gov/pmc/) an electronic version of their final, peer-reviewed manuscripts upon acceptance for publication, to be made publicly available no later than 12 months after the official date of publication. The NIH Public Access Policy ensures the public has access to the published results of NIH funded research. It requires investigators to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication. Further, the policy stipulates that these papers must be accessible to the public on PubMed Central no later than 12 months after publication.

Refer to:

- NIH Public Access Policy, http://publicaccess.nih.gov/
- NIH Office of Extramural Research (OER) Grants and Funding, http://grants.nih.gov/grants/oer.htm

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. A delay of up to 2 years is available for trials that meet certain criteria and have applied for certification of delayed posting.

As part of the result posting a copy of this protocol (and its amendments) and a copy of the Statistical Analysis Plan will be posted on ClincialTrials.gov.

For this trial the responsible party is DMID which will register the trial and post results.

The responsible party does not plan to request certification of delayed posting.

Refer to:

- Public Law 110-85, Section 801, Clinical Trial Databases

- 42CFR11
- NIH NOT-OD-16-149

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17 APPENDICES

Appendix A. Schedule of Activities

Study Visit	00	01	02	03	04	05	06	07	08	09	Unscheduled	Early Termination
Study day	-28 to –1	1	2	3	8	15	29	61	91	121		
Visit window (± number of days)					1	2	3	7	14	14		
Procedures												
Informed Consent	X											
Demographic Information	X											
Review Eligibility Criteria	X	X										
Medical History	X											
Concomitant Medications	X	X	X	X	X	X	X				X[a]	X[a]
Infusion		X										
Interim Medical History		X	X	X	X	X	X	X	X	X	X	X
Counsel on avoidance of pregnancy	X	X										
Full Physical Examination	X											
Focused Physical Examination		X	X	X	X						X[a]	X[a]
Symptom-driven Physical Examination						X	X	X	X	X	X	X

Vital Signs	X	X[b]	X	X	X	X	X				X[a]	X[a]
Oximetry Measurement		X[b]										
Cardiopulmonary Monitoring		X[c]										
Height and Weight (for BMI calculation)	X											
Weight (for dose calculation)		X										
Hematology[d]	X	X[e]	X	X	X		X					X[a]
Chemistry[d]	X	X[e]	X	X	X		X					X[a]
12-Lead ECG	X	X[f]	X									
Urine Pregnancy Test[g]	X	X										
Solicited AEs		Days 1-3										
Review e-Memory Aid			X	X	X							
Unsolicited AEs		Days 1-29										
SAEs, MAAEs, and NOCMCs		Days 1-121										
Update previous AE/SAEs (as needed)			X	X	X	X	X	X	X	X	X	X
PK samples [h]		X[i]	X[j]	X[j]	X	X	X	X	X	X		X
Serum samples for ADA (anti-drug antibodies)[h]		X[e]			X	X	X	X	X	X		X
Hypersensitivity Panel Sample[k]		X[k]										
Total amount of blood collected for visit (mL)	6	41 or 56[l]	11	11	16	10	16	10	10	10	0	16
Cumulative blood volume (ml) if there is no hypersensitivity reaction	6	47	58	69	85	95	111	121	131	141		
Cumulative blood volume (mL) if there is a hypersensitivity reaction	6	62	73	84	100	110	126	136	146	156		

a) Conducted if visit occurs within 7 days of infusion.

b) VS will be collected to confirm eligibility prior to randomization and will be repeated, along with oximetry measurements, at approximately 15 minutes prior to infusion, the end of infusion, and at 1-hour post-infusion. For infusions lasting at least 30 minutes, these will also be collected at approximately 15 minutes after the beginning of the infusion.
c) During infusion and up to one-hour post-infusion, continuous cardio-pulmonary monitoring will be conducted to assess for acute changes. If a CS change occurs, the monitoring will be continued until it has resolved and it is deemed safe to discontinue monitoring by the site PI or designee.
d) Clinical screening laboratory evaluations will include WBCs, Hgb, PLT, Cr, ALT, and T. Bili. Clinical safety laboratory evaluations obtained on Days 1, 2, 3, 8, and 29 will include WBCs, Hgb, PLTs, Cr, ALT, and T. Bili. Results of laboratory studies collected prior to infusion on Day 1 do not require review prior to infusion; rather, they serve as a baseline assessment for post-infusion safety assessment.
e) Day 1 blood draws for ADA, hematology and chemistry should be done at the same time as the first PK blood draw, within 15 minutes prior to the infusion
f) ECGs on Day 1 will occur approximately 15 minutes prior to infusion and 1-hour post-infusion.
gf) For women of childbearing potential, a urine pregnancy test at screening and on Day 1 with results confirmed as negative prior to infusion.
h) If an infusion is terminated early, the remaining Day 1 PK samples will not be drawn and the PK and ADA samples at follow-up visits will not be drawn.
i) PK samples on Day 1 will be obtained approximately 15 minutes prior to infusion, at end of infusion (within a + 5-minute window), and at 1-, 3-, and 5-hours post end of infusion, within a ± 15-minute window. Note, for infusions that last longer than 1-hour due to infusion related events or other disruptions in administration, see MOP for details on updating the PK collection timepoints.
j) PK sample will be collected within ±3-hour window of 24- and 48-hours after the end of infusion
k) All participants will have a baseline sample taken for hypersensitivity testing approximately 15 minutes prior to infusion. For any participant that has an anaphylactic or anaphylactoid-type reaction, three additional samples will be taken: 1) at the onset of symptoms, 2) at 2 or more hours after onset, 3) at the resolution of symptoms.
l) Larger amount will be collected only if there is a hypersensitivity reaction

Appendix B. Toxicity Tables

Protocol Assessment	Grade 1	Grade 2	Grade 3	Grade 4
Hematology				
Hemoglobin, Low (male) g/dL	12.0 - 13.0	10.0 - 11.9	8.0 - 9.9	<8.0
Hemoglobin, Low (female) g/dL	10.5 - 11.5	9.0 - 10.4	7.5 - 8.9	<7.5
WBC, Low mm^3	2,500 - 2,999	1,500 - 2,499	1,000 - 1,499	<1,000
WBC, High mm^3	11,600 - 15,000	15,001 - 20,000	20,001 - 25,000	>25,000
Platelets (Decrease) mm^3	125,000 - 139,000	100,000 - 124,000	25,000 - 99,000	<25,000
Chemistry				
ALT	2.0 - 3.5x ULN	>3.5 - 5.0x ULN	>5.0 - 10x ULN	>10x ULN
Creatinine	1.5 - 1.7x ULN	>1.7 - 2.0x ULN	>2.0 - 2.5x ULN	>2.5x ULN or requires dialysis
Bilirubin	1.5 - 2.0x ULN	>2.0 - 3.0x ULN	>3.0 - 5.0x ULN	>5.0x ULN
Vital Signs				
Hypertension (systolic) mmHg	141 - 150	151 - 155	>155	ER visit or hospitalization for management
Hypertension (diastolic) mmHg	91 - 95	96 - 100	>100	ER visit or hospitalization for management
Hypotension (systolic) mmHg	85-89	80-84	<80	ER visit or hospitalization for management
Bradycardia, baseline>60 bpm	50 - 54	45-49	<45	ER visit or hospitalization for management
Bradycardia, baseline <60 bpm	45-50	40-44	<40	ER visit or hospitalization for management
Tachycardia bpm	101 - 115	116 - 130	>130	ER visit or hospitalization for management
Temperature °C	38.0 - 38.4	38.5 - 38.9	39.0 - 40.0	>40