Clinical Trial Protocol

POX-MVA-031

A randomized, double-blind, multicenter Phase III trial to evaluate immunogenicity and safety of three consecutive production lots of a freeze-dried formulation of MVA-BN® smallpox vaccine in healthy, vaccinia-naïve subjects

Clinical Trial Protocol, Edition 3.0

22-Nov-2019

NCT 03699124

1 General Information

1.1 Investigator Signature Page

Herewith I agree that I have read and fully understand this protocol:

A randomized, double-blind, multicenter Phase III trial to evaluate immunogenicity and safety of three consecutive production lots of a freeze-dried formulation of MVA-BN smallpox vaccine in healthy, vaccinia-naive subjects.

This protocol describes the information necessary to conduct the trial. I agree that I will conduct the trial according to the instructions given within this protocol. Furthermore, I agree that I will conduct this trial according to International Conference of Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), Good Clinical Practice (GCP), the 2013 version of the Declaration of Helsinki, as well as applicable local legal and regulatory requirements in the respective countries. I agree that all information revealed in this protocol is handled strictly confidentially.

Additionally, I will permit trial related monitoring, audits, Institutional Review Board (IRB) / Independent Ethics Committee (IEC) review and regulatory inspections, providing direct access to source data/documents.

Investigator

(Date)	(Signature) [Name, Department]

1.2 Coordinating Investigator Signature Page

By signing the protocol:

A randomized, double-blind, multicenter Phase III trial to evaluate immunogenicity and safety of three consecutive production lots of a freeze-dried formulation of MVA-BN smallpox vaccine in healthy, vaccinia-naive subjects.

I agree, that the protocol was written according to international ethical and scientific quality standards (ICH-GCP), in compliance with the 2013 version of the Declaration of Helsinki and applicable local legal and regulatory requirements in the respective countries.

Coordinating Investigator

25 Nov 2019
(Date)

[redacted]
(Signature)

[redacted]

1.3 Sponsor Signature Page

By signing the protocol:

A randomized, double-blind, multicenter Phase III trial to evaluate immunogenicity and safety of three consecutive production lots of a freeze-dried formulation of MVA-BN smallpox vaccine in healthy, vaccinia-naive subjects,

the undersigning parties agree, that the protocol was written according to international ethical and scientific quality standards (ICH-GCP), in compliance with the 2013 version of the Declaration of Helsinki and applicable local legal and regulatory requirements in the respective countries.

Coordinating Author

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Vice President for Clinical Strategy

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1.4 Responsibilities

Trial Number	POX-MVA-031
Title	A randomized, double-blind, multicenter Phase III trial to evaluate immunogenicity and safety of three consecutive production lots of a freeze-dried formulation of MVA-BN smallpox vaccine in healthy, vaccinia-naive subjects
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List of Abbreviations

AD	Atopic Dermatitis
ADR	Adverse Drug Reaction
AE	Adverse Event
AESI	Adverse Event of Special Interest
ALT	Alanine Aminotransferase
ANC	Absolute Neutrophil Count
AST	Aspartate Aminotransferase
BARDA	Biomedical Advanced Research and Development Authority
BDS	Bulk Drug Substance
BLA	Biologics License Application
BN	Bavarian Nordic
CrCL	Creatinine Clearance
CRA	Clinical Research Associate
CRO	Contract Research Organization
CTS	Clinical Trial Site
CVA	Chorioallantois Vaccinia Virus Ankara
DSMB	Data Safety Monitoring Board
DTSG	Dextran Tris Sucrose Glutamate
ECG	Electrocardiogram
eCRF(s)	Electronic Case Report Form(s)
EDC	Electronic Data Capture
ELISA	Enzyme-linked Immunosorbent Assay
EU	European Union
FAS	Full Analysis Set
FD	Freeze-dried
FDA	United States Food and Drug Administration
FU	Follow-up
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
GMT	Geometric Mean Titer
HBV	Hepatitis B Virus
HCG	Human Choriogonadotropin
HCV	Hepatitis C Virus
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
IB	Investigator's Brochure
ICF	Informed Consent Form

ICH	International Conference of Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
IEC	Independent Ethical Committee
Inf.U	Infectious Units
IVRS	Interactive Voice Response System
IWRS	Interactive Web Response System
IM	Intramuscular
IMP	Investigational Medicinal Product
IRB	Institutional Review Board
LF	Liquid Frozen
LLOQ	Lower Limit of Quantitation
LV	Left Ventricular
MedDRA	Medical Dictionary for Regulatory Activities
MP	Medical Product
MPXV	Monkeypox Virus
MVA	Modified Vaccinia Ankara Strain
MVA-BN	Modified Vaccinia Ankara – Bavarian Nordic
NHP	Non-Human Primates
NIH	National Institutes of Health
NYCBH	New York City Board of Health
PEI	Paul Ehrlich Institut
PHI	Protected Health Information
PI	Principal Investigator
PPS	Per Protocol Set
PRNT	Plaque Reduction Neutralization Test
PV	Pharmacovigilance
SADR	Serious Adverse Drug Reaction
SAE	Serious Adverse Event
SC	Subcutaneous
SCR	Screening Visit
SD	Standard Deviation
$TCID_{50}$	Tissue Culture Infectious Dose 50%
ULN	Upper Limit of Normal
US(A)	United States (of America)
V	Visit
VRBPAC	Vaccines and Related Biological Products Advisory Committee
VV	Vaccinia Virus
VV-WR	Vaccinia Virus Western Reserve
WBC	White Blood Cell Count
WFI	Water for Injection
WHO	World Health Organization

WOCBP	Women of Childbearing Potential

1.5 Protocol Synopsis

Title	A randomized, double-blind, multicenter Phase III trial to evaluate immunogenicity and safety of three consecutive production lots of a freeze-dried formulation of MVA-BN smallpox vaccine in healthy, vaccinia-naive subjects
Clinical phase	Phase III
Sponsor	Bavarian Nordic A/S Hejreskovvej 10A DK-3490 Kvistgård, Denmark
Coordinating Investigator	[redacted]
Number of clinical trial sites and country/ies	Approximately 12 in the United States of America (USA)
Vaccination dose and schedule	Two vaccinations four weeks apart (at Day 1 and Day 29) each with 0.5 mL dose of vaccine containing at least 0.5 x 10^8 Infectious Units (Inf.U)
Route of administration	Each MVA-BN vaccine administration consists of one subcutaneous (SC) injection.
Trial duration	Up to 39 weeks for each subject.
Sample size	1110 (370 subjects per group).
Primary objective	To assess the consistency of three consecutively produced lots of a freeze-dried formulation of MVA-BN in terms of immunogenicity.
Secondary objectives	To assess uncommon adverse reactions. To collect vaccinia-specific humoral immune response data.

Primary endpoint	Geometric Mean Titers (GMTs) as measured by Plaque Reduction Neutralization Test (PRNT) 2 weeks following the second vaccination.

Secondary endpoints

Immunogenicity

- GMTs as measured by Enzyme-linked Immunosorbent Assay (ELISA) 2 weeks following the second vaccination.
- PRNT seroconversion rates 2 weeks following the second vaccination.
- ELISA seroconversion rates 2 weeks following the second vaccination.
- Pearson Correlation Coefficient between the $\log_{10}$ transformed PRNT titers and the $\log_{10}$ transformed ELISA titers 2 weeks following the second vaccination.

Safety and Reactogenicity

- Occurrence, relationship and intensity of any Serious Adverse Event (SAE) at any time during the trial.
- Occurrence, relationship and intensity of any cardiac sign or symptom indicating a case of myo-/pericarditis at any time during the trial.
- Occurrence of any Grade 3 or 4 adverse events (AEs) probably, possibly or definitely related to the trial vaccine within 28 days after each vaccination.
- Occurrence, relationship and intensity of unsolicited AEs within 28 days after each vaccination.
- Occurrence, intensity and duration of solicited local AEs (redness, swelling, induration, pruritus and pain) during the 8-day period (day of vaccination and the following 7 days) after each vaccination.
- Occurrence, relationship, intensity and duration of solicited general AEs (pyrexia, headache, myalgia, nausea, fatigue and chills) during the 8-day period (day of vaccination and the following 7 days) after each vaccination.

Trial design

Randomized, double-blind:

Group 1	370 subjects will receive two SC vaccinations with 0.5 mL MVA-BN Lot 1

Group 2	370 subjects will receive two SC vaccinations with 0.5 mL MVA-BN Lot 2
Group 3	370 subjects will receive two SC vaccinations with 0.5 mL MVA-BN Lot 3

Visit Schedule:

Visit (V)	Day	Target Week	Vaccination
SCR	Day -28 to - 1	-4	
V1	1	0	X
V2	V1 +12 - 16	2	
V3	V1 +28 - 35	4	X
V4	V3 +12 - 16	6	
V5	V3 +28 - 35	8	
FU Contact	V3 +182 - 210	32	

Subject entry criteria

Inclusion criteria

1. Healthy subjects, 18 to 45 years of age
2. The subject has read, signed and dated the informed consent form, having been advised of the risks and benefits of the trial in a language understood by the subject and prior to performance of any trial specific procedures
3. Body Mass Index ≥ 18.5 and < 35
4. Women of childbearing potential (WOCBP) must have used an acceptable method of contraception for 30 days prior to the first vaccination, must agree to use an acceptable method of contraception during the trial, and must avoid becoming pregnant for at least 28 days after the last vaccination. A woman is considered of childbearing potential unless post-menopausal (defined as ≥ 12 months without a menstrual period) or surgically sterilized. Acceptable contraception methods are restricted to abstinence, barrier contraceptives, intrauterine contraceptive devices or licensed hormonal products).

5. WOCBP must have a negative serum pregnancy test at screening (please note: a negative urine pregnancy test is required within 24 hours prior to each vaccination)
6. White blood cells ≥ 2500/mm^3 < ULN (Upper Limit of Normal)
7. Absolute neutrophil count (ANC) within normal limits
8. Hemoglobin within normal limits
9. Platelets within normal limits
10. Adequate renal function defined as a calculated Creatinine Clearance (CrCl) > 60 mL/min as estimated by the Cockcroft-Gault equation:
    - (140 – age in years) x (body weight in kg) ÷ (serum creatinine in mg/dL x 72) = CrCl (mL/min).
      For women the result, calculated with the above formula, has to be multiplied by 0.85 = CrCl (mL/min)
11. Adequate hepatic function defined as:
    - Total bilirubin ≤ 1.5 x ULN in the absence of other evidence of significant liver disease
    - Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase ≤ 1.5 x ULN
12. Electrocardiogram (ECG) without clinically significant findings (e.g. any kind of atrioventricular or intraventricular conditions or blocks such as complete left or right bundle branch block, AV node block, QTc or PR prolongation, premature atrial contractions or other atrial arrhythmia, sustained ventricular arrhythmia, two premature ventricular contractions in a row, ST elevation consistent with ischemia). For a more detailed guidance on interpretation of screening ECGs please refer to Appendix 3

Exclusion criteria

1. Typical vaccinia scar
2. Known or suspected history of smallpox vaccination
3. History of vaccination with any poxvirus-based vaccine
4. US Military service prior to 1991 or after January 2003
5. Pregnant or breast-feeding women

6. Uncontrolled serious infection, i.e. not responding to antimicrobial therapy
7. History of any serious medical condition, which in the opinion of the investigator would compromise the safety of the subject or would limit the subject's ability to complete the trial
8. History of or active autoimmune disease, persons with vitiligo or thyroid disease taking thyroid replacement are not excluded
9. Known or suspected impairment of immunologic function including, but not limited to, human immunodeficiency virus (HIV) Infection, clinically significant liver disease (including chronic active Hepatitis B Virus (HBV) or Hepatitis C Virus (HCV) infection), diabetes mellitus
10. History of malignancy other than squamous cell or basal cell skin cancer, unless there has been surgical excision that is considered to have achieved cure. Subjects with history of skin cancer must not be vaccinated at the previous tumor site.
11. History or clinical manifestation of clinically significant and severe hematological, pulmonary, central nervous, cardiovascular or gastrointestinal disorders
12. Clinically significant mental disorder not adequately controlled by medical treatment
13. History of coronary heart disease, myocardial infarction, angina pectoris, congestive heart failure, cardiomyopathy, stroke or transient ischemic attack, uncontrolled high blood pressure, or any other heart condition under the care of a doctor
14. Abnormal Troponin I level > ULN
15. Known history of an immediate family member (father, mother, brother, or sister) who has had onset of ischemic heart disease before age 50 years
16. Active or history of chronic alcohol abuse and/or intravenous and/or nasal drug abuse (within the past 6 months)
17. Known allergy to MVA-BN vaccine or any of its constituents, e.g. tris (hydroxymethyl)-amino methane, including known allergy to egg or aminoglycosides
18. History of anaphylaxis or severe allergic reaction to any vaccine

19. Acute disease (illness with or without a fever) at the time of enrollment
20. Body temperature ≥100.4°F (≥38.0°C) at the time of enrollment
21. Having received any vaccinations or planned vaccinations with a live vaccine within 30 days prior to or after trial vaccination
22. Having received any vaccinations or planned vaccinations with a killed/inactivated vaccine within 14 days prior to or after trial vaccination
23. Chronic systemic administration (defined as more than 14 days) of >5 mg prednisone (or equivalent)/day or any other immune-modifying drugs during a period starting from three months prior to administration of the vaccine and ending at last physical trial visit (Visit 5)
24. Post organ transplant subjects whether or not receiving chronic immunosuppressive therapy
25. Administration or planned administration of immunoglobulins and/or any blood products during a period starting from three months prior to administration of the vaccine and ending at last physical trial visit (Visit 5)
26. Use of any investigational or non-registered product within 30 days preceding the first dose of the trial vaccine, or planned administration of such a drug during the trial period
27. Trial personnel

1.6 Trial Procedure Schedule

Visit (V)	SCR	V1	V2	V3	V4	V5	FU Contact
Day / Visit +… D	**-28 - - 1**	**1**	**V1 +12-16**	**V1 +28-35**	**V3 +12-16**	**V3 +28-35**	**V3 +182-210**
Target week	**- 4**	**0**	**2**	**4**	**6**	**8**	**32**
Procedures							
Informed consent	X						
Inclusion/Exclusion criteria	X	X*					
Withdrawal criteria				X			
Medical History	X						
Smallpox vaccination/scar check	X						
Physical exam	X						
Vital signs	X	X	X	X	X	X	(X)[1]
Family cardiac risk factors	X						
Baseline signs and symptoms	X	X					
Targeted physical exam incl. auscultation of the heart and lung		X	X	X	X	X	(X)[1]
ECG[5]	X		(X)[2]		(X)[2]		
Prior/concomitant medications	X	X	X	X	X	X	
Pregnancy counseling for WOCBP[7]	X	X	X	X	X	X	
AE/SAE/AESI recording		X	X	X	X	X	X[3]
Laboratory Assessments							
Pregnancy test for WOCBP[4]	X	X		X		X	
Safety labs[5]	X		X		X		(X)[1]
Troponin I testing	X		(X)[2]		(X)[2]		(X)[2]
Antibody testing		X			X		
Vaccination							
Vaccine administration & subject observation (≥ 30 minutes)		X		X			
Immediate AEs		X		X			
Handout of memory aid		X		X			
Collection of memory aid			X		X		
Injection site exam			X		X		
Blood volume							
Appr. blood volume drawn (mL)[5, 6]	7	8.5	7	0	15.5	0	(7)[1]
Cumulative blood volume drawn (mL)[5]	7	15.5	22.5	22.5	38	38	(45)[1]

[1] If during the FU contact a serious condition is detected, the trial subject will be requested to return to the Clinical Trial Site (CTS) and the respective examinations will be performed.

[2] Only if clinically indicated, i.e. in the presence of cardiac signs or symptoms.

[3] New SAEs/AESIs and changes to SAEs/AESIs/AEs ongoing at V5 only.

[4] At Screening Visit (SCR), a serum test must be performed. At other visits, a urine pregnancy test will be performed.

[5] If clinically indicated, additional safety measures can be taken at any other trial visits or at unscheduled visits.

[6] Approximate amounts of single blood draws: Safety lab including all tests: 7 mL; antibody analysis: 8.5 mL

[7] Review of acceptable contraceptive methods and recent menstrual history with WOCBP.

(X) Only to be performed if clinically indicated.

[*] Once a subject has successfully met all eligibility requirements, then the subject will be randomized into the trial

2 Background Information and Scientific Rationale

2.1 Introduction

Despite the fact that the World Health Organization (WHO) officially declared successful global eradication of smallpox in 1980, the existence of variola stockpiles, the threat of bioterrorism and the risk of natural re-emergence of the virus means that the disease is still a threat and smallpox vaccine stockpiles are considered necessary as an effective countermeasure in case of an outbreak scenario. After the events of September 11th, 2001, concern over the use of bioweapons as agents of terrorism increased (McCurdy et al., 2004). The recently published *de novo* synthesis of horsepox may have direct implications for biosecurity by opening the door to potential synthesis of other orthopox viruses including variola and as such increasing the risk for reemergence of smallpox (Koblentz, 2017); (Noyce et al., 2018). As mass vaccination programs halted more than 40 years ago, it is estimated that the majority of the world population has no existing immunity to smallpox, and as such, the release of this highly contagious virus would have devastating effects. As a consequence, there is an urgent need for a safe and efficacious vaccine to protect the public against smallpox.

Bavarian Nordic A/S (BN), an international biopharmaceutical company, is developing a proprietary strain of Modified Vaccinia Ankara (MVA-BN) for use as a vaccine protecting against smallpox infection. A marketing authorization under exceptional circumstances in the European Union (EU) was granted in July 2013 under the name IMVANEX®. Authorization as an Emergency Use New Drug was granted by Health Canada in November 2013 under the name IMVAMUNE®. Authorization was granted by the US Food & Drug Administration in September 2019 under the name JYNNEOS® (Registered in the US and EU).

A liquid frozen (LF) formulation of MVA-BN has been stockpiled by the United States (US) government for potential use in HIV infected and atopic dermatitis (AD) subjects following a declared smallpox emergency. The current LF formulation offers several challenges for the US government, due to the requirement for a long-term stockpile of the vaccine as part of the national preparedness in case of a smallpox emergency. BN has therefore worked together with the US Department of Health and Human Services to develop, produce and supply a freeze-dried (FD) formulation of MVA-BN which is believed to offer many advantages in terms of life-cycle management and storage for stockpiling in a pre-outbreak situation and for potential use in a post-event scenario.

2.2 Traditional (Replicating) Smallpox Vaccines

The FDA has recently approved the anti-viral drug Tecovirimat for the treatment of smallpox. However, vaccination remains the best option for prevention of smallpox infection, as reflected by the continuous commitment of the US government to stockpile smallpox vaccines for the entire US population.

As part of national preparedness plans, the WHO and several nations have stockpiled smallpox vaccines as a countermeasure in case of accidental or deliberate release of variola. Most smallpox vaccines currently stockpiled by governments are derived from the historical vaccinia virus (VV) strains produced more than 40 years ago, e.g. Lister-Elstree strain recommended by the WHO and used primarily in Europe or the New York City Board of Health (NYCBH, Dryvax®) strain used in the United States. These vaccines were produced using outdated production methods (i.e. growing the virus on the skin of calves prior to harvest and lyophilization) and would not comply with today's current Good Manufacturing Practice (cGMP) requirements.

Traditionally, successful vaccination with a smallpox vaccine was assessed based on the formation of a vesicle ("take") at the inoculation site six to eight days after vaccination (Frey et al., 2002, Frey et al., 2007). Due to the replication of the VV and the formation of a virus containing pustule, replicating smallpox vaccines can cause severe and even fatal complications, particularly in individuals with immune deficiencies and skin disorders (CDC, 2001, Lane et al., 1969, Lane et al., 1970, Halsell et al., 2003). These rare but potentially life-threatening complications include:

- Inadvertent (auto-)inoculation of the vaccine recipient and/or their close contacts – spread of VV from the skin lesion to other parts of the body
- Eczema vaccinatum – spread of VV to areas of the body previously or presently afflicted by eczema
- Progressive Vaccinia – uncontrolled spread of VV to adjacent and underlying tissues resulting in tissue death
- Post-vaccinal encephalitis – spread of VV to the central nervous system
- Generalized Vaccinia – systemic spread of VV from the vaccination site
- Myo-/pericarditis – inflammation of the heart muscle or surrounding tissue

In the US Dryvax has been replaced by ACAM2000, a replicating smallpox vaccine based on the Dryvax NYCBH strain and manufactured in cell cultures according to cGMP standards (Monath et al., 2004). ACAM2000 was licensed following demonstration of non-inferiority compared to Dryvax based on the efficacy parameters of "take" rates 6 to 8 days after vaccination (in vaccinia-naïve subjects) or neutralizing antibody titers by PRNT (in vaccinia-experienced subjects). ACAM2000 is indicated for active immunization against smallpox disease for persons determined to be at risk for smallpox infection and is not commercially available to the public. The Prescribing Information for ACAM2000 (ACAM2000, 2018) issues a black box warning on the vaccine's risk profile and includes contraindications for persons including those with immune deficiencies (such as HIV infected individuals or people with AD) and cardiac disease, who are likely to be at a greater risk of developing adverse reactions which may result in severe disability, permanent neurological sequelae and/or death. Based on adverse event reporting in the Phase III clinical program, ACAM2000 has shown to be associated with an increased risk to develop myo-/pericarditis during the post vaccination period (5.73 events per thousand vaccinations in the

vaccinia-naïve population). Further prospective trials with ACAM2000 have confirmed the high incidence rate of myo-/pericarditis (Engler et al., 2015).

2.3 Non-replicating Smallpox Vaccines

MVA-BN is a non-replicating virus and has been developed as a safer alternative vaccine to the traditional replicating smallpox vaccines designed for use in the general population following either an accidental or deliberate release of variola, or as prophylactic vaccine in special populations, such as first line responders.

2.3.1 Origin and Characteristics of MVA-BN

VV is considered the best-known member of the poxvirus family and the prototype live viral smallpox vaccine. VV replicates in the cytoplasm of the host cell, its deoxyribonucleic acid does not integrate into the host cell genome and it is non-oncogenic.

Modified Vaccinia Ankara (MVA) was derived from the serial passage of chorioallantois vaccinia Ankara virus (CVA), a VV strain used during the smallpox eradication program. During this passaging, MVA underwent a multitude of mutations within its genome, including six major deletions, resulting in the loss of 15% (31 kbp) of original genetic information (Antoine et al., 1998). The deletions affected a number of virulence and host range genes (Antoine et al., 1998, Rosel et al., 1986, Meyer et al., 1991) and as a consequence, MVA exhibits a severely restricted host range in most mammalian cell types (Sutter and Moss, 1992, Carroll and Moss, 1997, Blanchard et al., 1998, Drexler et al., 1998). Although MVA exhibits a strongly attenuated replication in these cell types, its genes are efficiently transcribed with the block in viral replication being at the level of virus assembly and egress (Sutter and Moss, 1992, Carroll and Moss, 1997). Thus, a robust immune response is still induced despite the absence of viral replication.

MVA-BN has been derived from MVA-572 and is a highly attenuated, purified live vaccine produced under serum-free conditions in chicken embryo fibroblast cells. In contrast to replicating smallpox vaccines, such as Dryvax and ACAM2000, the non-replicating MVA-BN is not administered by scarification. The standard route and schedule of MVA-BN are two subcutaneous injections administered four weeks apart. Since the non-replicating MVA-BN does not induce a take (Mayr et al., 1975), BN has focused its strategy on comparability of immunological correlates of protection (neutralizing antibodies measured by PRNT and total antibodies measured by ELISA) that can be linked to clinical efficacy, as demonstrated for ACAM2000. During early preclinical and clinical development, two different formulations of MVA-BN have been investigated: an early FD formulation stabilized with Dextran, Tris, Sucrose and L-Glutamine (DTSG) and the LF formulation of MVA-BN. Although the early FD formulation of MVA-BN used a different Bulk Drug Substance (BDS) process (adherent CEF cells; sucrose cushion gradient purification; hereinafter referred to as early FD formulation of MVA-BN) to the LF formulation BDS using suspension cell cultures, purification by ultra- and

diafiltration; hereinafter referred to as LF formulation of MVA-BN), both formulations have shown to be highly comparable in terms of Chemical, Manufacturing and Controls and clinical safety and immunogenicity (Chaplin and Knappe, 2006).

In 2012, the manufacturing process of FD MVA-BN has been further developed on an industrial scale freeze-dryer using also DTSG as stabilizer as in the early development. The BDS used for the manufacture of FD MVA-BN is manufactured according to the same validated BDS process as used for the LF formulation. Comparability between this FD formulation and the LF formulation could be shown in several preclinical studies and in a clinical study.

This manufacturing process has been further optimized with a new state of the art filling line and freeze-dryer and led to a commercial and validated FD manufacturing process that is comparable to the industrial scale manufacturing process (Furness, 2017). The three consistently manufactured process performance qualification lots from the commercial process will be used in the present trial.

For further details on MVA-BN, please refer to the relevant sections in the Investigator's Brochure (IB).

2.4 Summary of Nonclinical Studies with MVA-BN

An extensive nonclinical development program has demonstrated the safety, efficacy and bio-equivalence of MVA-BN (both the LF and FD formulation) compared to other traditional smallpox vaccines.

The studies conducted demonstrated the superior attenuation profile of MVA-BN compared to conventional smallpox vaccines (e.g. ACAM2000, Dryvax) as well as to other MVA strains. In contrast to other strains, MVA-BN does not replicate in any of the human cell lines tested (Chaplin et al., 2002) and is not lethal for severely immune compromised animals (Suter et al., 2009). A bio-distribution study supports the in vitro finding that MVA-BN is replication deficient, since it only remained detectable for the first few days post administration.

Repeated administrations (SC or intramuscular (IM)) of MVA-BN at doses up to 4.9×10^8 Tissue Culture Infectious Dose 50% ($TCID_{50}$) resulted in injection site irritations and some lymphoid changes in Good Laboratory Practice (GLP) compliant toxicity studies in rats and rabbits; however, these effects were minimal and reversible and are therefore not considered to be dose-limiting.

Three developmental toxicity studies in rats and rabbits demonstrated that none of the tested doses of MVA-BN (1×10^7 $TCID_{50}$ or 1×10^8 $TCID_{50}$) were teratogenic or caused intrauterine toxicity to the fetuses. In a peri- and postnatal study in rats MVA-BN did not have any effect on the dams or the intrauterine development of the embryos. Furthermore, it did not have any effect on the lactating females or their developing offspring.

To assess pharmacodynamic properties, both the FD and LF formulations of MVA-BN have been assessed mainly in mice and non-human primates (NHP). Since smallpox was eradicated in 1980, the nonclinical testing strategy involved the demonstration of protective efficacy of MVA-BN in appropriate animal models in support of the clinical efficacy. Nonclinical studies on immunogenicity and efficacy demonstrated that MVA-BN smallpox vaccine induces a comparable immune response (antibody and T cells) as conventional smallpox vaccines (ACAM2000, Dryvax and Elstree) in both mice and NHP (Stittelaar et al., 2005). This immunogenicity translated into comparable protection of mice from lethal challenge with Vaccinia virus Western Reserve strain and Ectromelia virus, as well as protection of NHP from lethal challenge with monkeypox virus (MPXV).

Subsequently, immunogenicity and efficacy of the FD formulation (from the recently developed and validated process) was compared to MVA-BN LF formulation in GLP compliant mouse and NHP challenge studies: The FD formulation of MVA-BN was non-inferior to the LF formulation in terms of immunogenicity (total as well as neutralizing antibody responses) as well as protective efficacy in mice challenged with either Vaccinia virus Western Reserve strain or Ectromelia virus. Similarly, FD and LF MVA-BN induced comparable immune responses in NHP and equally fully protected NHP from an aerosol MPXV challenge or intravenously administered MPXV that causes severe disease or death in non-vaccinated animals.

For more detailed information on preclinical data please refer to the respective sections of the IB.

2.5 Clinical Profile of MVA-BN

BN has collected data of 22 completed clinical trials (16 sponsored by BN and 6 sponsored by the National Institutes of Health (NIH)) evaluating the safety and immunogenicity of MVA-BN in both healthy subjects and at-risk populations with contraindications to traditional smallpox vaccines; namely individuals diagnosed with AD or infected with HIV. More than 6500 subjects have been vaccinated with MVA-BN in 16 completed BN sponsored clinical trials and more than 1300 subjects have been vaccinated in 6 completed clinical trials sponsored and performed by the NIH. The trials were performed with both, the FD and the LF formulation of MVA-BN. An overview of the completed clinical trials is provided in Table 1.

Table 1: Overview of Clinical Trials with MVA-BN

Trial Identifier	Phase	Trial Sponsor	MVA-BN formulation	Reference
Dose finding clinical trials in healthy subjects				
POX-MVA-001	Phase I	BN	LF	(Vollmar et al., 2006)
POX-MVA-002	Phase I	US NIH	FD (pilot scale)	(Frey et al., 2007)

Trial Identifier	Phase	Trial Sponsor	MVA-BN formulation	Reference
POX-MVA-004	Phase II	BN	FD (early development)	(von Krempelhuber et al., 2010)
POX-MVA-009	Phase I/II	US NIH	LF	(Frey et al., 2013)
POX-MVA-036	Phase II	US NIH	FD (production scale)	(Jackson et al., 2017)
POX-MVA-028	Phase II	US NIH	LF	(Frey et al., 2014)
Clinical trial with direct comparison of MVA-BN to Dryvax				
POX-MVA-002	Phase I	US NIH	FD (pilot scale)	(Frey et al., 2007)
Clinical trials to evaluate the safety and immunogenicity of MVA-BN after primary and/or booster vaccinations in healthy subjects				
POX-MVA-005	Phase II	BN	LF	(Zitzmann-Roth et al., 2015)
POX-MVA-023	Phase II	BN	LF	Manuscript in preparation
POX-MVA-013	Phase III	BN	LF	(Overton et al., 2018)
Clinical trials to confirm the safety and immunogenicity in a variety of populations at increased risk or with contraindications to receive replicating smallpox vaccines				
POX-MVA-024	Phase II	BN	LF	(Greenberg et al., 2016)
POX-MVA-010	Phase I/II	BN	FD (pilot scale)	(Greenberg et al., 2013)
POX-MVA-011	Phase II	BN	LF	(Overton et al., 2015)
POX-MVA-007	Phase I	BN	FD (pilot scale)	(von Sonnenburg et al., 2014)
POX-MVA-008	Phase II	BN	LF	(Greenberg et al., 2015)
POX-MVA-030	Phase I	US NIH	LF	(Walsh et al., 2013)
POX-MVA-037	Phase II	BN	LF	
HIV-POL-002	Phase I/II	BN	LF	
HIV-NEF-004	Phase II	BN	FD (pilot scale)	
POX-MVA-03X	Special Access Program	BN	LF	

Trial Identifier	Phase	Trial Sponsor	MVA-BN formulation	Reference
Clinical trials to directly compare the FD to the LF formulation				
POX-MVA-029	Phase II	US NIH	FD (pilot scale) / LF	(Frey et al., 2015)
POX-MVA-027	Phase II	BN	FD (production scale) / LF	Manuscript in preparation
Clinical trial to demonstrate efficacy				
POX-MVA-006 (comparison to ACAM2000)	Phase III	BN	LF	(Pittman et al., 2019)

2.5.1 Safety Overview of MVA-BN

In all completed and ongoing clinical trials, vaccinations with MVA-BN have shown to be generally safe and well tolerated. No cases of death, assessed as being even possibly related, have been reported for a subject in a clinical trial using MVA-BN.

Serious Suspected Adverse Drug Reactions

A total of seven (7 out of 9784 vaccinated subjects = 0.07%) serious suspected Adverse Drug Reactions (ADRs) have been reported for MVA-BN so far (see Investigator Brochure, Ed. 23, Table 22, Section 7.2.10). All of them have been thoroughly reviewed by BN and the trial specific Data and Safety Monitoring Board (DSMB), who concluded that the continued use of MVA-BN in a clinical setting presented no increased risks to the subjects. BN assessed reactions as "possibly related" for which no medical cause or medical etiology is known to date and thus a relationship to the vaccine cannot be ruled out absolutely. No pattern regarding serious suspected ADRs has been identified.

Adverse Drug Reactions

Suspected ADRs, i.e. AEs for which there is a reasonable possible relationship to the trial vaccine, were reported in 22 completed clinical trials with MVA-BN to date.

For more detailed information on ADRs in the trials completed to date, and probability of occurrence please refer to the respective sections of the IB.

The majority of observed suspected ADRs represented local injection site reactions as well as common systemic reactions, typical for modern injectable vaccines and classified as being mild to moderate. More details on frequencies of suspected ADRs according to System of Organ Class and Preferred Term reported so far in completed MVA-BN clinical trials is provided in the current IB.

Cardiac Signs and Symptoms

Based on observations with first and second generation smallpox vaccines particular attention has been placed on monitoring for cardiac signs and symptoms in all recent clinical trials using MVA-BN.

Despite close cardiac monitoring, no signal of inflammatory cardiac disorders has been identified in the MVA-BN clinical development program. United States Food and Drug Administration (FDA) have agreed that routine post-vaccination ECG and Troponin I assessments may be omitted in the POX-MVA-031 clinical trial. However, should any subjects present with signs or symptoms potentially suggestive of cardiac adverse events, then evaluation should be performed according to the algorithm defined in section 8.2.7. Hence Adverse Events of Special Interest (AESI) (according to the definition in section 8.1.3.3) will be collected.

For further details about the cardiac safety profile of MVA-BN please refer to the IB.

2.5.2 Immunogenicity and Efficacy Overview of MVA-BN

Across all studies completed to date, MVA-BN has consistently demonstrated the ability to induce a fast and strong, vaccinia-specific immune response in all populations tested. This included healthy vaccinia-naïve and vaccinia-experienced populations (age range 18-80 years) as well as populations that are contraindicated to receive traditional smallpox vaccines, such as HIV infected, AD and previous HSCT patients (Frey et al., 2007, Frey et al., 2015, Frey et al., 2014, Frey et al., 2013, Greenberg et al., 2016, Greenberg et al., 2015, Greenberg et al., 2013, Walsh et al., 2013, von Sonnenburg et al., 2014, Overton et al., 2015, Zitzmann-Roth et al., 2015). The immunogenicity endpoints of these studies (seroconversion rates and GMTs by ELISA and PRNT) were based on historical (epidemiological and immunological) evidence that a measurable immune response against orthopox viruses is reliably correlated with the appearance of a take and/or protection and therefore likely to predict clinical benefit (Mack et al., 1972). The optimal dose of MVA-BN was selected based on results of the three dose ranging studies (Table

1) and is defined as 0.5 mL dose of vaccine containing at least 0.5 x 10^8 $TCID_{50}$ (nominal titer of 1 x 10^8 $TCID_{50}$) for subcutaneous administration (Vollmar et al., 2006, Frey et al., 2007, von Krempelhuber et al., 2010).

A Phase II trial (POX-MVA-027) comparing the LF and FD formulation confirmed the comparability between the 2 formulations with immune responses induced by the FD formulation being non-inferior to those induced by the LF formulation (see Table 1).

A pivotal efficacy Phase III trial (the POX-MVA-006) demonstrated non-inferiority of MVA-BN LF to ACAM2000 in terms of immunogenicity and efficacy. In this randomized, open label study in 440 volunteers, peak neutralizing antibodies induced by MVA-BN were shown to be 2-fold higher and more robust and durable than those stimulated by ACAM2000, which are considered protective. Efficacy parameters demonstrated that primary vaccination with MVA-BN resulted in a highly-attenuated take and prevented the vaccine take in the majority of subjects re-vaccinated with ACAM2000 (a historical measure of a protective immune response against smallpox) (see Table 1).

Additional detailed information on the clinical development of MVA-BN is provided in the IB.

2.6 Rationale

2.6.1 Rationale for Trial

Bavarian Nordic is developing MVA-BN as a safer smallpox vaccine suitable for the prevention of orthopox virus infections and protection against smallpox disease in the general population including those that are contraindicated for a traditional live replicating vaccine. During clinical development of MVA-BN, a LF and a FD formulation have been investigated in healthy and at-risk populations, including HIV infected subjects and people diagnosed with AD. Based on the available clinical data both formulations have shown to be safe and well tolerated and proved to induce comparable humoral immune responses in two non-inferiority trials.

While a LF product, which can be more rapidly manufactured, is advantageous during a smallpox outbreak situation, the FD formulation offers advantages in terms of virus titer stability. Guidelines for Industry published by the US FDA and the US Department of Health and Human Services for vaccines for prevention of infectious diseases (e.g. influenza) request lot consistency trials as part of the pivotal Phase III clinical development. These trials are designed to confirm that there are no significant differences in the biological attributes of various production lots and to bridge potency parameters to clinical immunogenicity.

The challenges imposed by the LF formulation for long-term storage of the vaccine led to the development of a freeze dried (FD) MVA-BN formulation. Two clinical trials were carried out to demonstrate the non-inferiority of immune responses induced by the FD formulation compared to the LF formulation (Table 1). The safety data collected in these trials confirmed the

comparability between both formulations, demonstrating that the FD formulation exhibits a similar safety profile to the LF formulation employed throughout the clinical development program of MVA-BN. For more detailed information on safety data please refer to the respective sections of the IB.

2.6.2 Rationale for Data Collected

Neutralizing antibodies are considered the gold standard for predicting protection against smallpox infection and disease and was one of the clinical endpoints accepted by the FDA for approval of the replicating smallpox vaccine ACAM2000. Likewise, non-inferiority of MVA-BN to ACAM2000 in terms of neutralizing antibodies (measured by PRNT) formed the basis for licensure of MVA-BN in the US, as agreed with the FDA (Phase III trial POX-MVA-006) (Table 1). Neutralizing antibody titers measured two weeks after the second MVA-BN vaccination (i.e. at the time of the expected peak antibody response) are therefore considered the most suitable measure to demonstrate lot consistency of consecutively produced MVA-BN lots. During clinical development of MVA-BN, lot consistency for the LF formulation was already demonstrated in the pivotal Phase III trial POX-MVA-013. A Biologics License Application (BLA) for the licensure of the LF formulation of MVA-BN was approved in September 2019 based on available data. Clinical trial POX-MVA-031 will generate the lot consistency data for the FD formulation; this data is planned for submission to FDA to apply for a license supplement to include the FD formulation in the MVA-BN LF BLA. The primary objective of POX-MVA-031 to assess consistency of three FD MVA-BN lots, will be established by measuring PRNT GMTs in vaccinia-naïve subjects receiving the standard regime (2 vaccinations of at least 0.5×10^8 Inf.U at Week 0 and 4 subcutaneously) two weeks following the second vaccination. Since preclinical data have indicated that there is not a single correlate of protection against lethal challenges with orthopox viruses, ELISA GMTs will be measured as a secondary endpoint. Additional humoral immunogenicity secondary endpoints include seroconversion rates by PRNT and ELISA, and a correlation analysis between the ELISA and PRNT antibody titers two weeks after the second vaccination.

In addition, POX-MVA-031 is designed to collect safety data in 1110 healthy, vaccinia-naïve individuals and therefore to expand the safety data set of MVA-BN FD obtained from previous clinical trials. Close monitoring of clinical and laboratory parameters will allow collection of a robust safety data set to confirm and support the so far excellent safety profile of MVA-BN FD.

2.7 Trial Population

Women and men aged 18 to 45 years who meet all the inclusion criteria and none of the exclusion criteria will be recruited for enrollment into this trial.

2.8 Risk/Benefit Assessment

2.8.1 Potential Risks

Blood drawing may cause local discomfort or bleeding under the skin from needle sticks, resulting in a localized hematoma that generally resolves within 1 to 2 weeks. A rare complication may be localized infection at the phlebotomy site or thrombophlebitis (swelling of a vein caused by a blood clot). This would be treated as medically indicated. Some subjects may feel lightheaded, queasy, or nauseated; have chills; develop a fast heartbeat; and/or faint during blood collection. These symptoms can be resolved by having the subject lie down and/or by stopping the procedure. Only qualified personnel will draw blood.

Preclinical data with MVA-BN in rats and rabbits have revealed no special hazard for humans based on conventional studies of safety.

Based on the present clinical experience with MVA-BN and MVA-based vaccines, adverse reactions to MVA-BN in this trial setting are expected to be comparable to adverse reactions previously reported for MVA-BN and/or those typically seen with other modern vaccines. Main risks involve the development of local reactions at the vaccination site, e.g. erythema, pain swelling and induration.

As with all injectable vaccines, there is a risk of an allergic reaction or an anaphylactic event. Clinical trial site staff will observe subjects for at least 30 minutes after each vaccination to identify any severe allergic reactions. In addition, appropriate medical treatment and supervision will be readily available.

The severe and life-threatening adverse reactions such as progressive vaccinia, eczema vaccinatum, generalized vaccinia and inadvertent inoculation that have been observed after the administration of conventional smallpox vaccines are due to the replication of the vaccinia strains. MVA-BN is replication incompetent in mammalian cells and consequently has a better safety and tolerability profile. Because it is replication incompetent, MVA-BN cannot induce the severe side effects listed above associated with replication competent vaccinia viruses. Apart from the better safety profile with regard to severe reactions, the available clinical experience with MVA-BN shows that it is generally better tolerated, for example with regard to local reactions, than conventional smallpox vaccines.

Since this trial enrolls only healthy subjects with no specific underlying disease(s), no subsequent treatment is necessary. Also, no consequent diseases are expected.

2.8.2 Benefits

Trial participants will contribute significantly to the development of a safer smallpox vaccine, which is a benefit to society in view of a potential threat following re-emergence or deliberate

release of smallpox virus. Based on the current immunogenicity and efficacy data collected for MVA-BN in preclinical and clinical studies, trial participants are expected to acquire protection against smallpox infection.

Future analysis of the samples collected will not directly benefit the subject. BN may learn more about smallpox and other diseases.

3 Objectives

Please refer to trial protocol synopsis (see section 1.5).

4 Trial Design

4.1 Experimental Design

This trial is a randomized, double-blind, multicenter Phase III trial to evaluate immunogenicity and safety of three consecutive production lots of a freeze-dried formulation of the MVA-BN smallpox vaccine in healthy, vaccinia-naïve subjects.

In total, approximately 1110 vaccinia-naïve subjects are to be enrolled in this trial. All subjects will be randomly assigned (1:1:1) to one of three MVA-BN groups (Groups 1-3) to receive two vaccinations each with FD MVA-BN administered in a double-blind manner.

Group 1: 370 subjects will receive two SC vaccinations with 0.5 mL FD MVA-BN Lot 1
Group 2: 370 subjects will receive two SC vaccinations with 0.5 mL FD MVA-BN Lot 2
Group 3: 370 subjects will receive two SC vaccinations with 0.5 mL FD MVA-BN Lot 3

4.2 Description of Trial Procedures

The trial procedures will be conducted according to section 1.6 and as described in this chapter. Visits should be scheduled within the given intervals / visit windows.

4.2.1 Screening Phase

Screening Visit (Day -28 to -1)

All subjects must be thoroughly informed of all aspects of the trial (e.g. trial visit schedule, required evaluations and procedures, risks and benefits) as described in the informed consent form (ICF). The ICF must be reviewed with the subject and signed and dated by the subject and the investigator or person designated by the investigator who conducted the informed consent discussion prior to the initiation of any evaluations or procedures required by the protocol.

After informed consent has been collected, subjects will enter a screening period of up to 28 days before the first vaccination.

Screening Visit (Days -28 to -1)

The following tasks will be performed:
• Subject to read, sign and date ICF
• Check vaccination history (previous smallpox vaccination or vaccination with a pox-virus-based vaccine) and absence of smallpox vaccine scar
• Check for all inclusion/exclusion criteria
• Obtain medical history and prior/concomitant medications
• Complete physical examination including auscultation of heart and lungs and measurement of body weight and height
• Evaluation of vital signs
• Evaluation of family cardiac risk factors
• Perform baseline ECG
• Counseling on avoidance of pregnancy: Review of acceptable contraceptive methods and recent menstrual history with WOCBP
• Blood draw for safety laboratory (7 mL) including
  o serum pregnancy test (WOCBP only)
  o troponin I
• Recording of baseline signs and symptoms

There may be situations where a subject is screened but cannot be randomized due to certain transient conditions (e.g. abnormal lab value due to an acute condition or a missing lab evaluation due to mishandling of the sample). If this occurs, the investigator may choose to "partially" re-screen the subject. This means the test in question may be repeated within the original 28-day screening window. A maximum of two "partial" re-screen visits will be allowed per investigator discretion. The relevant forms in the electronic case report form(s) eCRF will need to be completed.

In the event a subject is screened but cannot be randomized within the 28-day screening window due to other circumstances (e.g. completion of a wash-out period for a medication or vaccine not allowed during the trial), then the investigator may choose to "fully" re-screen a subject. This means the subject will be assigned a new subject number, a new set of eCRFs will be completed, and the 28-day screening window is reset once the subject starts the re-screening visit. Each subject will be allowed one "full" re-screen visit. The initial eCRF record will reflect the subject is a screen failure, and both subject numbers will be linked for tracking purposes.

4.2.2 Active Trial Phase

After successfully completing the screening assessments the eligible subject will enter the active trial phase (Visit 1 to Visit 5) starting with Visit 1.

Randomized treatment assignments for the subjects will be done at Visit 1 after re-confirmation of subject's eligibility. The randomization scheme is 1:1:1 (three FD MVA-BN production lot groups). Randomization will be stratified by Clinical Trial Site (CTS). An automated randomization system will be used. The detailed process will be described in a trial specific document.

The procedures performed at Visit 1 and all following visits are listed below. **Blood draws and all other examinations listed above the vaccination events must always be performed prior to vaccination.**

At Visit 1, subjects will receive the first of two SC vaccinations with one standard dose of MVA-BN (0.5 mL vaccine containing a nominal titer of 1 x 10^8 Inf.U) in the non-dominant upper arm (deltoid region).

Following vaccination, subjects will be kept under close observation by the CTS staff for at least 30 minutes with appropriate medical treatment readily available in case of an unexpected anaphylactic reaction following administration of the vaccine. Any AEs that occur during or after vaccination will be recorded.

Reactogenicity will be collected on a subject memory aid by having the subject record daily maximum temperatures and solicited AEs for an 8-day period (Days 1-8), beginning with the day of vaccination. The memory aid will be returned to the clinic staff at the following visit. If symptoms persist at Day 7, daily symptoms and temperature will continue to be measured each day until resolved and the last day of symptoms and maximum intensity is recorded on the memory aid. AEs will be assessed at all active trial phase visits (Visit 1 to Visit 5).

Visit 1 (Day 1)
Task to be performed prior to randomization and vaccination: • (Re-) Check of inclusion / exclusion criteria • Targeted physical examination including auscultation of the heart and lungs • Evaluation of vital signs • Recording of concomitant medications • Recording of baseline signs and symptoms • Counseling on avoidance of becoming pregnant: Review of acceptable contraceptive methods and recent menstrual history with WOCBP • Urine pregnancy test (WOCBP only) • Blood draw (serum collection, 8.5 mL) for baseline antibody testing **Temporary deferral of vaccination**: If an acute illness is present, the subject may be vaccinated at a later date within the accepted time window. The vaccine can be administered to persons with a minor illness such as diarrhea, mild upper respiratory infection, or any other mild condition with or without low-grade febrile illness, i.e. oral temperature < 100.4°F (< 38.0°C). **Blood draw and all tasks mentioned above must <u>always</u> be performed prior to vaccination. If the subject is still eligible for participation in this trial the subject will be randomized. The following tasks will be performed after randomization:** • Administration of first trial vaccination (FD MVA-BN SC) • Handout of memory aid, ruler and thermometer • Subject observation by CTS staff for at least 30 minutes after vaccination • Recording of immediate AEs/ Adverse Events of Special Interest (AESIs)/SAEs

Visit 2 (Visit 1 + 12-16 days)
The following tasks will be performed: • Targeted physical examination including auscultation of the heart and lungs • Evaluation of vital signs • Examination of the injection site • Counseling on avoidance of becoming pregnant: Review of acceptable contraceptive methods and recent menstrual history with WOCBP • Collection of the memory aid handed out at Visit 1, review with subject • Blood draw for safety laboratory (7 mL) • Troponin I testing (optional) if clinically indicated • Recording of AEs/SAEs/AESI and concomitant medication • ECG (optional) if clinically indicated

Visit 3 (Visit 1 + 28-35 days)
Tasks to be performed prior to vaccination: • Check withdrawal criteria • Targeted physical examination including auscultation of the heart and lungs • Evaluation of vital signs • Counseling on avoidance of becoming pregnant: Review of acceptable contraceptive methods and recent menstrual history with WOCBP • Urine pregnancy test (WOCBP only) • Recording of AEs/SAEs/AESIs and concomitant medications **Temporary deferral of second vaccination**: If an acute illness is present, the subject may be vaccinated at a later date within the accepted time window. The vaccine can be administered to persons with a minor illness such as diarrhea, mild upper respiratory infection, or any other mild condition with or without low-grade febrile illness, i.e. oral temperature < 100.4°F (< 38.0°C). **All tasks mentioned above must <u>always</u> be performed prior to vaccination.** **The following tasks will be performed after vaccination:** • Administration of second trial vaccination (FD MVA-BN SC). • Handout of memory aid • Subject observation by CTS staff for at least 30 minutes after vaccination • Recording of immediate AEs/AESIs/SAEs

Visit 4 (Visit 3 + 12-16 days)
The following tasks will be performed: • Targeted physical examination including auscultation of the heart and lungs • Evaluation of vital signs • Examination of the injection site • Counseling on avoidance of becoming pregnant: Review of acceptable contraceptive methods and recent menstrual history with WOCBP • Blood draw for safety laboratory (7 mL) • Troponin I testing (optional) if clinically indicated • Blood draw (serum collection, 8.5 mL) for antibody testing • Recording of AEs/SAEs/AESIs and concomitant medications • Collection of the Memory aid handed out at Visit 3, review with subject • ECG (optional) if clinically indicated

Visit 5 (Visit 3 + 28-35 days)
• Targeted physical examination including auscultation of the heart and lungs • Evaluation of vital signs • Counseling on avoidance of becoming pregnant: Review of acceptable contraceptive methods and recent menstrual history with WOCBP • Urine pregnancy test (WOCBP only) • Recording of AEs/SAEs/AESIs and concomitant medications

4.2.3 Follow-Up (FU) Phase

To monitor long-term safety, the CTS will contact the subject by phone, email, or text to inquire whether an SAE /AESI might have occurred since the last trial visit and if there is any new information on SAEs/AESIs/AEs ongoing at last trial visit. In cases where a potential relevant condition is detected, the trial subject will be requested to return for a physical examination and further work-up at the CTS.

For subjects who were withdrawn from the 2nd vaccination, the FU contact will be performed 6 months after the 1st vaccination (see section 4.2.5. withdrawal from 2nd vaccination).

FU Contact (Visit 3 + 182-210 days)
• Recording of new SAEs/AESIs and follow-up on ongoing SAEs/AESIs/AEs **If a physical visit at the CTS is deemed necessary, the following should be performed:** • Targeted physical examination including auscultation of the heart and lungs • Evaluation of vital signs • Blood draw for safety laboratory (7 mL), if required • Other safety evaluations, if required

4.2.4 Unscheduled Visits

If clinically indicated, additional visits may be necessary between scheduled visits. Unscheduled visits may be scheduled to repeat laboratory testing or physical exams due to a new development. Examinations performed at unscheduled visits will be documented in the source documents as well as on the respective eCRF pages for unscheduled visits.

4.2.5 Withdrawal from Second Vaccination

The decision not to administer the second vaccination can be made by the investigator or by the subject.

Criteria:

The following criteria should be checked prior to second vaccination.
If any are applicable, the subject should not receive the second vaccination:

- Any clinically significant cardiac sign and symptom (i.e. AESI) defined in section 8.1.3.3.
- An AE that, in the opinion of the investigator, makes it unsafe for the subject to receive the second vaccination. In this case, the appropriate measures will be taken.
- Anaphylactic reaction following the administration of any vaccine(s).
- Administration of a licensed vaccine not foreseen by the clinical trial protocol.
- Start of chronic administration (defined as more than 14 days) of > 5 mg prednisone (or equivalent) per day or any other immune-modifying drugs.
- Administration of immunoglobulins and/or any blood products.
- Clinical need for concomitant or ancillary therapy not permitted in the trial.
- Use of any investigational or non-registered drug or vaccine other than the trial vaccine.

- Any condition which contradicts administration of the second vaccination in the opinion of the investigator.
- Pregnancy.
- Subject refuses to receive second vaccination.

Procedure:

If the subject did not receive the second trial vaccination the reason for this decision must be recorded in the eCRF and in the subject's medical record. Visit 3 and Visit 4 are not required; and the procedures below should be followed:

- If Visit 3 has not already occurred, then Visit 5 procedures should be performed within 28 to 35 days after Visit 1.
- FU contact should be performed 182 to 210 days after Visit 1.

4.2.6 Premature Discontinuation

The trial may be discontinued prematurely for a subject at any time. The decision to discontinue the trial for a subject prematurely can be made by the investigator as well as by the subject himself. Reasons for discontinuing the trial prematurely may include, but are not limited to the following:

Criteria:

- Subject's request to discontinue prematurely (withdrawal of informed consent).
- Subject unwilling or unable to comply with trial requirements.
- Any reason that, in the opinion of the investigator, precludes the subject's further participation in the trial.
- Discontinuation due to an AE.

Procedure:

If a subject discontinues prematurely, the reason for this decision must be recorded in the eCRF and in the subject's medical record. If the subject is unable or not willing to attend all planned visits, every attempt should be made to perform at least a concluding safety visit. For WOCBP a pregnancy test should be performed during this safety visit. If the subject is not willing to undergo any further trial procedure (withdrawal of consent), "withdrawal of consent" needs to be documented as reason for premature discontinuation.

4.2.7 Emergency Unblinding

In case of emergency which makes unblinding necessary (i.e. the subject's safety is dependent on unblinding) a mechanism that permits rapid unblinding but does not permit undetectable breaks of the blinding will allow the investigator the possibility of learning the treatment assignment for a subject. When emergency unblinding is performed via electronic systems such as Interactive Voice Response System (IVRS) and Interactive Web Response System (IWRS) a backup system enabling unblinding of treatment will be provided.

If the blind is prematurely broken, it is the responsibility of the investigator to promptly document and explain any unblinding to the sponsor.

The detailed process for emergency unblinding will be described in a trial specific procedure.

4.3 Trial Duration

The total duration of the trial for each subject including the screening period and FU contact will be up to 39 weeks. The duration of the trial as a whole is dependent on the recruitment period.

4.4 Data Safety Monitoring Board

The DSMB is an independent board that oversees the safety of subjects participating in the trial. The members of the DSMB are independent, i.e. not involved as investigators in any MVA-BN trials and have no direct or indirect financial interests in BN or the contract research organization (CRO) managing the trial. The primary responsibilities of the DSMB are to periodically review and evaluate the accumulated trial data for participant safety, trial conduct and progress, and make recommendations to BN and the Coordinating Investigator and Principal Investigators (PIs) concerning the continuation, modification, or termination of the trial. The DSMB considers trial specific data as well as relevant background knowledge about the disease, test agent, and subject population under trial. DSMB meetings may consist of open sessions (blinded and unblinded participants), closed sessions (unblinded participants only) and executive sessions (DSMB members only). A separate charter describes in detail relevant operational procedures, communication pathways, roles and responsibilities of the DSMB.

If an event occurs which fulfils the trial halting rules the DSMB will review the event in a timely manner and give a recommendation to BN and the Coordinating Investigator and PIs to halt, resume or terminate the trial participation of the affected subject and/or the trial as a whole.

4.5 Trial Halting Rules

A temporary halting or termination for the trial as a whole can be decided in case of an occurrence of:

- an SAE with an at least reasonable possibility of a causal relationship to the administration of MVA-BN
- an unexpected Grade 3 or higher systemic reaction or lab toxicity (Appendix 1) with an at least reasonable possibility of a causal relationship to the administration of MVA-BN
- These parameters are not all-inclusive. Other AEs could occur that would trigger a DSMB review. Any member of the DSMB, the PI and/or the BN Medical Monitor could request a DSMB review based on any observation.

If an event fulfilling the trial halting criteria reaches the investigator's attention, the investigator has the liability to alert the responsible Pharmacovigilance (PV) Department immediately (within 24 hours) and provide a comprehensive documentation of the event. Contact details of the responsible PV Department are provided in section 8.3.1.

5 Selection of Subjects

Each investigator will keep a log of subjects screened for the trial and provide the reason in case of exclusion. Information about every subject entering the trial will be collected in CTS source documentation and the eCRF and will be provided for review to BN and its designees, regulatory agencies, and IRBs.

Recruitment Procedure

Subjects will be recruited actively. Recruitment strategies, including IRB approved paid advertisements, will be evaluated by the sponsor.

It is planned to randomize one thousand, one hundred and ten (1110) subjects. After signing the ICF, subjects undergo screening procedures to check eligibility according to the inclusion/exclusion criteria. In the event of a screening failure due to mild or limited acute illness or abnormal laboratory values, the subject may be re-screened after resolution of the event. Re-screening may require only an additional blood draw or a complete re-screening evaluation, depending on the circumstances of and the time interval since the initial screening failure. See also section 4.2.1.

5.1 Inclusion Criteria

Please refer to trial protocol synopsis (see section1.5.)

5.2 Exclusion Criteria

Please refer to trial protocol synopsis (see section 1.5.)

6 Investigational Product

MVA-BN is a highly attenuated live VV. It will be provided in a freeze-dried formulation. The vaccine is filled with a nominal virus titer of 1 x 10^8 Inf.U/0.5 mL dose. One standard dose (0.5 mL) of the MVA-BN reconstituted smallpox vaccine contains at least 0.5 x 10^8 Inf.U/0.5 mL dose throughout its shelf life. MVA-BN will be administered SC.

In 2017, the MVA-BN potency assay changed from a $TCID_{50}$ based assay to a flow cytometry based assay, and the units changed from $TCID_{50}$ to Inf. It should be emphasized that although the method of the potency assay changed from $TCID_{50}$ to flow cytometry and the unit changed from $TCID_{50}$ to Inf.U, the conversion factor was 1:1, therefore the established limits and specifications remain unchanged. FD MVA-BN will be reconstituted with 0.65 mL Water for Injection (WFI) prior to use. For further details see current version of the IB and the current WFI Product Specifications Document.

6.1 Production, Packaging and Labeling

MVA-BN:
The bulk drug substance MVA-BN is produced at Bavarian Nordic A/S and the final drug product MVA-BN is filled and labeled at the contract manufacturer IDT Biologika GmbH.

Addresses:

Bavarian Nordic A/S
Hejreskovvej 10A
3490 Kvistgård, Denmark
Phone: +45 3326 8383

IDT Biologika GmbH
Am Pharmapark
06861 Dessau-Rosslau, Germany
Phone: +49 34901 885 0

All packages and vials are labeled with the respective label required by the regulatory authorities of the countries in which the trial will be performed.

6.2 Shipment, Storage and Handling

MVA-BN is packaged in an open-labeled manner. The vaccine will be shipped temperature controlled from a warehouse to the CTS. The package is handed over to the unblinded personnel in charge of vaccine preparation, e.g. the pharmacist.

At the CTS, only the unblinded personnel have access to the information which of the three MVA-BN lots is allocated to a subject. They are not allowed to disclose this information.

After receipt of vaccine, the unblinded personnel are responsible for proper storage.

The FD MVA-BN has to be stored at -13°F to + 5°F (-25°C to -15°C) and avoiding direct light. Additional details regarding shipment, storage, and handling are provided in a separate pharmacy manual.

6.3 Preparation, Administration and Dosage

The preparation of the vaccine will be performed by unblinded personnel only. The unblinded personnel must not be involved in the trial treatment and/or the evaluation of the trial subjects.

Detailed trial-specific instructions on the preparation and administration of MVA-BN are provided in a separate pharmacy manual supplied to each clinical trial site.

6.4 Accountability and Disposal

Used (if allowed by institutional policy) and unused vials of all investigational medicinal product (IMP) need to be retained in a place with limited access until appropriate drug accountability has been performed. Drug accountability must be documented whenever the IMP is either prepared or administered.

BN will provide a Drug Accountability Log for recording receipt, dispensation, and destruction of IMP (see Pharmacy Manual). Alternative systems used to track drug accountability are acceptable for use in the trial provided the aforementioned items are adequately captured and records are available for review during scheduled monitoring visits to the site.

After drug accountability has been performed, used and unused vials should either be returned to BN, to the designated drug depot, or discarded according to local regulations.

Destruction or return of IMP must be agreed upon with BN and appropriately documented. Documentation should be reviewed and signed off by the pharmacist and Clinical Research Associate (CRA) assigned to monitor the site.

Sites are responsible for the proper destruction and disposal of used needles and syringes and this should be done according to local regulations. If local disposal is not possible, used clinical supplies may be returned to the Sponsor or to the designated drug depot after prior consultation with BN.

7 Assessment of Immunogenicity

Immunogenicity testing will be performed on samples drawn at trial Visit 1 (baseline before first vaccination) and Visit 4 (expected peak antibody titer).

The methods of collection, storage and shipment of specimens for immunogenicity testing will be specified in study specific instructions and the Central Laboratory's Manual, which will be provided to the investigators before enrollment commences. Additionally, training will be provided on the procedures during the Investigator Meeting and/or at the initiation visit.

7.1 Immunogenicity Testing

Vaccinia-specific antibody levels will be measured by means of a validated vaccinia-specific PRNT [using Vaccinia Virus Western Reserve (VV-WR)] and a validated vaccinia-specific ELISA (using MVA-BN as antigen). Testing will be performed at Bavarian Nordic GmbH, Martinsried, Germany. All testing personnel will be blinded to subject randomization details.

Testing SOPs, effective at the time of testing will be filed in the Trial Master File.

A vaccinia-specific PRNT will be performed to determine neutralizing antibody titers according to Bavarian Nordic SOPs.

A vaccinia-specific ELISA will be performed to determine total antibody titers according to Bavarian Nordic SOPs.

The GMT is calculated per visit by taking the antilogarithm of the mean of the $\log_{10}$ titer transformations. Antibody titers below the lower limit of quantitation (LLOQ) will be given a value of half the LLOQ for the purpose of calculation.

The seroconversion rate (applicable only to post-baseline Visit 4) is calculated and is defined as the percentage of subjects who seroconverted based on the total number of subjects with test results available (at Visits 1 and 4).

A subject's seroconversion status is assessed with regards to the corresponding baseline (Visit 1) test result.

Seroconversion is defined as:

- Appearance of antibody titers ≥ LLOQ for subjects with a pre-vaccination titer < LLOQ.
- An at least two-fold increase of the antibody titer compared to the pre-existing baseline titer (Visit 1) for subjects with a pre-vaccination antibody titer of ≥ LLOQ.

7.2 Future Use of Lab Specimen

Serum specimens remaining after completion of immunogenicity testing as per clinical trial protocol will be stored for possible future research and analysis supporting the licensure path of MVA-BN and recombinant MVA-BN vaccines. Subjects will be asked to consent to storage / future use of samples and will be informed about data protection measures. Specimens will be stored in Bavarian Nordic's secured laboratory area or at an external storage facility in a coded, pseudonymized manner to ensure data protection. Genetic testing will not be performed.

8 Safety and Reactogenicity

Taking into account the medical history of the subject, safety will be monitored by performing physical examinations including vital signs, routine laboratory measurements and ECGs as well as by evaluating local and general solicited AEs and unsolicited AEs.

Using replication-competent vaccinia-based smallpox vaccines during smallpox vaccination programs in the USA during the last years, cases of acute myocarditis and pericarditis were observed (Cassimatis et al., 2004). Although no such cases have been observed for MVA-BN, cardiac monitoring assessments will be performed post-vaccination should any subjects present with signs or symptoms potentially suggestive of cardiac adverse events. AESIs (according to the definition in section 8.1.3.3) will be recorded.

8.1 Definitions

8.1.1 Medical History

Symptoms present before ICF signature will be documented on the medical history eCRF page.

8.1.2 Baseline Signs and Symptoms

Any new signs, symptoms or changes in health that occur after ICF signature and before the first vaccination will be recorded in the baseline signs and symptoms sections of the eCRF and in the subject's medical record. Baseline signs and symptoms meeting criteria of an SAE will be reported as outlined in section 8.3.1.

8.1.3 AE

New signs, symptoms or changes in health starting after the first vaccination are documented in the AE section. AEs are recorded based on unsolicited and solicited questioning.

8.1.3.1 Unsolicited AE

Unsolicited AEs are defined as any untoward (undesirable) occurrence of a medical event in a clinical trial subject temporally associated with the administration of an IMP or a medical product (MP) which does not necessarily have a causal relationship with this IMP/MP. Up to Visit 5 all AEs (e.g. feeling of ill-health, subjective symptoms and objective signs, intercurrent diseases, accidents, etc.) observed by the investigator and/or reported by the subject must be recorded in the eCRF and in the subject's medical record regardless of the assessment of causality in relationship with the IMP/MP.

Abnormal laboratory values assessed as being clinically significant by the investigator are to be documented as AEs. In addition, abnormal laboratory values fulfilling the Grade 3 or Grade 4

criterion according to the toxicity scale (Appendix 1) are to be documented as AE in the eCRF and in the subject's medical record, regardless of whether they are considered clinically relevant or not. Toxicity grade and seriousness of an AE will be assessed separately, i.e. a Grade 3 or Grade 4 AE will not automatically be regarded as serious.

The investigator should ask the subject if they have experienced any AEs since their last visit. All intercurrent diseases reported by the subject need to be recorded by the investigator in the appropriate page of the eCRF and in the subject's medical record.

8.1.3.2 Solicited AE

Within this clinical trial protocol solicited AEs are defined as all symptoms specifically listed in the memory aid provided to the subjects following each vaccination. The subjects are requested to monitor and record local symptoms in the memory aid, i.e. erythema, swelling, induration, pruritus and pain at the site of injection as well as general symptoms, i.e. body temperature, headache, chills, myalgia, nausea and fatigue daily for the day of vaccination and the following 7 days (Days 1-8, 8-day duration).

8.1.3.3 AESI

An AESI is defined in this trial as:

- Any cardiac sign or symptom developed since the first vaccination
- ECG changes determined to be clinically significant
- Cardiac enzyme troponin I > ULN (≥ Grade 1; see toxicity scale, Appendix 1)

8.1.3.4 SAE

An SAE is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening
  The term "life-threatening" in the definition of "serious" refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death, if it were more severe
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity
- Results in a congenital anomaly or birth defect
- or is an otherwise important medical event, e.g.
  - leads to suspicion of transmission of an infectious agent

- suggests lack of efficacy of the product
- documents an overdose of the product

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious.

Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

8.2 Assessment

8.2.1 Relevant Medical History

Relevant medical history will be documented at the Screening Visit (SCR) and will focus particularly on any important diseases and in case of infections or tumors, the pathogen involved or the pathological diagnosis, if available. Special attention should be given to history of prior allergic reactions, especially to vaccines.

In addition, smallpox vaccination history must be checked (check for a smallpox vaccine scar and any documentation of previous smallpox vaccination, if available; Note: check also for smallpox vaccination programs during military service or for smallpox response teams or participation in other pox-virus based vaccine trials).

8.2.2 Prior and Concomitant Medications

All concomitant (ongoing) medications except homeopathic substances and dietary supplements must be recorded in the eCRF and in the subject's medical record including information about the indication, dosage regimen, and the onset and end of treatment. Assessment of concomitant medications will occur at SCR and at all visits during the active trial phase (Visit 1 to Visit 5).

The following medications, taken within three months prior to screening, will also be recorded in the eCRF and in the subject's medical record: Vaccines, corticosteroids (via any route of administration), other immune-modulating drugs, immunoglobulins and/or any blood products, investigational drugs and depot preparations which are still active at the date of screening.

8.2.3 Physical Examination

Complete physical examination:

A complete physical examination will be performed at the SCR. The examination includes a review of major organ systems as well as height and weight. The examination should be directed at finding evidence of any infections, tumors and lymphadenopathy (a grading scale for lymphadenopathy is included in Appendix 4). In addition, auscultation of the heart and lungs to check specifically for signs of any heart condition will be performed.

Targeted physical examination:

A targeted physical examination, guided by any signs or symptoms previously identified or any new symptoms that the subject has experienced since the last visit, is required at all visits during the active trial phase (Visit 1 to Visit 5). Auscultation of the heart and lungs will be performed.

A targeted physical examination at the FU Visit is only required if a subject reports during the phone, email, or text FU contact any potentially relevant condition (such as SAE/AESI) which will trigger that the trial subject be requested to return for a physical examination and further work-up to the CTS; see section 4.2.3.

8.2.4 Vital Signs

Evaluation of vital signs will be performed at Screening and at all visits during the active trial phase (Visit 1 to Visit 5). Vital signs at the FU Visit will only be performed if a subject reports during the phone, email, or text FU contact any potentially relevant condition (such as SAE/AESI) which will trigger that the trial subject be requested to return for a physical examination and further work-up to the CTS; see section 4.2.3. Blood pressure and pulse rate will be taken after the subject has been sitting for at least two minutes. Body temperature will be measured orally.

8.2.5 Unsolicited AE

All intercurrent diseases reported when the investigator actively inquires the subject will be documented in the source and all required details (e.g. start and stop date, severity) will be assessed. Unsolicited AEs will be reported in the respective section of the eCRF.

AEs will be assessed and documented at all visits of the active trial phase (Visit 1 to Visit 5) and if ongoing at Visit 5 until resolution or until the FU contact at the latest.

SAEs and AESIs will be assessed and documented at all trial visits, including the FU contact. Ongoing AESIs and SAEs will be followed up until resolution or achievement of stable clinical conditions.

Assessment of Intensity

For all unsolicited AEs not represented in the Toxicity Scale for Laboratory Values, grading will be based on maximum intensity (see Appendix 1).

Assessment of Causality

The relationship between the occurrence of an AE and the IMP will be assessed using the categories presented below. For expedited reporting and all other purposes, the categories "none" and "unlikely" will represent no evidence or argument to suggest a causal relationship, while "possible", "probable" and "definite" will be seen to convey that there is evidence or argument to suggest a causal relationship.

None	The time interval between the administration of the IMP and the occurrence or worsening of the AE rules out a relationship, **and/or** another cause is established and there is no evidence of a (concomitant) causal connection with or worsening caused by the IMP.
Unlikely	The time interval between administration of the IMP and the occurrence or worsening of the AE makes a causal relationship unlikely, **and/or** the known effects of the IMP or substance class provide no indication of a (concomitant) causal connection with or worsening caused by the IMP and there is another cause which serves as an adequate explanation, **and/or** although the known effects of the IMP or substance class make it possible to derive a plausible causal chain with regard to a (concomitant) causal connection or worsening, however, another cause is considerably more likely, **and/or** another cause of the AE has been identified and a (concomitant) causal connection with or worsening caused by the IMP is unlikely.
Possible	A plausible causal chain with regard to a (concomitant) causal connection with / worsening of the AE can be derived from the pharmacological properties of the IMP or substance class. However, other approximately equally likely causes are known, **or** although the pharmacological properties of the IMP or substance class provide no indication of a (concomitant) causal connection with / worsening of the AE, there is no other known cause which provides an adequate explanation.
Probable	The pharmacological properties of the IMP or substance class, **and/or** the course of the AE after discontinuation of the IMP and possible subsequent re-exposure, **and/or** specific findings (e.g. positive allergy test or antibodies against the trial drug / metabolites) suggest a (concomitant) causal connection with / worsening of the AE resulting from the IMP, however another cause cannot completely be ruled out.
Definite	The pharmacological properties of the IMP or substance class **and/or**

	the course of the AE after discontinuation of the IMP and possible subsequent re-exposure, **and/or** specific findings (e.g. positive allergy test or antibodies against the trial drug / metabolites) definitely indicate that there is a (concomitant) causal connection with / worsening of the AE resulting from the IMP and there are no indications of other causes.

8.2.6 Solicited AE

After each vaccination subjects receive a memory aid to record solicited local and general AEs most likely to occur on the day of vaccination and the following 7 days (Days 1-8, 8-day period).

All solicited symptoms observed after vaccination with details concerning the intensity and the course of the reaction should be documented there. The investigator will collect this information during the following scheduled visits and transfer it to the eCRF. Local and general reactions still ongoing after 7 days will be measured or examined each day until resolution or until no further change can reasonably be expected, and the last day of symptoms and maximum intensity will be documented in the memory aid.

In case of severe and unexpected local and/or general reactions, the subject should be instructed to contact the trial physician outside of scheduled trial visits.

8.2.6.1 Solicited Local AE

The solicited local symptoms erythema, swelling, induration, pruritus and pain at the injection site are to be documented in the memory aid by the subject.

To standardize procedures, uniform rulers will be handed out to all subjects for measurement of erythema, swelling and induration diameters.

Assessment of Intensity

Injection site erythema	size measured in diameter
Injection site swelling	size measured in diameter
Injection site induration	size measured in diameter

The maximum severity will be scored as follows:

0	=	0
1	=	< 30 mm
2	=	≥ 30 – < 100 mm
3	=	≥ 100 mm

Injection site pruritus

0	=	No symptoms
1	=	Mild - routine daily activities not impaired
2	=	Moderate - routine daily activities impaired
3	=	Severe - prevents routine daily activities

Injection site pain:

0	=	No pain
1	=	Painful on touch
2	=	Painful when moving the limb
3	=	Spontaneously painful / prevents normal activity

Assessment of Causality

Solicited local AEs are defined as being related to the trial vaccine.

8.2.6.2 Solicited General AE

The solicited general symptoms body temperature, headache, myalgia, nausea, chills and fatigue are to be documented in the memory aid by the subject.

To standardize procedures, digital thermometers will be handed out to all subjects for oral measurements of body temperature.

Assessment of Intensity

Subjects are asked to document the solicited general AEs in the memory aid as described in Table 2 below. In the subject's memory aid, the grading of maximum symptom intensity is described in basic, easily understood language based on the following descriptions:

Table 2: Grading of General Symptoms from the Subject's Memory Aid

Medical Dictionary for Regulatory Activities (MedDRA) coded Preferred Term General AEs	Grade	Maximum Severity
Body temperature*	0	< 99.5°F (< 37.5°C)
	1	≥ 99.5 – < 100.4°F (≥ 37.5 – < 38.0°C)
	2	≥ 100.4 – < 102.2°F (≥ 38.0 – < 39.0°C)
	3	≥ 102.2 – < 104°F (≥ 39.0 – < 40.0°C)
	4	≥ 104°F (≥ 40.0°C)
Headache, Myalgia, Nausea, Chills and Fatigue	0	No symptoms
	1	Mild: routine daily activities not impaired
	2	Moderate: routine daily activities impaired
	3	Severe: prevents routine daily activities

*Pyrexia is defined as oral temperature ≥ 100.4°F (≥ 38.0 C).

Assessment of Causality

Causal relationship between solicited general AEs and the vaccine will be assessed by the investigator using the same categories as for unsolicited AEs (see section 8.2.5).

8.2.7 Cardiac Assessment

To evaluate the cardiac profile of MVA-BN, targeted physical exams including auscultation of the heart and lung will be performed. Any kind of cardiac signs (i.e. discovered by the physician during examination of the subject) or symptom(s) (i.e. experienced and reported by the subject) detected during the trial such as but not limited to chest pain, dyspnea, arrhythmia or edema are recorded.

ECG

A standard 12-lead ECG will be taken at SCR. At Visit 2 and Visit 4 an ECG is only done if clinically indicated. An ECG can be performed at any time in case of an AESI, see Figure 1. ECGs will be evaluated by a centralized ECG service provider. The final decision about clinical significance of ECG findings is with the investigator.

Cardiac risk factors

Family cardiac risk factor is evaluated at SCR. Subjects with an immediate family member (father, mother, brother, or sister) who has had onset of ischemic heart diseases before 50 years of age are excluded from trial participation.

Troponin I

Troponin I will be measured at the SCR. At Visit 2, Visit 4, and the Follow-up Visit, troponin I measurement is only done if clinically indicated. Troponin I can be measured at any time in case of an AESI, see Figure 1.

Cardiac events fulfill the definition of an AESI as described under section 8.1.3.3. The investigator will be asked to assess the clinical significance of the case.

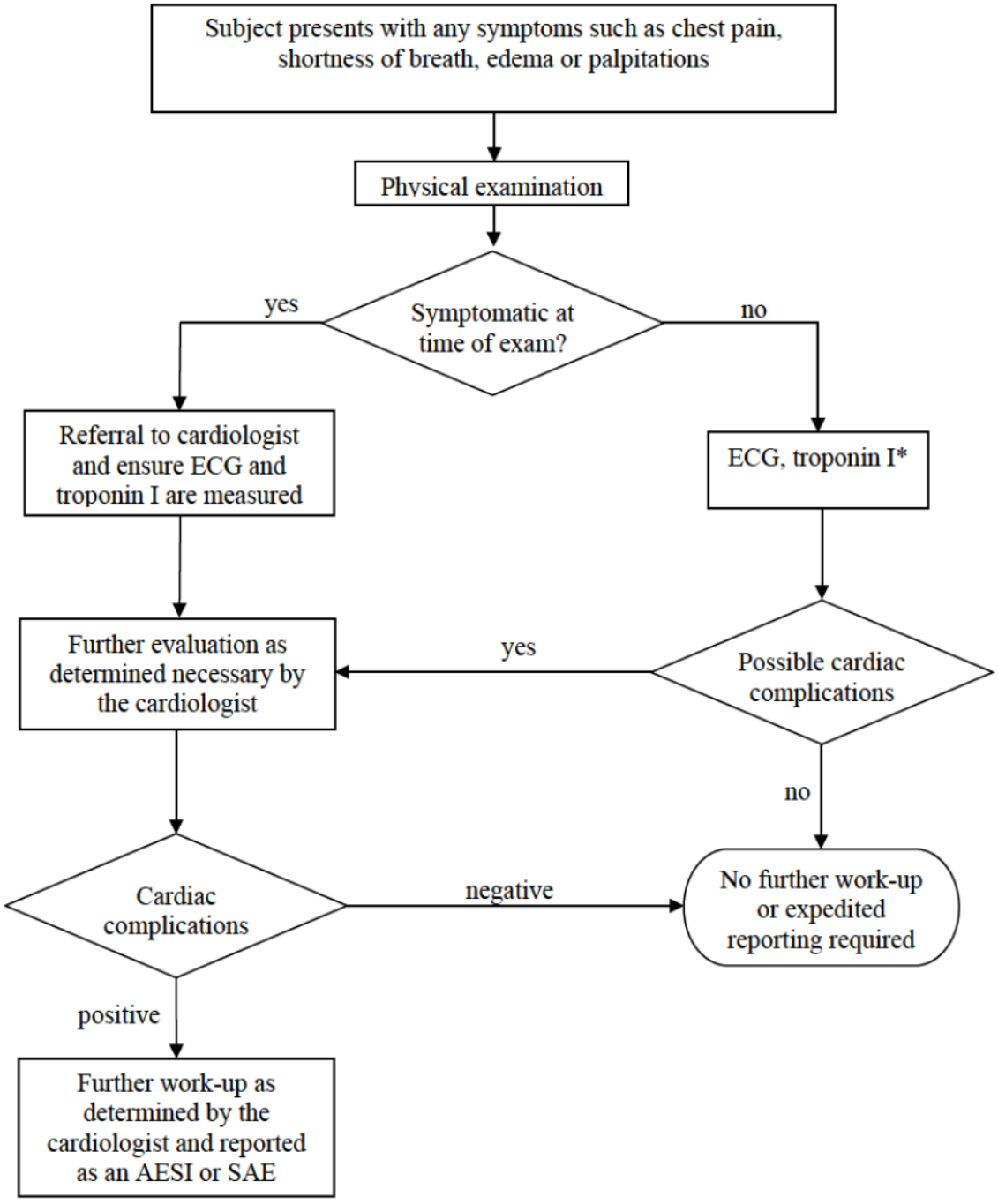
Case definitions as published by the Centers of Disease Control and Prevention ("Update: Cardiac-Related Events During the Civilian Smallpox Vaccination Program --- United States, MMWR May 30, 2003, Vol. 52, No. 21, p. 494") are provided in Appendix 2 in order to:

- help investigators to recognize possible events of acute myocarditis and/or pericarditis and
- distinguish from unspecific and isolated ECG changes without or with unclear clinical significance.

Subjects who develop any kind of cardiac signs or symptoms during the trial such as but not limited to chest pain, dyspnea, arrhythmia or edema are referred to a local cardiologist for cardiac evaluation such as (treadmill) ECG, cardiac enzymes and/or echocardiogram. Depending on the results of these evaluations, further diagnostic tests will be done as recommended by the cardiologist and subjects will be followed up at a frequency determined by the cardiologist. AESIs will be followed up until complete resolution or until the sequelae are stable and considered to be permanent.

Figure 1 outlines the algorithm for assessment of cardiac events.

Figure 1: Algorithm for Assessment of Cardiac Events



*At any (clinically indicated) ECG and/or troponin I abnormality, the algorithm will begin at this point.

8.2.8 Safety Laboratory Measurements

The intensity of laboratory / systemic quantitatively measured toxicities will be graded according to the toxicity scale in Appendix 1. These grading scales include the laboratory values determined with the routine safety parameters. In case of other laboratory values not included in the routine safety laboratory and not listed in Appendix 1, the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials, published September 2007 (FDA, 2007) will be used for grading of laboratory toxicities.

Safety laboratory tests are completed at SCR, Visit 2 and Visit 4 and at any other visit(s) if clinically indicated. The safety laboratory measurements are performed at a central laboratory. Laboratory normal ranges are provided by the central laboratory and filed in the Investigator File. Safety laboratory parameters to be evaluated are:

Hematology:

Red blood cell count, hemoglobin, total and differential white blood cell count (WBC), platelet count

Serum chemistry:

Total bilirubin, alkaline phosphatase, AST, ALT, serum creatinine (for calculation of CrCl at SCR), sodium, potassium, calcium, troponin I (troponin I mandatory at the SCR and in addition at Visit 2, Visit 4, and at the Follow-up visit if clinically indicated).

Pregnancy test:

A β-human choriogonadotropin (HCG) pregnancy test will be conducted for all WOCBP at SCR, within 24 hours prior to each vaccination (Visits 1, 3) and at Visit 5 (respectively the individual last active trial phase visit). At screening, a serum β-HCG pregnancy test will be performed; all other pregnancy tests will be conducted as urine β-HCG tests.

8.2.9 Pregnancy

As per inclusion criteria, women of childbearing potential must have a negative serum pregnancy test at screening and a negative urine pregnancy test within 24 hours prior to each vaccination. In addition, they must have used an acceptable method of contraception for 30 days prior to the first vaccination, must agree to use an acceptable method of contraception during the trial, and must avoid becoming pregnant for at least 28 days after the last vaccination. Nevertheless, IMP exposed pregnancies cannot be excluded with certainty. Subjects who become pregnant prior to the first vaccination will be excluded from the trial and are regarded as screening failures. Subjects who become pregnant during the active trial phase (up to and including one month [minimum 28 days] after receiving a dose of vaccine) must not receive additional doses of

vaccine but may continue other trial procedures at the discretion of the investigator (Withdrawal from Further Trial Vaccination see section 4.2.5). All IMP exposed pregnancies should be followed up until delivery.

Subjects should be instructed to notify the investigator if it is determined after completion of the trial that they became pregnant either during the trial or within one month (minimum 28 days) after receiving the last vaccine dose.

8.3 Reporting

8.3.1 Reporting of SAEs

All SAEs occurring throughout the entire course of the trial have to be reported to the BN PV Department. The CTS will complete the SAE form located in the electronic data capture system (EDC) within 24 hours of becoming aware of the AE. Paper SAE forms should only be completed in the event the EDC system is not operational. Paper SAE forms will be sent by email or fax to the BN PV Department within 24 hours of becoming aware of the AE.

Bavarian Nordic PV contact information:

Bavarian Nordic Pharmacovigilance
fax: +49 89 255 446 419 (Germany)
email: drug.safety@bavarian-nordic.com

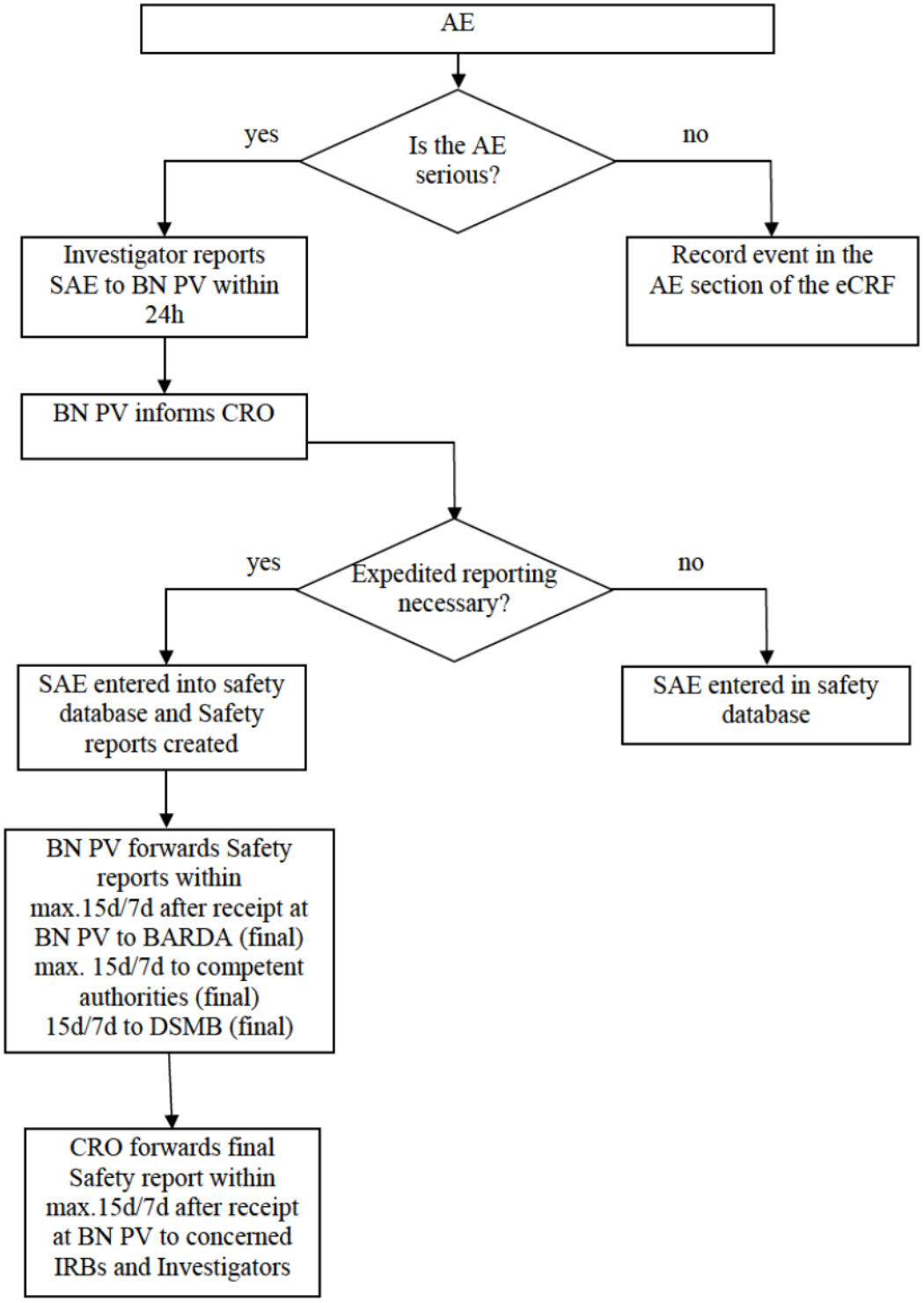
The investigator should not delay reporting because of missing information. Nonetheless, the report should be as complete as possible. This initial notification should include, as a minimum, sufficient information to permit identification of the following:

- the reporter (investigator's name and contact information)
- the subject
- involved trial medication
- AE(s)
- Seriousness criterion and/or criterion for AESI
- date of onset

The BN PV Department informs the CRO of all SAEs. BN is responsible for expedited as well as periodic reporting to the involved regulatory authorities (e.g. FDA, Paul Ehrlich Institute (PEI)) according to applicable laws and guidelines. Regulatory authorities will be notified as soon as possible but no later than 7 days after first knowledge of fatal or life-threatening unexpected SAE with an at least possible relationship to the IMP (serious adverse drug reaction [SADR]) and no later than 15 days after knowledge of any other unexpected SADR. In addition, BN will report the SAEs to the responsible Biomedical Advanced Research and Development Authority

(BARDA) representative when applicable and forward them to the DSMB, while the investigator or the CRO is responsible for reporting to the IECs or IRBs. Figure 2 outlines the reporting process and timelines for SAEs.

Figure 2: Algorithm for Reporting of SAEs



8.3.2 Reporting of AESI

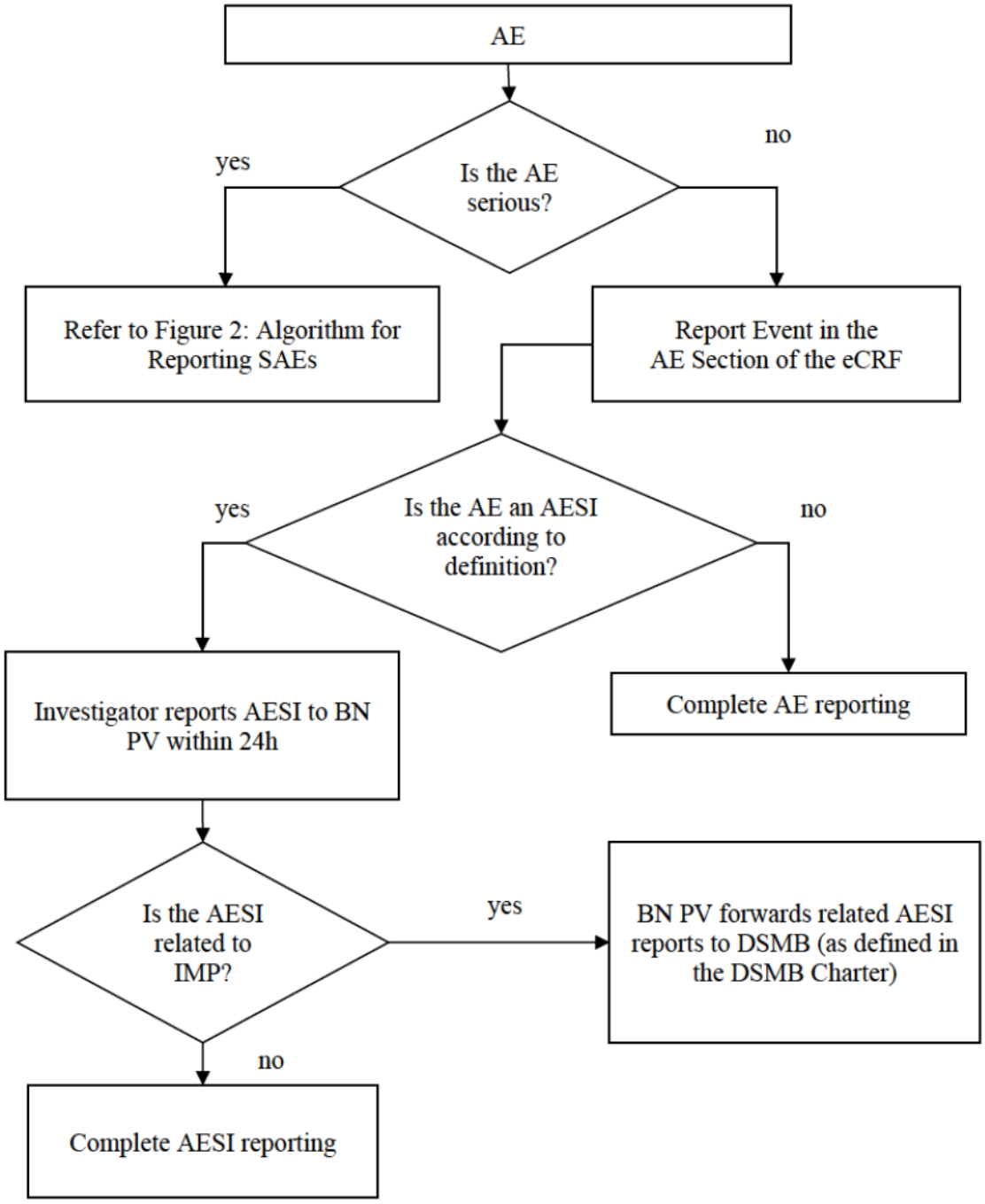
All AESIs occurring throughout the entire course of the trial have to be reported to the BN PV Department. The CTS will complete the appropriate form in the EDC within 24 hours of becoming aware of the AESI. Paper forms should only be completed in the event the EDC system is not operational. Paper forms will be sent by email or fax to the BN PV Department within 24 hours of becoming aware of the AESI.

Bavarian Nordic PV contact information:

Bavarian Nordic Pharmacovigilance
fax: +49 89 255 446 419 (Germany)
email: drug.safety@bavarian-nordic.com

A periodic report for AESIs will be provided from the BN PV to the CRO. Figure 3 outlines the reporting process and timelines for AESIs.

Figure 3: Algorithm for Reporting of AESIs



8.3.3 Reporting of Pregnancies

If a subject becomes pregnant during the active trial phase (up to and including one month [minimum 28 days] after receiving a dose of vaccine) this must be reported to BN on a Pregnancy Report Form within 24 hours of the investigator's becoming aware of the event, using the same contact details as provided in section 8.3.1.

A pregnancy should be followed to term, any premature terminations reported, and the health status of the mother and child including date of delivery and the child's gender and weight should be reported to BN after delivery.

Any event during pregnancy fulfilling the criteria for an SAE will be reported as SAE to BN PV. However, hospitalization for delivery is a prospectively planned hospitalization and is not considered a SAE per se.

9 Statistical Considerations

9.1 Primary Trial Hypothesis

The primary objective of the trial is to show that the humoral immune responses elicited by three consecutively produced MVA-BN lots are statistically equivalent. The humoral immune response is defined as the within-group GMT of the individual subject PRNT titers measured at Visit 4 (i.e., 2 weeks after the second vaccination). As the titers are log-normally distributed, the endpoint will be tested on the $\log_{10}$ scale. The primary endpoint will be met if the means of the $\log_{10}$ titers are equivalent within a pre-specified amount. This amount is called the margin of equivalence (Δ).

Suppose m_1 is the mean of the $\log_{10}$ titers in Group 1, m_2 is the mean of the $\log_{10}$ titers in Group 2 and m_3 is the mean of the $\log_{10}$ titers in Group 3. The test on equivalence will be applied for the following hypothesis:

$$H_0: |m_1-m_2| \geq \Delta \text{ OR } |m_1-m_3| \geq \Delta \text{ OR } |m_2-m_3| \geq \Delta$$

versus

$$H_A: |m_1-m_2| < \Delta \text{ AND } |m_1-m_3| < \Delta \text{ AND } |m_2-m_3| < \Delta$$

Δ is set at 0.301 for the $\log_{10}$ PRNT titers, equivalent to a factor of 2 for the GMT.

Assuming the $\log_{10}$ titers are approximately normally distributed, the above hypothesis will be tested based on the difference of the $\log_{10}$ titer means. Specifically, the two-sided 95% confidence interval limits for the difference of $\log_{10}$ titer means will be calculated. If all of the lower confidence interval limits for the three lot-group differences are above $-\Delta$, and all of the

upper confidence interval limits for the three lot-group differences below Δ, the null-hypothesis will be rejected in favor of the alternative hypothesis of equivalence.

9.2 Endpoints

Please refer to trial protocol synopsis (see section 1.5).

9.3 Sample Size Calculation

The primary objective of the trial is to demonstrate equivalence of three consecutively produced lots of MVA-BN in terms of the primary endpoint, the GMT measured by PRNT 2 weeks following the second vaccination.

In order to consider that small variations in the virus titer of the produced lots may influence the power to reject the null hypothesis (Ganju et al., 2008), a simulation study was performed to calculate the required number of analyzable subjects per group based on the following underlying assumptions:

- Significance level: 5% (two-sided) i.e. 95% CIs (two-sided)
- Power: 90%
- Within-lot variability in PRNT: $SD_{within} = 0.45$ on $\log_{10}$ transformed antibody titers (based on previous trials i.e. POX-MVA-006 and POX-MVA-013)
- Between-lot variability in PRNT: $SD_{between} = 0.075$ on $\log_{10}$ transformed antibody titers. This is derived as follows:
  - the SD of the dose of the produced lots is not larger than 0.13 (based on the upper 95% CI of $\log_{10}$ transformed virus titers observed in 8 released lots of MVA-BN FD)
  - the slope of the dose-response curve on the $\log_{10}$ scale is about 0.57 for the dose used in this study (based on the data of study POX-MVA-004)
  - these 2 assumptions translate to about $0.13 \times 0.57 \sim 0.074$ which was conservatively rounded to $SD_{between} = 0.075$
- Equivalence margin: $\Delta = 0.301$ on the $\log_{10}$ scale

The simulations showed that an analyzable sample size of 315 in each group yields a power of at least 90% of showing equivalence for all three MVA-BN groups.

In order to account for a dropout rate of about 15%, which has been observed in previous MVA-BN trials, a total of 370 subjects will be randomized in each group.

9.4 Trial Cohorts/Datasets to be Evaluated

Full Analysis Set (FAS):

The FAS includes all subjects who were randomized and received at least one dose of trial vaccine, regardless of the occurrence of protocol deviations.

The analysis of safety will be performed on this analysis set.

Per Protocol Set (PPS):

The PPS is a subset of the FAS including all subjects without major protocol deviations i.e. protocol deviations that might have a substantial impact on the immunogenicity results.

The decision whether a protocol deviation is major or not for the classification of subjects in the PPS will be made case-by-case in a blinded data review meeting before database lock.

The primary analysis of immunogenicity will be performed on the PPS. In particular, the primary trial hypothesis will be confirmatory tested on this analysis set. All analyses of immunogenicity will be repeated for the FAS to assess the robustness of the results.

9.5 Biometrical Evaluation

As soon as the last subject has completed the FU contact and after any necessary settlement of queries etc. in the eCRFs, the database will be locked. A full analysis of the data available will be performed.

All data obtained in this trial and documented in the eCRFs will be listed. For parameters of interest, summary tables with descriptive group statistics for continuous variables will be prepared. For categorical / dichotomous variables summary tables showing the absolute and relative count in each category will be prepared. Summaries will be presented for Groups 1, 2, and 3 separately and for Groups 1, 2 and 3 combined.

Full details of the analyses will be defined in a Statistical Analysis Plan which will be finalized prior to database lock and unblinding.

The CRO will be responsible for data management and statistical evaluation. Data will be analyzed using SAS® software. The procedure for accounting for missing, unused and spurious data will be given in the Statistical Analysis Plan.

All statistical tests for secondary endpoints and comparisons are regarded as descriptive and no adjustment for multiple testing will therefore be done.

The occurrence of solicited local and general AEs at the day of vaccination and the following 7 days (Days 1–8 = 8-day period) will be summarized on a per subject and per vaccination basis.

Unsolicited AEs will be coded using MedDRA coding terminology. The intensity of AEs will be graded according to section 8.2.5.

SAEs will be listed separately. Each SAE will be described individually in detail.

Clinical laboratory test results will be marked whether the result is below, within or above the respective reference range. The number of values outside of the corresponding reference range will be counted.

All ECGs will be evaluated by the central reader and by the respective investigator and the assessments will be summarized.

10 Ethical Aspects

10.1 Ethical and Legal Regulations

This trial will be conducted in a manner consistent with the principles that have their origin in the Declaration of Helsinki and in accordance with FDA regulations (21 CFR § 11, 50, 54, 56, and 312), with the ICH GCP guidelines (ICH E6), as well as with any and all applicable federal, state and/or local laws and regulations.

10.2 Approval by an IEC / IRB

Before enrollment of subjects into the clinical trial, as required by federal regulations (21 CFR § 56), ICH GCP and local regulations, the current protocol and ICF will be reviewed and approved by an appropriate IRB. A letter documenting the IRB or IEC approval must be received by the sponsor before the initiation of the trial at a clinical site. Amendments to the protocol will be subject to the same requirements as the original protocol.

If one of the investigators is a member of one of these committees, he/she may not vote on any aspect of the review of this protocol.

The Investigator will submit a progress report at least once yearly to the IRB. However, the frequency of these reports will depend on IRB requirements. As soon as possible after completion or termination of the trial, the Investigator will submit a final report to the IRB per the IRB requirements, and in compliance with FDA regulations and ICH GCPs.

The Investigator, the sponsor, or designee shall promptly notify the IRB or IEC of any SAEs, or any other information that may affect the safe use of the trial drug during the course of the trial, per the IRB local requirements, and in compliance with FDA regulations and ICH GCPs.

Copies of all correspondence between the investigator and the IRB must be forwarded immediately to the Sponsor or designee. In case of withdrawal of IRB approval of the trial, the Sponsor or designee has to be contacted immediately by facsimile, email or telephone.

10.3 Confidentiality and Data Protection

Information on maintaining subject confidentiality in accordance with individual local and national subject privacy regulations must be provided to each subject as part of the informed consent process either as part of the ICF or as a separate signed document (for example, in the US, a site-specific Health Insurance Portability and Accountability Act [HIPAA] consent may be used).

The Investigator or designee must explain to each subject that for the evaluation of trial results, the subject's protected health information (PHI) obtained during the trial may be shared with BN and its designees, regulatory agencies, and IRBs. As the trial Sponsor, BN will not use the subject's PHI or disclose it to a third party without applicable subject authorization. It is the Investigator's or designee's responsibility to obtain written permission to use PHI from each subject. If a subject withdraws permission to use PHI, it is the Investigator's responsibility to obtain the withdrawal request in writing from the subject and to ensure that no further data will be collected from the subject. Any data collected on the subject before withdrawal will be used in the analysis of trial results.

During the review of source documents by the monitors or auditors, the confidentiality of the subject will be respected with strict adherence to professional standards and regulations.

11 Informed Consent

The investigator is responsible for ensuring that the subject understands the potential risks and benefits of participating in the trial, including answering any questions the subject may have throughout the trial and sharing in a timely manner any new information that may be relevant to the subject's willingness to continue his or her participation in the trial.

No subject can participate in the trial without first having given informed consent in writing. The investigator or his delegate will inform the subject clearly and completely, verbally and in writing, about the purpose, procedures and the potential benefits and risks of participation in the trial prior to the initiation of any trial specific procedure. The subject will also be informed of the potential future use of specimens collected during the trial.

The ICF will be used to explain to the subject in simple terms the potential risks and benefits of trial participation and to document that the subject is satisfied with his or her understanding of the risks and benefits of participating in the trial.

The investigator is responsible for ensuring that informed consent to participate in the trial is given by each subject (or their legal representative). This includes obtaining the appropriate signatures and dates on the ICF prior to the performance of any protocol procedures and prior to the administration of trial drug.

One signed copy of the ICF (including HIPAA) must be given to each subject and one signed copy must remain in the Investigator Site File and be available for verification by the monitor, Sponsor/CRO auditor or competent regulatory authorities at any time.

Subjects must be informed unequivocally that they may refuse participation in the trial and that they may withdraw from the trial at any time and for whatever reason and that withdrawal of consent will not affect their subsequent medical treatment or relationship with the treating physician.

Subjects also consent to authorize the monitor, quality assurance personnel and regulatory authorities to inspect source documents for data verification and quality assurance purposes. Such verifications will always be conducted at the clinical trial site and under the ethical supervision of the investigator. To the degree possible, confidentiality of the subject's PHI will be maintained.

The Informed Consent will be prepared in accordance with ICH GCP guidelines and must be approved by the appropriate IRB.

12 eCRFs and Retention of Records

12.1 eCRF

In this trial, an eCRF will be used.

eCRFs will be used to collect the clinical trial data and must be completed for each enrolled subject. All data should be accurately recorded such that the information matches the data contained in subject's medical records (e.g., physicians' notes, nurses' notes, clinic charts and other trial-specific source documents). Authorized trial site personnel (i.e., listed on the Delegation of Authority form) will complete eCRFs designed for this trial according to the eCRF Completion Guidelines (provided as a separate document). The Investigator will ensure that the eCRFs are accurate and completed within 5 days of each subject's visit. At all times, the Investigator has final responsibility for the accuracy and authenticity of all clinical data.

The eCRFs exists within an EDC system with controlled access managed by BN or its authorized representative for this trial. Trial staff will be appropriately trained in the use of eCRFs and application of electronic signatures before the start of the trial and before being given access to the EDC system. Original data and any changes of data will be recorded using the EDC system, with all changes tracked by the system and recorded in an electronic audit trail. The Investigator attests that the information contained in the eCRFs is true by providing electronic signature

within the EDC system. After database lock, the Investigator will receive a copy of the subject data (e.g., paper, CD-ROM or other appropriate media) for archiving at the clinical trial site.

12.2 Retention of Records

The Investigator/trial staff must maintain adequate and accurate records to enable the conduct of the trial to be fully documented and the trial data to be subsequently verified. All essential documents, as listed in ICH GCP guidelines, will be retained by the Investigator for at least 2 years after the date the last marketing application is approved for the drug in the indication being investigated and until there are no pending or contemplated marketing applications; or, if no application is to be filed or if the application is not approved for such indication, until 2 years after formal discontinuation of clinical development of the drug.

The Investigator must notify and obtain written approval from BN before destroying any clinical trial documents or images (e.g., scan, radiograph, ECG tracing) at any time. The Sponsor will inform the Investigator of the date that the trial records may be destroyed or returned to BN.

Should an Investigator wish to assign trial records to another party, advance written notice must be given to the Sponsor. BN must also be notified in advance and provide express written approval of any change in the maintenance of clinical trial documents, should the Investigator choose to move trial records to another location.

If the Investigator cannot guarantee the aforementioned archiving requirements at the clinical trial site for all such documents, special arrangements must be made between the Investigator and BN to store these documents in secure sealed containers away from the clinical trial site. These documents must be able to be returned in their secure sealed containers to the clinical trial site for auditing/inspection purposes.

13 Monitoring of the Trial

Representatives of BN or its designee (e.g., CRO) will monitor this trial until completion. Monitoring will be conducted according to the monitoring plan which must be approved by BN. The monitoring plan will specify in detail the items for source data verification and other tasks, to be performed by the CRA during the clinical trial site visit.

Monitoring will be conducted through personal visits with the Investigator and site staff, remote monitoring, as well as any appropriate communications by mail, fax, email, or telephone. The purpose of monitoring is to ensure that the trial is conducted in compliance with the protocol, SOPs, and other written instructions and regulatory guidelines, and to ensure the quality and integrity of the data. This trial is also subject to reviews or audits.

To assure the accuracy of data collected in the eCRFs, it is mandatory that the monitor have access to all original source documents, including all electronic medical records at reasonable

times and upon reasonable notice. During the review of source documents, every effort will be made to maintain the anonymity and confidentiality of all subjects involved in this clinical trial.

14 Audits and Inspections

Site audits may be carried out at any time during or after completion of this trial by the Quality Assurance Department at Bavarian Nordic or designee. All trial-related documentation must be made available to the designated auditor. The Investigator agrees to allow the IRB, representatives of BN, its designated agents and authorized employees from local, state, or Regulatory Authority (e.g., FDA) to inspect the facilities used in this clinical trial at any time before, during, or after completion of the clinical trial and, for purposes of verification, allow direct access to the hospital or clinic records of all randomized subjects. A statement to this effect will be included in the informed consent and permission form authorizing the use of protected health information. In the event of such an inspection, BN will be available to assist in the preparation. All pertinent trial data should be made available as requested for verification, audit, or inspection purposes.

15 Responsibilities of the Investigator

The PI agrees to carry out the trial in accordance with the guidelines and procedures outlined in this clinical trial protocol. The PI especially consents to strictly adhere to the ethical principles (see section 10 of this protocol).

Changes to the protocol require written "Amendments to the protocol" and written approval by the IRB, the Coordinating Investigator, and the PI of the respective CTS. Changes are allowed only if trial value is not reduced and if they are ethically justifiable. The amendment must be passed on to all participating PIs with the obligation to adhere to its provisions. If warranted, the subject information has to be changed accordingly.

It is within the responsibility of the PI that the eCRF is completed in a timely manner after each subject visit and electronically signed after the subject has finished the trial for each subject participating in the trial.

At the conclusion of the trial, the investigator will return all partly used, unused and empty vaccine vials to the Sponsor, the designated drug depot, or the vaccine vials will be destroyed by the CTS.

The investigator may ask to terminate the trial due to administrative or other reasons. If this should be the case, appropriate measures which safeguard the interests of the participating subjects must be taken after verification and consultation with the PI.

Each investigator will maintain appropriate medical and research records for this trial, in compliance with ICH E6 (R1) Guideline for GCP, Section 4.9, and regulatory and institutional

requirements for the protection of confidentiality of subjects. The investigator will permit authorized representatives of the sponsor and regulatory agencies to review (and, when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits, and evaluation of the trial safety and progress.

The PI agrees to follow the detailed publication policy included in the clinical trial agreement.

By signing this protocol, the PI confirms that he/she has read the entire clinical trial protocol, agrees to its procedures, and will comply strictly with the formulated guidelines.

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Appendices

Appendix 1: Toxicity Scale for Laboratory Values

Grade 1 or Grade 2 toxicity is only graded according to Table 3 and Table 4, if the value is outside of the institutional normal range applicable for this trial.

Estimating severity grade
For abnormalities NOT found elsewhere in the Toxicity Tables use the scale below to estimate grade of severity:

Grade 1	An AE which is easily tolerated by the subject, causing minimal discomfort and not interfering with daily activities.
Grade 2	An AE which is sufficiently discomforting to interfere with daily activities.
Grade 3	An AE which prevents daily activities. Such an AE would, for example, prevent attendance at work and would necessitate the administration of corrective therapy.
Grade 4	Life-threatening or disabling

Serious or life-threatening AEs
ANY clinical event deemed by the clinician to be serious or life-threatening should be considered a Grade 4 event. Clinical events considered to be serious or life-threatening include, but are not limited to: Seizures, coma, tetany, diabetic ketoacidosis, disseminated intravascular coagulation, diffuse petechiae, paralysis, acute psychosis, severe depression.

Table 3: Toxicity Scale for Serum Chemistry

Lab Value, Serum*	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)**
Sodium – Hyponatremia mEq/L	132 - 134	130 - 131	125 - 129	< 125
Sodium – Hypernatremia mEq/L	144 - 145	146 - 147	148 - 150	> 150
Potassium – Hyperkalemia mEq/L	5.1 - 5.2	5.3 - 5.4	5.5 - 5.6	> 5.6
Potassium – Hypokalemia mEq/L	3.5 – 3.6	3.3 – 3.4	3.1 - 3.2	< 3.1
Calcium – Hypercalcaemia mg/dL	10.5 – 11.0	11.1 - 11.5	11.6 - 12.0	> 12.0
Calcium- Hypocalcaemia mg/dL	8.0 - 8.4	7.5 - 7.9	7.0 - 7.4	< 7.0
Creatinine mg/dL	1.5 - 1.7	1.8 - 2.0	2.1 - 2.5	> 2.5 or requires dialysis
Alkaline Phosphatase increase by factor	1.1 - 2.0 x ULN***	2.1 - 3.0 x ULN	3.1 - 10.0 x ULN	> 10.0 x ULN
ALT (SGPT) and AST (SGOT) increase by factor	1.1 - 2.5 x ULN	2.6 - 5.0 x ULN	5.1 - 10.0 x ULN	> 10.0 x ULN
Bilirubin – when accompanied by any increase in Liver Function Test; increase by factor	1.1 - 1.25 x ULN	1.26 - 1.50 x ULN	1.51 - 1.75 x ULN	> 1.75 x ULN
Bilirubin – when Liver Function Test is normal; increase by factor	1.1 - 1.5 x ULN	1.6 - 2.0 x ULN	2.1 - 3.0 x ULN	> 3.0 x ULN

* The laboratory values provided in the table serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

** The clinical signs or symptoms associated with laboratory abnormalities might result in characterization of the laboratory abnormalities as Potentially Life Threatening (Grade 4). For example, a low sodium value that falls within a Grade 3 parameter (125 - 129 mEq/L) should be recorded as a Grade 4 hyponatremia event if the subject had a new seizure associated with the low sodium value.

***"ULN" is the upper limit of the normal range.

Table 4: Toxicity Scale for Hematology

Lab Value, Hematology*	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Hemoglobin (Female) – gm/dL	11.0 - 12.0	9.5 - 10.9	8.0 - 9.4	< 8.0
Hemoglobin (Male) – gm/dL	12.5 - 13.5	10.5 - 12.4	8.5 - 10.4	< 8.5
WBC Increase cell/mm^3	10,800 - 15,000	15,001 - 20,000	20,001 - 25,000	> 25,000
WBC Decrease cell/mm^3	2500 - 3500	1500 - 2499	1000 - 1499	< 1000
Lymphocytes Decrease cell/mm^3	750 - 1000	500 - 749	250 - 499	< 250
Neutrophils Decrease cell/mm^3	1500 - 2000	1000 - 1499	500 - 999	< 500
Platelets Decrease cell/mm^3	125,000 - 140,000	100,000 - 124,000	25,000 - 99,000	< 25,000

* The laboratory values provided in the table serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

Table 5: Grading for Troponin I

Lab Value	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Cardiac troponin I	> ULN - < 2 x ULN	≥ 2 - < 5 x ULN	≥ 5 x ULN	N/A

"ULN" is the upper limit of the normal range.

Appendix 2: Case Definitions Acute Myocarditis / Pericarditis

Case Definition for Acute Myocarditis

A possible case of acute myocarditis is defined by the following criteria and the absence of evidence of any other likely cause of symptoms:

- Presence of dyspnea, palpitations, or chest pain of probable cardiac origin in a subject with either one of the following:
  - ECG abnormalities beyond normal variants, not documented previously, including
    - ST-segment or T-wave abnormalities,
    - Paroxysmal or sustained atrial or ventricular arrhythmias,
    - AV nodal conduction delays or intraventricular conduction defects, or
    - Continuous ambulatory electrocardiographic monitoring that detects frequent atrial or ventricular ectopy, **or**
    - Evidence of focal or diffuse depressed leftventricular (LV) function of indeterminate age identified by an imaging trial (e.g., echocardiography or radionuclide ventriculography).

A probable case of acute myocarditis, in addition to the above symptoms and in the absence of evidence of any other likely cause of symptoms, has one of the following:

- Elevated cardiac enzymes, specifically, abnormal levels of cardiac troponin I, troponin T, or creatine kinase myocardial band (a troponin test is preferred),
- Evidence of focal or diffuse depressed LV function identified by an imaging trial (e.g., echocardiography or radionuclide ventriculography) that is documented to be of new onset or of increased degree of severity (in the absence of a previous trial, findings of depressed LV function are considered of new onset if, on follow-up studies, these findings resolve, improve, or worsen), or
- Abnormal result of cardiac radionuclide imaging (e.g., cardiac magnetic resonance imaging with gadolinium or gallium-67 imaging) indicating myocardial inflammation.

A case of acute myocarditis is confirmed if histopathologic evidence of myocardial inflammation is found at endomyocardial biopsy or autopsy.

Case Definition for Acute Pericarditis

A possible case of acute pericarditis is defined by the presence of:

- Typical chest pain (i.e., pain made worse by lying down and relieved by sitting up and/or leaning forward) and no evidence of any other likely cause of such chest pain.

A probable case of acute pericarditis is a possible case of pericarditis, or a case in a person with pleuritic or other chest pain not characteristic of any other disease, that, in addition, has one or more of the following:

- Pericardial rub, an auscultatory sign with one to three components per beat,
- ECG with diffuse ST-segment elevations or PR depressions without reciprocal ST depressions that are not previously documented, or
- Echocardiogram indicating the presence of an abnormal collection of pericardial fluid (e.g., anterior and posterior pericardial effusion or a large posterior pericardial effusion alone).

A case of acute pericarditis is confirmed if histopathologic evidence of pericardial inflammation is evident from pericardial tissue obtained at surgery or autopsy.

Appendix 3: Interpretation Support for Assessment of Screening ECGs

For a clearer and mutual understanding of inclusion criterion #12, the following provides clarifying explanations and examples pertaining to eligibility for enrollment.

Examples of subjects **eligible for enrollment**:

- Non-specific ST and T wave changes are not considered clinically significant and subject can be enrolled.
- Sinus bradycardia which does not require clinical intervention is not considered clinically significant and subject can be enrolled.
- Subjects who present with atrial disease which do not require clinical intervention, e.g. a pacemaker or drug treatment are allowed to be enrolled, as these can be considered not clinically significant. Examples are premature atrial contractions or ectopic atrial beats.
- Occasional premature ventricular contractions which do not require clinical intervention are not considered clinically significant and subject can be enrolled.
- First degree AV block or PR interval prolongations are also acceptable as long as they do not require clinical intervention, i.e. do not represent an indication for a pacemaker, and therefore the condition can be classified as not clinically significant.
- Right or left axis deviation which does not require clinical intervention is not considered clinically significant and subject can be enrolled.
- QTc prolongations < 500 ms which do not require clinical intervention are not considered clinically significant and subject can be enrolled. QTc prolongations > 500 ms which do not require clinical intervention should be discussed with the Medical Monitor before enrollment.

Examples of subjects **NOT eligible for enrollment**:

- Second or third degree atrioventricular block could represent significant heart disease and subject should not be enrolled.
- Incomplete left bundle branch blocks could represent significant heart disease and subject should not be enrolled.
- Significant ventricular disease represented by complete intraventricular conduction defects (complete left or right bundle branch block) must be considered clinically significant and subjects presenting with any such condition should not be enrolled. Left anterior or

posterior intraventricular fascicular blocks or hemiblock could represent ventricular disease and subject should not be enrolled.

- ST elevation consistent with ischemia, subject should not be enrolled.
- Two premature ventricular contractions in a row, subject should not be enrolled.

Appendix 4: Grading Scale for Lymphadenopathy

Grade 0 (normal finding):	No palpable lymph nodes or lymph nodes up to a diameter of 1 cm, soft, non-tender
Grade 1 (mild):	Slightly palpable lymph nodes or lymph nodes up to a diameter of 1 cm, bilaterally enlarged lymph nodes, signs of tenderness
Grade 2 (moderate):	Markedly palpable lymph nodes or lymph node diameter exceeds 1 cm, bilaterally enlarged lymph nodes, pain, skin redness, warmth, limiting instrumental daily life activities
Grade 3 (severe):	Markedly palpable lymph nodes or lymph node diameter exceeds 2 cm, generalized enlargement of lymph nodes, severe pain, general symptoms like fever and sweating limiting self-care daily activities

Appendix 5: Protocol Changes

Protocol Edition 3.0

Section 1.3 – Sponsor Signature Page

Team member change Coordinating Author: [redacted]

Section 1.4 – Responsibilities

Team member change Project Leader:

[redacted] to [redacted]

Team member position update:

Medical Monitor ~~/ Director Pharmacovigilance~~ [redacted]

List of Abbreviations

Updated to include **Lower Limit of Quantitation (LLOQ)**

Rationale: Lower Limit of Quantitation was not defined in Protocol Edition 2.0

Section 2.1 Introduction

Authorization was granted by the US Food & Drug Administration in September 2019 under the name JYNNEOS® (Registered in the US and EU).

Rationale: Updated to include the Biologics License Application approval under the name JYNNEOS®.

Section 2.5 – Clinical Profile of MVA-BN
Table 1 Overview of Clinical Trials with MVA-BN

POX-MVA-006 Reference changed from ~~Manuscript in preparation~~ to **Pittman et al., 2019**.

Rationale: Manuscript has been published since last protocol update.

Section 2.5.1 – Safety Overview of MVA-BN – Serious Suspected Adverse Drug Reactions

A total of seven (7 out of ~~7871~~ **9784** vaccinated subjects = 0.07~~9~~%) serious suspected Adverse Drug Reactions (ADRs) have been reported for MVA-BN so far (see Investigator Brochure, Ed.2~~1~~, Table22, Section 7.2.~~9~~10).

Rationale: Sentence and reference updated based on current MVA-BN IB edition.

Section 2.5.1 – Safety Overview of MVA-BN – Adverse Drug Reactions

Suspected ADRs, i.e. AEs for which there is a reasonable possible relationship to the trial vaccine, were reported in **22** ~~POX-MVA~~ **completed** clinical trials **with MVA-BN**~~completed~~ to date.

Rationale: Number of relevant clinical trials added, and sentence rephrased for clarity.

Section 2.5.1 – Safety Overview of MVA-BN – Cardiac Signs and Symptoms

Despite close cardiac monitoring, no ~~confirmed event of myo-/pericarditis~~ **signal of inflammatory cardiac disorders** has been ~~observed~~ identified in ~~any~~ **the** MVA-BN ~~trial~~ **clinical development program**.

Rationale: Statement updated to align with FDA requirements.

Section 2.5.2 Immunogenicity and Efficacy Overview of MVA-BN

The optimal dose of MVA-BN was selected based on results of the three ~~BN-sponsored~~ dose ranging studies (Table 1) and is defined as 0.5 mL dose of vaccine containing at least 0.5×10^8 $TCID_{50}$ (nominal titer of 1×10^8 $TCID_{50}$) for subcutaneous administration.

Rationale: Sentence corrected since two of the three dose finding studies were BN sponsored and one was sponsored by NIH.

Section 2.6.2 – Rationale for Data Collected

A Biologics License Application (BLA) for the licensure of the LF formulation of MVA-BN was ~~submitted~~ **approved** on ~~25Oct2018~~ **24Sep2019** based on available data.

Rationale: Statement updated to reflect the status of the LF BLA.

Section 7.1 – Immunogenicity Testing

Original Text:

The GMT is calculated per visit by taking the antilogarithm of the mean of the $\log_{10}$ titer transformations. *Antibody titers below the detection limit will be given an arbitrary value of one (1) for the purpose of calculation.*

…

Seroconversion is defined as:

- *Appearance of antibody titers ≥ assay detection limit for initially seronegative subjects.*
- *An at least two-fold increase of the antibody titer compared to the pre-existing baseline titer (at Visit 1) for subjects with a pre-existing antibody titer.*

New Text:

The GMT is calculated per visit by taking the antilogarithm of the mean of the $\log_{10}$ titer transformations. *Antibody titers below the lower limit of quantitation (LLOQ) will be given a value of half the LLOQ for the purpose of calculation.*

…

Seroconversion is defined as:

- *Appearance of antibody titers ≥ LLOQ for subjects with pre-vaccination titers below the LLOQ.*
- *An at least two-fold increase of the antibody titer compared to the pre-existing baseline titer (Visit 1) for subjects with a pre-existing antibody titer of at least the LLOQ.*

Rationale: Changes to the imputation of titer values below the LLOQ in the vaccinia-specific PRNT and ELISA were made based on a request from the Center for Biologics Evaluation and Research at the Food and Drug Administration with regard to the Biological License Application assessment for the liquid frozen formulation of MVA-BN, JYNNEOS® on 14 August, 2019.

Appendix 1: Toxicity Scale for Laboratory, Table 3 Toxicity Scale for Serum Chemistry

Grade 3 toxicity grading for *Bilirubin – when Liver Function Test is normal* was updated to 2.~~0~~ **1**- 3.0 x ULN.

Rationale: Table has been updated to reflect correct Grade 3 toxicity grading for Bilirubin – when Liver Function Test is normal.

Several typographical updates have been made throughout the document.

Protocol Edition 2.0

Section 1.3 – Sponsor Signature Page, Vice President for Clinical Strategy

Team member change: [redacted] to [redacted]

Section 1.4 – Responsibilities

Team member change Vice President for Clinical Strategy:

[redacted] -to [redacted]

List of Abbreviations

Updated to include **Electronic Data Capture (EDC)**

Rationale: Electronic Data Capture was not defined in Protocol Edition 1.0

Section 1.5 – Protocol Synopsis

Exclusion Criteria updated to include **Abnormal Troponin I level > ULN**

Rationale: Based on FDA feedback

Follow up (FU) Visit name changed from ~~FU Phone~~ to **FU Contact**

Rationale: Contact broadens the communication options for contacting the subjects

(The FU contact change was also made in Section 1.6 – Trial Procedure Schedule, Section 4.2.5 – Withdrawal from Second Vaccination, Section 4.3 – Trial Duration, Section 8.2.5 – Unsolicited AE, and Section 9.5 – Biometrical Evaluation.)

Section 2.5 – Clinical Profile of MVA-BN
Table 1 Overview of Clinical Trials with MVA-BN

~~IMVAMUNE~~ Formulation changes to **MVA-BN** Formulation

Rationale: Changed to MVA-BN for consistency throughout the document

Section 2.6.2 – Rationale for Data Collected

A Biologics License Application (BLA) for the licensure of the LF formulation of MVA-BN ~~is planned to be~~ **was** submitted **on 25Oct2018** based on ~~the already~~ available data.

Rationale: Statement updated to reflect the status of the LF BLA

Section 4.2.3 – Follow-Up (FU) Phase

Follow up Visit contact options updated from ~~phone~~ to **phone, email, or text**

Rationale: Multiple communication options increase the opportunity to obtain follow up information from subjects
(The FU contact change was also made in Section 8.2.3 – Physical Exam and Section 8.2.4 – Vital Signs.)

Section 8.1.3.3 – AESI

AESI definition for cardiac enzyme troponin I has been updated from ~~≥ 2 x ULN~~ to **> ULN** (≥ Grade **1**)

Rationale: Based on FDA feedback

Section 8.2.3 – Physical Exam ~~and Vital Signs~~

Vital signs removed from section title

Rationale: Vital signs information is located in Section 8.2.4

Section 8.2.8 – Safety Laboratory Measurements

Reference changed from ~~National Cancer Institute Common Toxicity Criteria Table~~ to **Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials**

Rationale: Section updated to reflect the correct toxicity grading reference

Section 8.3.1 – Reporting of SAEs

Text has been updated to reflect the current SAE reporting process.

All SAEs occurring throughout the entire course of the trial have to be reported to the BN PV Department. The CTS **will** ~~has to send the~~ **completed the SAE form located in the electronic data capture system (EDC) within 24 hours of becoming aware of the AE. Paper SAE forms should only be completed in the event the EDC system is not operational. Paper SAE forms will be sent** by email or fax to the BN PV Department within 24 hours of becoming aware of the AE

Rationale: The preferred SAE reporting process via the EDC system was not included in Protocol Edition 1.0.

Section 8.3.2 – Reporting of AESIs

Text has been updated to reflect the current AESI reporting process.

All AESIs occurring throughout the entire course of the trial have to be reported to the BN PV Department. The CTS ~~has to send the~~ **will completed the ~~AESI~~ appropriate form in the EDC within 24 hours of becoming aware of the AESI. Paper forms should only be completed in the event the EDC system is not operational. Paper forms will be sent** by email or fax to the BN PV Department within 24 hours of becoming aware of the ~~AE~~ AESI.

Rationale: Language has been updated to reflect the correct AESI reporting process.

Section 9.5 – Biometrical Evaluation

Text has been updated to reflect that solicited local and general AEs will begin on **Day 1**, day of vaccination, for an 8-day period.

Rationale: Inconsistency corrected with the day of first vaccination change from ~~Day 0~~ to **Day 1. Day 1** is used for compliance with FDA data submission standards.

Appendix 1: Toxicity Scale for Laboratory, Table 3 Toxicity Scale for Serum Chemistry

Table 3 has been updated to reflect the correct toxicity grading

Rationale: Table has been updated to reflect toxicity grading based on Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials reference

Appendix 1: Toxicity Scale for Laboratory, Table 4 Toxicity Scale for Hematology

Table 4 has been updated to reflect the correct toxicity grading

Rationale: Table has been updated to reflect toxicity grading based on Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials reference