



DEPARTMENT OF HEALTH & HUMAN SERVICES

**Public Health Service
Food and Drug Administration**

Memorandum

Date: August 23, 2019

To: The File STN 125678 / 0

From: Alonzo García, DVP
CMC/Product reviewer

Through: Jerry Weir, DVP / Division Director

Keith Peden, DVP / Branch/Lab Chief

Anissa Cheung, DVP
CMC/Product reviewer

Cc: Bharat Khurana, DVRPA,
Chair of the Review Committee

Sudhakar Agnihothram, DVRPA
Josephine Resnick, DVRPA

Sponsor: Bavarian Nordic A/S (BN-K)

Subject: CMC / Product Review of Original BLA STN 125678

Product: Smallpox (Modified Vaccinia Ankara) Vaccine, Live, Non-replicating
(JYNNEOS, also referred to as MVA-BN)

Submission Date: October 25, 2018

Action Due Date: September 24, 2019

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1. **BLA#:** STN 125678/0
2. **APPLICANT NAME AND LICENSE NUMBER:** Bavarian Nordic A/S
3. **PRODUCT NAME/PRODUCT TYPE**
 - Non-Proprietary/Proper/USAN: Smallpox (Modified Vaccinia Ankara) Vaccine, Live, Non-replicating
 - Proprietary Name: JYNNEOS
4. **GENERAL DESCRIPTION OF THE FINAL PRODUCT**
 - *Pharmacological category:* TBD
 - *Dosage form:* Suspension for injection
 - *Strength/Potency [the concentration of drug product, type of potency assay(s)]:* 0.5×10⁸ infectious units (Inf. U) per 0.5 mL dose (b) (4) - based assay]
 - *Route of administration:* Subcutaneous
 - *Indication(s):* Active immunization against smallpox in adults aged 18 years and older
5. **MAJOR MILESTONES**

Filing Meeting: December 7, 2018
Filing Action: December 24, 2018
Mid-Cycle Communication: February 14, 2019
Late-Cycle Meeting with the Sponsor: July 23, 2019
BLA Action Due Date: September 24, 2019

6. **CMC/QUALITY REVIEW TEAM**

Reviewer/Affiliation	Section/Subject Matter
Clement Meseda, OVRD/DVP	Nonclinical studies in the non-human primate (cynomolgus macaques) challenge models with monkeypox (Section 4.2.1.1)
Anissa Cheung, OVRD/DVP	Drug Substance and Drug Product Manufacture, Adventitious Agents Safety, and Clinical Assays
Lei Huang, DB/OBE/VEB	PRNT and ELISA (Section 5.3.1.4)
Most Nahid Parvin, OCBQ/DBSQC Salil Ghosh, OCBQ/DBSQC/ LACBRP	Release assays Release assays

7. **INTER-CENTER CONSULTS REQUESTED**

Reviewer/Affiliation	Section/Topic	In agreement with consult recommendations (Yes/No)
none	N/A	N/A

8. SUBMISSION(S) REVIEWED

Date Received	Submission	Comments/ Status
October 25, 2018	STN 125678.0	
November 23, 2018	STN 125678.003	New gentamicin ELISA method acceptable
January 9, 2019	STN 125678.009 (response to IR #8)	Acceptable
February 22, 2019	STN 125678.019	120-Day stability data for (b) (4) DP acceptable
February 12, 2019	STN 125678.021 (response to IR #13)	PRNT assay LLOQ
February 15, 2019	STN 125678.022 (response to IR #15)	Acceptable
March 20, 2019	STN 125678.026 (response to IR #19)	Updated stability data
April 12, 2019	STN 125678.030 (response to FDA form 483, Observation 2)	Gentamicin assay specification acceptable
May 08, 2019	STN 125678.036 (response to IR #23)	Revised labels for package and single-vial
May 23, 2019	STN 125678.038 (response to IR #25)	Acceptable
July 5, 2019	STN 125678.042 (Follow-up response to IR #25, comment 7a)	Acceptable
July 31, 2019	STN 125678.047 (response to IR #30)	Acceptable

9. REFERENCED REGULATORY SUBMISSIONS (e.g., IND, BLA, 510K, MASTER FILE, etc.)

Submission Type & #	Holder	Referenced Item	Letter of Cross-Reference	Comments/Status
none	N/A	N/A	N/A	N/A

10. REVIEWER SUMMARY AND RECOMMENDATION

A. EXECUTIVE SUMMARY

This BLA (STN 125678) is for a Smallpox (Modified Vaccinia Virus Ankara) live, non-replicating, vaccine. The proprietary name for this vaccine is JYNNEOS, and is also referred to as MVA-BN in this memo. JYNNEOS consists of purified Modified Vaccinia Virus Ankara-Bavarian Nordic (MVA-BN) in Trometamol-buffered saline and is presented in a single-dose glass vial as a liquid frozen formulation with at least 0.5×10^8 Inf. U/mL per 0.5 mL. At the time of administration, JYNNEOS is allowed to thaw to obtain a vaccine suspension for injection. The JYNNEOS vaccine is indicated for active immunization against variola virus, the causative agent of smallpox disease, and monkeypox virus disease. The MVA-BN vaccine virus is non-replicating in human cell lines (as well as other mammalian cells) and is shown to be avirulent in immune-compromised mice.

This review covers: (i) all relevant quality-related information contained in the BLA (Sections 3.2S, 3.2.P, 3.2.A.2 and 3.2.R eCTD); (ii) clinical assays (including Vaccinia-specific ELISA and Vaccinia-specific PRNT described in BLA Section 5.3.1.4 (entitled, *Report of bioanalytical and analytical methods for human studies*); (iii) CMC-related nonclinical study reports described Section 4.2.1.1 (including murine models based on intranasal challenge with vaccinia virus WR strain [vv-WR] and Ectromelia virus [ECTV]); and (iv) CMC-related parts of Amendments 3, 9, 19, 21, 22, 26, 30, 36, 38, 42 and 47. Nonclinical studies conducted in three (3) non-human primate (cynomolgus macaques) models using intravenous, intratracheal and aerosol challenge with monkeypox (MPXV) were evaluated in a separate review by another DVP CMC/product reviewer (Dr. Clement Meseda).

The drug substance (purified MVA-BN) manufacturing is performed at the Bavarian Nordic A/S facility (BN-K) located in Kvistgaard, Denmark, and the drug product manufacturing is performed at the (b) (4) facility, (b) (4). Release of drug substance (DS) and drug product (DP) is performed at Bavarian Nordic A/S facility in Kvistgaard, Denmark.

The current commercial-scale manufacturing process of the drug substance (purified DS bulk) is a (b) (4) process, which can be divided into (b) (4) main steps, (b) (4)

The MVA-BN in the (b) (4) is concentrated and purified by (b) (4) steps performed with a tangential-flow filtration (TFF) unit (equipped with a (b) (4)). The purification of MVA-BN is accompanied by a Benzonase digestion of host-cell DNA followed by a (b) (4) (with Tris - Sodium chloride [NaCl] buffer) and (b) (4) concentration step by TFF. Here, MVA-BN is purified by removal of (b) (4) in the homogenate. The purified DS bulk is collected into (b) (4) and distributed into (b) (4) and stored at (b) (4) prior to formulation. A full batch size of the purified DS bulk is (b) (4) per manufacturing process run.

The manufacturing process of the MVA-BN drug product (DP) at (b) (4) can be divided into two main steps: (1) (b) (4) formulation of final bulk by (b) (4) Formulation Buffer (*i.e.*, Tris-sodium chloride buffer) and DS to obtain the target titer (3.8×10^8 Inf. U/mL) and, (2) aseptic filling and assembly of the filled final vial container (comprised of a sterile 2 mL (b) (4) glass vial, stopper, cap and crimp). The final vial DP containers are inspected, labeled and packaged into folding boxes. The entire DP lot is placed into a freezing chamber and, afterwards transferred to a storage freezer (at either -20°C (b) (4)). The expiration date of the MVA-BN DP final container is dependent on the storage condition as follows: 36 months at -20°C ± 5°C (b) (4).

The MVA-BN (b) (4) drug product are manufactured under cGMP conditions. The development of the manufacturing process experienced three (3) major process upgrades, namely: (1) early development/pilot scale ((b) (4) process); (2) industrial-scale (at (b) (4)); and (3) commercial-scale (at BN-K). During different stages of development, the production scale of the (b) (4) increased from (b) (4).

(b) (4). The filling lot sizes for the DP were (b) (4) vials (b) (4); frozen liquid formulation), (b) (4) vials (b) (4) process; (b) (4) formulation), (b) (4) vials (b) (4) formulation), (b) (4) vials (industrial-scale process, using (b) (4) batch(es) per DP filling; frozen liquid formulation), and (b) (4) vials (commercial-scale process, using (b) (4) batches per DP filling). The industrial (b) (4) manufacture process (in 2003 – 2005) was supported by the 2005 validation at (b) (4) and later at BN-K, after the (b) (4) process was transferred to the Bavarian Nordic A/S facility (in 2005) to increase the commercial supply of the vaccine product. Consequently, several changes were made to optimize and standardize the commercial-scale (b) (4) process to improve (b) (4).

(b) (4) which were supported in process validation and qualification studies of 2007, 2008, and 2013. In 2016, the CEF cell preparation step was changed from a (b) (4).


(b) (4) (The former (b) (4) CEF cell process distributed the CEFs into (b) (4)). In 2017, the (b) (4).

(b) (4) to improve the DS manufacturing yield. Clinical trial material for Phase I/II studies was obtained from the pilot scale process up to the commercial-scale (b) (4) manufacturing (b) (4) process. The initially validated DP formulation and filling process (on Line (b) (4) at (b) (4)) for industrial- and commercial-scale production of the DP was supported up to 2007. The DP process was transferred to a different filling line (Line (b) (4) at (b) (4)) essentially changed and was subsequently revalidated in 2007 and 2008. Clinical trial DP materials for the lot-consistency study (POX-MVA-013) and the Phase III efficacy study (POX-MVA-006) were obtained from the commercial-scale (b) (4) manufacturing (b) (4) process supported by the 2008 (b) (4) DP PV studies and 2013 (b) (4) PV study, respectively. The product quality of the MVA-BN (b) (4) drug product were shown to remain comparable at different stages of development based on quantitative in-process controls (IPCs), release test analytical parameters, safety test results, impact assessment studies, stability studies, and preclinical efficacy and immunogenicity studies. Critical-process parameters (CPPs) and critical quality attributes (CQAs) were

identified during the development and validation of the (b) (4) DP manufacturing process using a risk-based assessment of the impact of various CPPs on the CAQs as it relates to safety of humans.

The ability of the manufacturing process to produce MVA-BN (b) (4) DP of consistent quality was assessed using comparability study data that were generated through quantitative IPC and analytical release tests for determination of cell count, virus titer (potency) by TCID₅₀ assay, process-related impurity content (e.g., Benzonase and gentamicin), host cell-related impurity content (e.g., host cell DNA, host cell protein and total protein), microbiological purity (e.g., sterility, (b) (4) and bacterial endotoxins), adventitious agents (by conventional *in vitro* and *in vivo* tests), (b) (4) activity by (b) (4) assay, and appearance by visual inspection. Quality of the (b) (4) DP is also evaluated in the long-term and accelerated stability study data generated from the following test parameters: virus titer (by TCID₅₀ assay and (b) (4)-based assay), appearance, pH, sterility, genetic identity (by (b) (4) analysis), and container closure integrity. The assigned shelf life of 36 months at -20°C for the MVA-BN DP is supported by several primary stability DP lots obtained from three (3) PV studies (conducted in 2008, 2013 and 2014) and the Phase III DP lots used for the clinical lot consistency study and the efficacy study. All five (5) stability studies performed at -20°C with the primary stability DP lots are completed except for stability testing of the 2014 PV DP lots, which is ongoing and up to (b) (4) months of stability results were shown to be satisfactory. The proposed shelf life of (b) (4) months at (b) (4)°C for the DP is currently supported by three (3) primary stability DP lots manufactured in the 2014 PV campaign and statistical analysis of available virus titer data. The stability study conducted with the 2014 PV lots at (b) (4)°C is ongoing, and up to (b) (4)-months of stability results were shown to be satisfactory. Also, the enclosed statistical evaluation of a shelf life for virus titer projected a shelf life of (b) (4) at (b) (4)°C. Therefore, the proposed shelf life of (b) (4) months at the intended storage temperature of (b) (4)°C is reasonable.

(b) (4)



(b) (4)

The following review issues were encountered and resolved:

A number of issues occurred in the validation of the vaccinia-specific PRNT:

- The *precision* of the very low (VL) titer (at GMT of ~ (b) (4)) was found to be incorrectly assessed by the biostatistical reviewer. In the evaluation of precision according to (b) (4) titer range of (b) (4), experimental titer values occurring below a titer of (b) (4) were excluded from the analyses (according to the Validation Protocol). Upon reviewing the precision datasets comprised of a total (b) (4) test values, it was noted that a substantial number of test values (*i.e.*, N = (b) (4)) with a titer of (b) (4) were excluded from the precision analyses. The removal of these low titer results from the precision datasets was found to decrease the estimated variability of the assay around the (b) (4) range and thus potentially resulting in an erroneous interpretation that the assay is sufficiently precise within the titer range of (b) (4). This discrepancy was explained to BN in communication (enclosed in IR #13; dated 16-JAN-2019). In Amendment 21 (*submission date: 12-FEB-2019*), BN concurred with the Agency's evaluation and acknowledged that it was questionable to ignore test values at a titer of (b) (4) of the LOD in the precision analyses.
- The proposed LOD and LLOQ at a titer of (b) (4), respectively, (for PRNT versions (b) (4)) were identified as another issue by the biostatistical reviewer. Both LOD and LLOQ values were estimated using an extrapolation method of the dilutional-linearity plot generated from test values of (b) (4) dilutions of (b) (4) (*i.e.*, from (b) (4)). Although the ICH Q2(R1) guidance document indicates that extrapolation can be used for estimating the LOD (which may be subsequently validated by an independent analysis of samples known to be near or prepared at the DL), this practice does not necessarily apply to estimating the LLOQ. Therefore, this discrepancy of setting the LLOQ based on extrapolation was explained in a communication (enclosed in IR #13; dated, 16-JAN-2019) to BN, whereas the LOD at a titer of (b) (4) was considered likely based on the nature of the titer determination of the PRNT. In addition, this communication (IR #13) recommended that the limits should be validated by the testing of a suitable number of samples prepared at or near the LOD in order to validate the LLOQ. In Amendment 21 (*submission date: 12-FEB-2019*), BN provided a more detailed explanation in support of the extrapolation process used to estimate the LLOQ (including the LOD) for the PRNT. However, the review of the extrapolation approach for estimating LLOQ and LOD along with BN's rationale did not sufficiently address the discrepancy concerning the setting of the proposed LLOQ. Therefore, a separate communication (enclosed in IR #25; dated, 23-MAY-2019) was sent to BN to reiterate the discrepancy associated with setting the LLOQ to a titer of (b) (4) and to recommend that the provisional LLOQ can be established based on confirmatory experimental data, involving the testing of a

suitable number of samples with titers (at or near the target range of the LLOQ) to evaluate the relative accuracy and precision at the proposed LLOQ or to adjust the LLOQ accordingly.

In a follow-up response (to IR #25) submitted to Amendment 42 (submission date: 05-JUL-2019), BN indicated that they extended the linearity study of the PRNT (assay (b) (4)) down to titers at the LLOQ of (b) (4). The linearity testing was performed whereby log10 titer values from a total of (b) (4) fold dilutions of (b) (4) (from (b) (4)) were plotted against (b) (4) on a regression line. While data points from neat to (b) (4) dilution sample of (b) (4) were plotted on the regression line, the highest sample dilution (i.e., (b) (4)) was not on the regression line but had a median GMT of (b) (4), which was calculated in a range assessed to be less precise. Consequently, a hypothetical change of the LLOQ at a titer 20 was proposed by BN and evaluated in terms of the impact the change had on recalculating PRNT (assay (b) (4)) titer results in the pivotal clinical trials (i.e., POX-MVA-006 and POX-MVA-013). As reported by BN, the shift of the LLOQ to 20 did not lead to a change in the conclusions of the two pivotal clinical studies. The biostatistical review of BN's expanded linearity test results determined that LLOQ at a titer of (b) (4) resulted in substantial assay bias, whereas the (b) (4) dilution sample (i.e., GMT of ~20) exhibited lower assay bias (of (b) (4)), which was considered more appropriate to set the LLOQ value at a titer of ~20. In addition, the LLOQ value of 20 was determined to be suitable for PRNT (assay (b) (4)).

To receive concurrence from BN regarding the proposed LLOQ set at a titer of 20 for the PRNT, a communication (enclosed in IR #30; dated, 15-JUL-2019) was sent to BN. In Amendment 47 (submission date: 31-JUL-2019), BN agreed to revising the immunogenicity data enclosed in the Prescribing Information (PI) to reflect the change of the LLOQ.

B. RECOMMENDATION

- **APPROVAL**

I recommend approval of this BLA.

- **COMPLETE RESPONSE (CR)**

No deficiencies or CRs to be communicated to Bavarian Nordic (BN-K).

III. SIGNATURE BLOCK

Reviewer/Title/Affiliation	Concurrence	Signature and Date
Alonzo Garcia, DVP /CMC reviewer	Concur	
Keith Peden, DVP / Branch/Lab Chief	Concur	
Jerry Weir, DVP / Division Director	Concur	

3.2.S DRUG SUBSTANCE [Substance - Manufacturer]

3.2.S.1.1 - 1.3 Nomenclature, Structure and General Properties

Nomenclature:

The active substance of the MVA-BN Smallpox vaccine is the live purified Modified Vaccinia Ankara-Bavarian Nordic (MVA-BN). Other nomenclatures of the vaccine are MVA-BN and IMVAMUNE. The proprietary name proposed for this submission is JYNNEOS.


Structure:

The MVA-BN vaccine virus is a member of the *Orthopoxvirus* genus (of the *Poxviridae* family). Orthopoxviruses possess the following characteristics:

- Virions are large and have an asymmetrical brick shape (or flatten-barrel shape) appearance with dimensions of 360 nm × 270 nm × 250 nm based on electron microscopy data.
- Virions contain outer envelope structure (1 to 3 membranes) derived from the host cell but contain virus-specific proteins. The internal cylindrical viral core contains the genome DNA and viral proteins that are organized to form a nucleoprotein complex.
- The poxvirus genome consists of a linear double-stranded DNA of 130 to 230 kbp (kilo-base pairs) in size with covalently closed hairpin ends.
- The genomic DNA in MVA-BN is comprised of (b) (4) nucleotides. Overall, the MVA-BN genome consists of (b) (4) open reading frames (ORFs) that encode for polypeptide proteins spanning more than (b) (4) amino acids in size.
- In comparison with the parental Vaccinia Virus Ankara strain (*Chorioallantois Vaccinia Virus Ankara*, CVA), the viral genome lost (b) (4) bp due to multiple passaging of CVA in chicken embryo fibroblasts (CEF) cells. Thus, MVA-BN has (b) (4) deletions totaling over (b) (4) bp that resulted in deletion/truncation of (b) (4) ORFs as well as multiple short deletions, insertions and point mutations, which resulted in gene fragmentation/truncation, short internal deletions, and amino acid exchanges.

General Properties:

(b) (4)



(b) (4)

3.2.S.2 Manufacture

3.2.S.2.1 Manufacturer(s) and Contract Laboratories

List of Manufacturer(s) and facilities:

Drug Substance (DS) is manufactured by:

Bavarian Nordic A/S
Hejreskovvej 10A
3490 Kvistgaard, Denmark

Master Seed Virus (MSV) stock and Working Seed Virus (WSV) were manufactured according to cGMP by:

(b) (4)

The Drug Substance is released by:

Bavarian Nordic A/S
Hejreskovvej 10A
DK-3490 Kvistgaard, Denmark

Testing of the MSV, WSV and DS intermediates is performed either in-house or by approved Contract Research Organizations (CRO). Tabulated overview of the name, address and responsibility of the testing facilities are provided below:

Testing facilities involved in DS release testing

Name and Address	Responsibility
Bavarian Nordic A/S Hejreskovvej 10A 3490 Kvistgaard, Denmark	DS, process intermediate and stability testing: (b) (4)
Bavarian Nordic (b) (4)	DS, process intermediate and stability testing: (b) (4)

(b) (4)

Testing facilities involved in WSV characterization

Name and Address	Responsibility
Bavarian Nordic A/S Hejreskovvej 10A 3490 Kvistgaard, Denmark	Stability testing: (b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

Storage and Shipping

The DS is shipped frozen to the drug product manufacturing site located in (b) (4). Information regarding the shipment qualification and validation is enclosed in Section 3.2.S.2.5.

3.2.S.2.3 Control of Materials

All commercial starting materials, raw materials and reagents used in the DS manufacturing process including the generation of MSV/WSV are documented with Certificate of Analysis (CoA) along with origin and source information from suppliers. The enclosed CoA documents provide adequate information certifying that all materials meet specifications for release testing and identity testing with their respective specifications. If applicable to the raw materials, CoAs provide a brief statement regarding animal-component-free (ACF) status and TSE/BSE risks of the starting

materials. A list of ingredients from the BITS-ABC database has been reviewed and compared with a summary of ingredients listed in a *Components Information Table* (CIT) (enclosed in **Appendix I** of this memo), and the documented information was found to be acceptable.

Control of Raw Materials NOT of Biological Origin

A tabular summary of ancillary and raw materials not derived of biological origin or sources, their intended use(s) and the process step(s) where they are used in the process is provided in **Table 4**.

(b) (4)

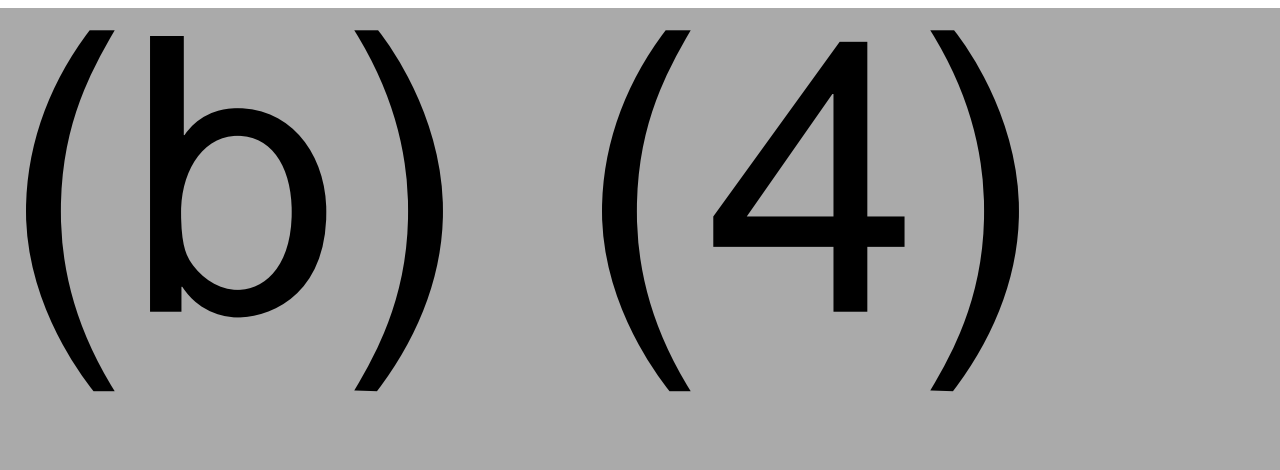
Reviewer's comments:

All materials (i.e., material, raw material, and ancillary reagent) listed in **Table 4** are found to be appropriate for their intended use in the DS manufacturing process. The controls on all input materials are documented in their respective CoA reports, which indicate release and confirmatory tests to demonstrate quality, (b) (4) and identity of all materials according to test specifications. In addition, in-house release testing by BN of all incoming raw materials (apart from (b) (4)) is shown in their respective CoA documents. The source report on raw materials used to produce the (b) (4) medium is provided in the supplier's CoA document (3.2.A.2-Appendix 9). Although (b) (4) medium is declared a raw material comprised of components with no biological origin, it contains (b) (4) components that are manufactured chemically, biosynthetically (e.g., (b) (4)) or obtained from mineral sources. However, (b) (4) components (b) (4), which are indicated to be potential process-related impurities in the DS product in Module 3.2.S.3.2. Also, until 2013, (b) (4) source report of raw material for in (b) (4) identified (b) (4) components (i.e., (b) (4)) that were manufactured with materials of secondary animal origin (e.g., (b) (4)).

The (b) (4) DS manufacturing process step is intended to (b) (4). Among the input materials, gentamicin and Benzonase are process-related impurities that are tested for in the (b) (4) release testing program.

Control of Raw Materials of Biological Origin

A tabular overview of input raw materials derived from biological origins or sources, their intended use(s) and the process step(s) where they are used is provided in **Table 5**

**Reviewer's comments:**

supplied from (b) (4) chicken flocks from suppliers (b) (4)

chicken flocks ((b) (4)) are certified

to be (b) (4) for the production and quality control of vaccines.

(b) (4) eggs from chicken flocks are (b) (4)

Master and Working Viral Seeds

(b) (4)

			(b) (4)
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Reviewer's comments and Information Request:

- This Section described aspects of the overall control strategy which includes release tests of the DS harvest, control tests of in-process material (IPC), and Process Monitoring Tests (PMTs) in combination with defined Process Parameters. From a CMC perspective, the release tests, IPC tests and PMTs are appropriate features in the overall control strategy DS, as described in **Module 3.2.R-Control Strategy**.
- (b) (4)
- The release testing approach for production CEF cells appeared inconsistent as applied to (b) (4) CEF cell preparation stream (b) (4)

. Briefly, (b) (4)

Secondly, BN clarified that (b) (4) cells, obtained from the production CEF cells, cannot be cultured and handled in the same manner as suspension culture for the following reasons:

(b) (4)

(b) (4)

- The following minor discrepancy was noticed in the set of in-process control (IPC) tests in the DS process (Table 7). Module 3.2.S.2.2 indicated that (b) (4)

(b) (4)

not included in Module 3.2.S.2.4 (Table 4). This discrepancy led to an IR comment which requested the submission of an updated version of Table 4 in Module 3.2.S.2.4 to include the (b) (4) test in the CEF cell preparation step.

- Section 3.2.S.2.4 does not provide detailed information regarding the storage conditions (i.e., container closure, storage duration and temperature) for the purified MVA-BN DS product. Also, information on the stability of critical intermediate(s) is not provided in the Section.
- The 483 inspectional observation report of the BN-K DS facility (dated, 01-MAR-2019) found that the investigation of sterility of the CEF cells was not thorough to prevent reoccurrence of microbial contamination. This led to the implementation of setting a (b) (4)

Thus, a (b) (4) (this change is dated, 30-JUN-2019).

Analytical procedures (Section 2):

This Section provides the method descriptions of analytical procedures used in the testing of critical steps and intermediates. As indicated, the analytical test procedures described in this Section have undergone validation evaluation.

(b) (4)

(b) (4)



3.2.S.2.5 Process Validation and/or Evaluation

General validation approach:

An overview of the process validation (PV) used to evaluate the control of the commercial-scale DS manufacturing process to adequately and consistently produce DS product is described. The PV evaluation is performed according to predetermined acceptance criteria for in-process control (IPC) tests, established critical quality attributes (CQAs), and other product-related characteristics. Thus, during each PV study, a minimum of (b) (4) DS batches is manufactured, which is planned and executed according to process validation protocols (PVPs). After the PV, the process performance and product quality are monitored to ensure that the DS manufacturing process remains in a state of control over time as part of a continued process verification (CPV) program. Three types of parameters controlled are indicated during the DS manufacturing process: *(i)* In-process controls (IPCs), *(ii)* Process Monitoring Tests (PMTs) and *(iii)* release test. (Both IPC and release tests comprise the analytical output parameters of the PV).

The development of the control strategy for the MVA-BN DS manufacturing process is described in BLA Section **3.2.R-Control Strategy**. The control strategy includes several Critical Quality Attributes (CQAs), that are associated with DS quality, and Critical-process parameters (CPPs, provided in **Appendix C** of this memo), which can

have an impact on the CQA of the DS. A total of thirty-one (31) Quality Attributes (QA) were identified, of which 21 of them were assessed to be critical QAs, and the remaining QAs were assessed to be non-Critical QAs, which are provided in **Table 10**. The criticality of each QA is based on risk assessments that is intended to rate the levels of severity of the QAs and the consequences of failure of each QA to humans, as described in BLA Section **3.2.R-Control Strategy**.

(b) (4)

(b) (4)

N/A: not applicable

Process Validation summary (Sections 1.3 and 1.4):

A summary of the development, establishment, and characterization of the DS manufacturing process as a “(b) (4)” process leading to the development of the industrial-scale (at (b) (4)) and commercial-scale (at BN-K) manufacturing process is provided. BLA Section 3.2.S.2.5 describes the process validation studies performed at (b) (4) during 2005 and at BN-K during 2007, 2008, 2013, 2016 and 2017.

2005 Process Validation (PV) of DS process at (b) (4) (Section 1.3):

The industrial-scale DS manufacturing process (initially developed at (b) (4)) incorporated (b) (4) and a purification process step by tangential flow filtration (TFF). The PV evaluation of the industrial-scale process, during OCT/NOV-2005, scheduled the manufacture of (b) (4) DS batches (*PV Batch No.* (b) (4)). (The DS batches were subsequently used for validation of the drug product (DP) process at (b) (4)). The PV study was performed with pre-defined operational process parameters (PP) according to “set point” and acceptance criteria for IPC and release testing.

Review comments:

The enclosed 2005 Process Validation information showed that the industrial-scale DS process (at (b) (4)) can consistently produce the MVA-BN DS. Quantitative analyses data from IPC and release testing of output quality parameters of the (b) (4) PV DS batches were shown to fulfill the acceptance specification criteria. Therefore, the DS manufacturing process was considered validated.

2007 Process Validation (PV) of MVA-BN DS process at BN-K (Section 1.4):

In 2005, the DS process was transferred to the Bavarian Nordic production facility (in Kvistgaard, Denmark; BN-K) to increase the commercial supply of the MVA-BN vaccine product. Due to the higher capacity of the DS process, a few adaptations were introduced during the re-optimization of the DS process. Thus, the DS manufacturing process was re-validated at BN-K during FEB/MAR-2007 by the manufacture of (b) (4) PV batches (*PV batch* (b) (4); production scale: (b) (4)). The PV was performed using pre-defined operational process parameters according to “set points” and acceptance criteria for IPC and release testing defined in the Process Validation Protocol (PVP) and against revised acceptance criteria. (Revised acceptance criteria were determined from (b) (4) demonstration DS batches manufactured using the optimized process. The mean values and ranges [mean \pm (b) (4) SD] were calculated for (b) (4) DS demonstration batches (summarized in this BLA Section). The product/CMC information for the 2007 PV study has been submitted to Amendment 85 of IND 11596 (submission date: 17-DEC-2007), and a full review memo was prepared.

Review comments:

The enclosed 2007 Process Validation study information indicated that the commercial-scale DS process (developed at BN-K) can consistently produce MVA-BN

DS. Process parameters were maintained within pre-defined ranges, and IPC and release test results were shown to meet the acceptance specification criteria, except for a few minor IPC deviation results as indicated:

- At the TFF (b) (4) process step, (b) (4) PV batches (i.e., (b) (4)) displayed deviations in (b) (4) results that occurred above the original acceptance range as defined in the PVP, and a (b) (4) OOS result occurred in PV batch (b) (4) .
- At the TFF (b) (4) process step, the same (b) (4) PV batches displayed deviations in (b) (4) results that occurred above the original acceptance range as defined in the PVP.

These deviation results were re-evaluated according to revised acceptance criteria (shown in Table 20 of the BLA Section) which were established based on mean values and ranges [mean \pm (b) (4) SD] that were calculated from manufacturing data of (b) (4) DS demonstration batches. Thus, it was concluded that the DS manufacturing process at BN-K was validated.

2008 Process Validation (PV) of MVA-BN DS process at BN-K (Section 1.5):

During the 2007 and 2008 period, several process steps were adapted at BN-K to improve and maintain high DS product yields. The process changes were intended to enhance the (b) (4) process and to limit (b) (4)

Thus, the revalidation of the DS process in SEPT/OCT-2008 was conducted through the manufacturing of (b) (4) PV batches (PV batch (b) (4)

manufacture scale: (b) (4)). The 2008 PV DS batches were subsequently placed into the stability program as stability indicating batches, as described in Module 3.2.S.7.3. In addition, the 2008 PV DS batches were used in the validation activities of the Drug Product (DP) process at (b) (4), which is covered in Module 3.2.P.3.5.). The validation status of the DS manufacturing process was considered unchanged up to 2013. The process validation since 2008 supported the DS manufacturing process used for the production of clinical trial material for the Phase III lot-consistency study (POX-MVA-013).

Process validation procedure and results (Section 1.5.3):

The process validation study was performed with pre-defined process parameters according to set point. The IPC and release testing acceptance criteria in the PVP were implemented based on the evaluation of (b) (4) technical characterization batches (including (b) (4) PV batches) manufactured using the validated DS process at BN-K. The PV data evaluation focused on the ability of the process parameters and product attributes to fulfill pre-defined acceptance ranges and criteria. The 2008 PV evaluation covered the following:

- Operational process parameters (i.e., input parameters such as (b) (4)).
- Product attributes (i.e., quantitative output parameters such as (b) (4)).
- Quality parameters (or safety parameters) as described above.

Production data (Section 1.5.3.1):

The production data consisted of process parameters and results obtained from the manufacture of (b) (4) PV batches, according to the (b) (4) manufacturing operational units (MOUs): (b) (4)

The (b) (4) PV batches were manufactured while maintaining process parameters within pre-defined set points with some minor deviations. Nonetheless, the production data indicated a consistent manufacturing process despite the minor deviation observed in some process parameters. The reported deviations were assessed to not be critical for product quality or for the process validation status. Overall, the IPC and releases test results were shown to meet their corresponding acceptance specification criteria. All safety test parameters were shown to conform to their corresponding acceptance criteria (summarized in this BLA Section). Thus, based on the qualitative analyses data, the PV DS batches were considered to have consistent quality.


Additional studies performed during 2008 PV study – (b) (4) (Section 1.5.4.1):

(b) (4)

2013 Process Validation (PV) of MVA-BN DS process at BN-K (Section 1.6):

Multiple changes were implemented following the revalidation of the commercial-scale DS process at BK-K in 2008 (these changes are summarized in this BLA Section). The individual changes on their own were not considered significant to initiate a revalidation action. Nevertheless, the validation status of the DS process was considered acceptable. Thus, the revalidation of the DS process was performed during

(b) (4)



Reviewer's comments:

The 2013 Process Validation study information on the production of (b) (4) PV DS batches indicated that the DS process consistently produced the MVA-BN DS. Process parameters were maintained within pre-defined ranges, and IPC and release test results were shown to meet the acceptance specification criteria. Thus, the documented changes implemented in the DS process were considered to have a minor impact on the ability of this process to produce DS of consistent quality.

2016 Process Validation (PV) of MVA-BN DS process at BN-K (Section 1.7):

In APR/MAY-2016, the MVA-BN DS process underwent re-re-validation following several changes implemented to the process. Thus, the following changes were evaluated:

(b) (4)

[REDACTED]

The (b) (4) process was implemented to further improve the process handling of the CEF cells and reduce the risk of loss of an entire DS batches due to (b) (4)

[REDACTED]

The 2016 PV study information was submitted to Amendment 343 of IND 11596 (submission date: 16-MAR-2017) and a full review memo was prepared.

The evaluation of the changes in the DS process was performed by originally manufacturing (b) (4) PV batches ((b) (4)) scheduled for full-scale batch (b) (4) production (according to the PV protocol). However, due to several Out-of-Specification (OOS) (b) (4) results that occurred in three process steps (i.e., TFF (b) (4), TFF (b) (4), and DS), the PV study evaluated an additional (b) (4) full-scale DS batches ((b) (4)). Incidentally, PV batch (b) (4) was excluded from the PV study due to a (b) (4) test failure (most likely caused by (b) (4)). Thus, (b) (4) batches were used in the PV evaluation.

The pre-defined key process parameters (i.e., (b) (4)) for the PV batches are summarized in this BLA Section. All other process parameters were maintained according to set points.

Process validation results (Section 1.7.4):

All (b) (4) PV batches were executed with key process parameters at pre-defined set points (batch summaries are summarized in the BLA). However, for each batch, the number of deviations was indicated. All deviations were assessed to have no impact on product quality of the DS material nor were they associated with the process changes or process performance.

Evaluation of CEF cell preparation and yield:

CEF cell yield measurements from each preparation (b) (4) (i.e., (b) (4)) of the (b) (4) PV batches are summarized in this BLA Section. Overall, the (b) (4)

(b) (4)

Evaluation of process holding times:

The potential impact of extended hold times on the production of the MVA-BN DS in the (b) (4) process was evaluated in the following process steps:

(b) (4)

The various hold times at the different process steps were analyzed during the PV study (are summarized in this BLA Section). While the process and hold times for the (b) (4) individual preparation (b) (4) are changed, the results were assessed to be consistent within each stream and data indicated that product was not impacted.

Quality data (Section 1.7.4.2):

The quantitative analyses results for the (b) (4) PV batches are provided according to process steps (i.e., (b) (4)) and are summarized in this BLA Section. Overall, IPC and release test results were shown to be within the acceptance specifications for the final PV DS batches. However, the PV study reported Out-of-Trend (OOT) results for (b) (4) levels at process steps

(b) (4) in the DS. (More details on the root cause investigations are presented in this BLA Section).

- The OOT observations pertaining to the significant declining trend in (b) (4) levels (for batches (b) (4) and 2016 PV DS batches) were identified during a root cause assessment to be caused by a drift in the (b) (4) positive controls used in the assay for (b) (4). Correction of these OOT assay results was based on previous assay results from 2014 and 2015, indicating that none of these (b) (4) results exceeded the acceptance limit (*specification limit*: (b) (4)). Thus, the drift in the assay for (b) (4) was not considered to impact the affected batches or the PV.
- The OOT observations pertaining to a significant (b) (4) content in the DS production during 2016 (in comparison with results from 2014 and 2015) were the subject of a root cause assessment. No plausible root cause was identified in the (b) (4) levels. Nonetheless, the (b) (4) content is not considered to be critical for the quality of the DS batch material since (b) (4) is an unwanted impurity.

In addition, an investigation was conducted to address several (b) (4) OOS results in the DS process steps, (b) (4) and DS (in affected batches (b) (4)), and the details on the root cause investigation are covered in this BLA Section. In short, the investigation confirmed that the (b) (4) OOS results were caused by a (b) (4)

Lastly, all safety test parameters were shown to be compliant with their corresponding acceptance criteria (as summarized in this BLA Section).

Additional testing during the PV (Section 1.7.5):

(b) (4)

(b) (4)

Reviewer's comments and Information Request:

The review of the enclosed 2016 Process Validation study report of the DS process noticed that the (b) (4) OOS and (b) (4) OOT results were presented in a summary format. To gain further information regarding the OOS and OOT occurrences, an Information Request (IR #15) was communicated to BN on 15-FEB-2019 to request the Formal Investigation (FI) Reports for the (b) (4) OOS and the (b) (4) OOT on the PV DS batches. In Amendment 22 (dated, 01-MAR-2019), the following report documents were provided:

- Formal Investigation Report, (b) (4) OOS on MVA-BN BDS batches, Doc. No. 31000057
- The Out-of-Trend report for (b) (4) (K16-OOT0002)

The FI report for the (b) (4) OOS occurrence in the affected DS batches adequately recapitulated the findings as described in the 2016 PV Section. In addition, FI report indicated that the (b) (4) OOS results are limited to samples only, whereas actual DS products identified as affected batches were not impacted. Therefore, the FI report on the (b) (4) OOS is acceptable. The (b) (4) OOT investigation report (# K16-OOT0002) adequately recapitulated the findings as described in the 2016 PV Section. The most plausible root cause for the declining shift in assay results is supported in three observations outlined in the report. Therefore, the (b) (4) OOT report was found to be acceptable.

2017 Process Validation (PV) of MVA-BN DS process at BN-K (Section 1.8):

A revalidation of the DS manufacturing process was performed during FEB-2017 to evaluate an (b) (4)

This change was evaluated through the manufacture of (b) (4) PV batches ((b) (4)), which were scheduled for full-scale batch (b) (4) production and pre-defined for holding times at process steps (b) (4)

The 2017 PV study information was also submitted to Amendment 359 of IND 11596 (submission date: 06-FEB-2018) and a full review memo was prepared.

The pre-defined key process parameters (i.e., (b) (4))

for the PV batches are summarized in this BLA Section. All other process parameters were maintained according to set points (set points and normal ranges are summarized in this BLA Section).

Further, supportive data indicated that (b) (4). However, this data indicated a (b) (4) at the various intermediate process steps. Thus, this PV study planned additional testing for (b) (4).

Process validation results (Section 1.8.3):

(b) (4)

Quality data (Section 1.8.3.2):

The quantitative analyses result for the (b) (4) PV batches are presented according to process steps (i.e., (b) (4)) and are summarized this BLA Section. Overall, IPC and release test results were shown to meet the acceptance specifications in the DS production and final DS batches. (b) (4)

(b) (4) were shown to be within their corresponding acceptance specification limits and levels appeared comparable. In addition, safety parameters were shown to be compliant with their respect acceptance criteria (as summarized this BLA Section). No process and product quality-related deviations were reported in this Section.

Reviewer's comments:

The indicated change to the (b) (4) time was validated based on the successful manufacture of (b) (4) PV DS batches. All process parameters were maintained within pre-defined ranges. Quantitative IPC and release test results showed that the DS process can consistently manufacture the MVA-BN DS product. Deviations associated with this PV were investigated and assessed to have no impact on the quality of the DS batches.

The PV summary mentioned that the 2017 PV batches contained (b) (4)

Based on the 2017 PV and the manufacture of (b) (4) DS batches, the acceptance criteria for (b) (4) at the intermediates were re-evaluated and slightly adjusted (as described in Section 3.2.S.2.4), which was considered reasonable given that the limit for (b) (4) was updated from

(b) (4) . Otherwise, the acceptance criteria for (b) (4) for the final DS were not affected by the change to the DS process.

Holding time studies for process intermediates (Section 2.0):

The hold times for the DS process intermediates (i.e., (b) (4)) were investigated as part of the process validation. The established hold times are provided below in tabular format (**Table 11**). This Section provides an overview of the supportive holding time studies.

(b) (4)

3.2.S.2.6 Manufacturing Process Development

The BLA indicates that the MVA-BN (b) (4) DP manufacturing process has experienced three (3) major process changes in terms of the process scale in the following stages: (1) Early-development ((b) (4))/ pilot-scale process (at (b) (4)), (2) Industrial-scale process (at (b) (4)) and (3) Commercial-scale process (at BN-K). The optimizations and validations of the industrial- and commercial scale (b) (4) manufacturing process was covered under Section 3.2.S.2.5 of this review memo. Therefore, this section will concentrate on the comparability of the (b) (4) manufacturing process following different process validation studies; specifically, between 2008 PV and 2013 PV and between 2013 PV and 2016 PV/2017 PV. Briefly, the 2008 PV was performed to support the (b) (4) process used for production of the clinical trial material (CTM) for the Phase III lot-consistency study (POX-MVA-013). The 2013 PV was performed to support the DS process used for the production of the CTM for the Phase III pivotal efficacy study (POX-MVA-006). The 2016 PV and 2017 PV studies are associated with the licensed commercial-scale (b) (4) process.

(b) (4)



(b) (4)

Reviewer's comments: The MVA-BN coding sequence (165,041 bp) is available in GenBank (Accession No. DQ983238). This Section indicated that MVA-BN is identical in coding sequence to that of the (b) (4) vaccine virus, which was (b) (4)

Also, the identity of the coding region of the MVA-BN MSV was confirmed by sequence analysis.

(b) (4)

(b) (4)



3.2.S.5 Reference Standards or Materials

Internationally approved standards are not available for determination of (b) (4). Thus, in-house reference materials were developed for in-process and release of the DS. Otherwise, reference material used for testing of impurity and contaminants (e.g., adventitious agents and microbial contamination) are commercially available. **Table 24** in the memo provides a list of reference standards/material used for in-process and release testing of the DS.

Table 24: Overview of reference standards / materials used for DS release and in-process testing

(b) (4)

(b) (4)

(b) (4)

3.2.P DRUG PRODUCT [Product - Dosage Form - Manufacturer]

3.2.P.1 Description and Composition of the Drug Product

The MVA-BN Drug Product (DP) is a liquid-frozen suspension with at least 0.5×10^8 Inf. Units of MVA-BN in a dose volume of 0.5 mL supplied in a single-use, 2 mL glass vial, which is closed with a bromobutyl rubber stopper, crimped with an aluminum cap and covered with a polypropylene closure. The MVA-BN liquid suspension is for subcutaneous injection. The DP does not contain an adjuvant or preservative but contains trace amounts of residual host cell protein and DNA, Benzonase and the antibiotic gentamicin. A tabulated summary of the composition of the MVA-BN DP (Liquid-Frozen formulation) is provided in **Table 27**. MVA-BN is the active ingredient and the other components are inactive ingredients.

Table 27: Composition of the MVA-BN Drug Product

Component	Quantity per Dose (0.5 mL)	Function	Quality Demand / Specification
Active Substance			
MVA-BN	At least 0.5×10^8 Inf. U	Active substance immunizing antigen	In-house specification
Excipients			
Tris (Tris-hydroxymethyl-aminomethane, Trometamol)	(b) (4)	Buffering agent	(b) (4)
Sodium chloride	(b) (4)	Saline	(b) (4)
Water for injection	q.s.	Solvent	(b) (4)

3.2.P.2 Pharmaceutical Development

3.2.P.2.1 Components of the Drug Product

3.2.P.2.1.1 Drug Substance

The MVA-BN Drug Substance (DS) consists of purified MVA-BN virus in Trometamol-buffered saline (*10 mM Tris, 140 mM NaCl, pH 7.7*). Further details regarding the physicochemical and biological properties of the MVA-BN DS are provided in BLA Module 3.2.S.3.1.

3.2.P.2.1.2 Excipients

The BLA lists the following excipients as components of the formulation buffer shown in **Table 28**.

Table 28: Composition of the formulation buffer

Ingredients	Quantity per Dose (0.5 mL)	Quality Demand
Tris (Tris-hydroxymethyl-aminomethane, Trometamol)	(b) (4)	(b) (4)
Sodium chloride	(b) (4)	(b) (4)
Water for injection (WFI)	q.s.	(b) (4)

All excipients are compliant with (b) (4)

The excipients were selected based on formulation development studies, which are described in more detail in BLA Module 3.2.P.2.2.1. None of the excipients used in the manufacturing of the DP are of human and animal origin.

3.2.P.2.2 Drug Product

3.2.P.2.2.1 Formulation Development

The formulation development for the DP was undertaken with the objective of obtaining a liquid solution suspension for subcutaneous injection. The formulation buffers obtained during different development stages of the MVA-BN DP are summarized:

- In 1999, the sponsors developed a liquid frozen formulation for the DP using a Tris-NaCl formulation buffer (*10 mM Tris, 140 mM NaCl (pH (b) (4))*), which, as a biological buffer, was used to emulate physiological conditions.
- As part of the continual development of the early process at (b) (4), the formulation buffer was changed to a (b) (4) formulation using the same Tris-NaCl buffer components, but included (b) (4).
- As part of the development of industrial-scale process (at (b) (4)), the liquid frozen (LF) formulation in Tris-NaCl buffer (pH 7.7) was adopted in the manufacturing of MVA-BN FDP for the NIAID/DMID Request for Proposal (RFP).

Impact of pH on DP formulation: The selection of formulation buffer pH of (b) (4) 7.7 was based on an evaluation on the effect of pH on the DP virus titer over a range of (b) (4) pH values. The results indicated that pH values between (b) (4) resulted in poorer stability, while pH above (b) (4) resulted in a more stable preparation. Based on these results, it was initially decided to use pH (b) (4) as set point for the formulation buffer, which was later changed to pH 7.7.

Target formulation titer: The development of DP manufacturing process experienced minor changes in the target titer. The present target formulation titer of 3.8×10^8 TCID₅₀/mL was implemented starting from the manufacturing campaign of AUG-2011. Based on the evaluation of (b) (4) DP lots (# (b) (4)), the release titer for all lots have been within the release titer specification range of (b) (4) – $8.9 \log_{10}$ TCID₅₀/mL.

3.2.P.2.2.2 Overages

The formulating of the MVA-BN DP is set to a target formulation titer of 3.8×10^8 Inf. U/mL that is slightly higher than the lower release specification ((b) (4) Inf. U/mL). This was adopted to adjust for potential loss of potency during long-term storage of the DP. A target filling volume of (b) (4) mL for 2 mL vials was established to ensure an extractable volume of ≥ 0.50 mL. The fill overage was assessed to assure that the single dose volume of 0.5 mL can be delivered upon administration.

3.2.P.2.2.3 Physicochemical and Biological Properties

The BLA provides information related to the quality target production profile (QTPP) of MVA-BN that is intended to ensure desired quality based on safety, efficacy, route of administration, dosage form, strength, and stability. The most relevant product parameters are provided in **Table 29**.

Table 29: Quality target production profile (QTPP) of MVA-BN.

Parameters	Target
INN	Modified Vaccinia Ankara Virus
Mechanism of action	MVA-BN is indicated for active immunization against smallpox and monkeypox virus
Strength of MVA-BN DP	One dose (0.5 mL) contains at least 0.5×10^8 Inf. U
Container closure system	0.5 mL suspension in a 2 (b) (4) vial ((b) (4) glass) with stopper (bromobutyl rubber) and flip off/tear off cap closure.
Dosage form	Suspension of injection
Route of administration	Subcutaneous
Drug product matrix	Single dose vial

Physicochemical properties: The physical properties of MVA-BN DP are represented according to the following properties:

- **Physical appearance:** The DP is a pale white to light yellow colored milky suspension.
- **pH:** The pH range is 7.7 (b) (4)
- (b) (4)

Biological properties: The biological activity of the DP is characterized by its immunological properties. The efficacy and comparability of the MVA-BN smallpox vaccine with traditional smallpox vaccines was evaluated in mouse and non-human primate models. The immunogenicity and protective efficacy of MVA-BN vaccine was shown to be comparable with traditional smallpox vaccines. Safety and toxicity of the MVA-BN DP was evaluated in several repeat dose studies in rats and rabbits.

Criticality Assessment of Quality Attributes: Critical quality attributes (CQA) refer to physical, chemical, biological or microbiological properties that should meet appropriate limits, ranges, or distributions to ensure the desired product quality. The CQA for the DP was assessed based on identifying attributes for the product. Each quality attribute was systematically evaluated for level of criticality (ranging from *Negligible*, *Minimal*, *Medium*, *Significant* to *Catastrophic*), as part of a risk analysis approach, which assesses the possible impact of each attribute on safety and efficacy. The risk analysis approach of the quality attributes for MVA-BN DS and DP was taken into consideration for the overall control strategy for MVA-BN DS and DP, covered in BLA Section **3.2.R-Control Strategy**.

3.2.P.2.3 Manufacturing Process Development

The development of the MVA-BN DS and DP manufacturing process was initiated at (b) (4), where the DS and DP manufacturing process underwent two major process upgrades related to process scale: (1) Early-development ((b) (4)) / pilot-scale process (during 2000 – 2003) and (2) Industrial-scale process (2003 – 2005). In 2005, the DS manufacturing process was transferred to the BN-K production facility (Kvistgaard, Denmark) to increase the commercial supply of the MVA-BN vaccine DP. (The development, significant changes and validation of the MVA-BN DS manufacturing process are detailed in Sections 3.2.S.2.5 and 3.2.S.2.6 of the BLA).

During the early pilot-scale development process, (b) (4) and liquid-frozen (LF) formulations of the DP were evaluated. However, only the LF formulation of the DP is the subject of this BLA. The DP manufacturing process, including optimization and validation of commercial-scale process, was performed at (b) (4) in conjunction with BN-K.

Early development process: The first MVA-BN DP lot (# (b) (4)) manufactured by the pilot-scale process ((b) (4); during 1999-2000) was formulated in a Tris-buffered saline buffer (LF formulation). During further development of the pilot-scale process ((b) (4)), the DP formulation was changed to a (b) (4) formulation ([10 mM Tris-140 NaCl, pH (b) (4)] (b) (4)). Across development stages of the pilot-scale process, the following Phase I and II clinical trial MVA-BN DP lots were manufactured:

- Early development-I ((b) (4)) produced DP lot # 021100 (LF formulation) that was used in non-IND Phase I clinical study (POX-MVA-001).
- (b) (4) produced DP lot # 080902 ((b) (4) formulation) that was used in a non-IND Phase 2 clinical dose range finding study (POX-MVA-004).
- (b) (4) produced DP lot # 130303 ((b) (4) formulation) that was used in three (3) Phase I clinical studies: POX-MVA-002, -007 and -010.

Reviewer's comments:

While the three clinical lots represent the advancing stages in the manufacturing process development (Section 3.2.S.2.6), the BLA mentioned that no relevant differences in safety or immunogenicity were observed in the clinical development studies.

Industrial-scale DP process: The development of the industrial-scale MVA-BN DS process (at (b) (4) during 2003 – 2005) increased the production scale of the DP. In addition, the formulation was changed back to the Tris-NaCl buffer for the liquid frozen (LF) MVA-BN product. As part of the characterization of the DS process, several process test runs (PTR) were performed in 2005, whereby DP lot # (b) (4) was manufactured ((b) (4)). DP # (b) (4) was used to support non-clinical safety and efficacy studies (BN-PRE-05-032, -05-21, -06-004 and -06-005) and Phase I/II clinical studies (POX-MVA-011, -008 and -005). DP lot # (b) (4) is representative of the initial industrial-scale process.

Reviewer's comments:

The comparability the pilot scale (DP (b) (4) batches) and industrial scale (DP lot (b) (4) batch) was evaluated based on identity, impurity levels, stability and infectious virus titer (potency) results. Quality test parameter data obtained from both processes were considered comparable. Pre-clinical data of the pilot-scale DP batch and industrial-scale DP batch revealed little difference in immunogenicity and efficacy results.

Process validation of the industrial-scale process: The validation activities in 2005 produced (b) (4) DP consistency lots ((b) (4)). The only changes introduced in DP consistency lots compared with the DP lot (b) (4) was the use of different DS batches to increase the lot size and consistency of the DP process.

Reviewer's comments:

The comparability of the DP lot (b) (4) and the (b) (4) PV DP consistency lots was evaluated based on quality data. The release results of the lot (b) (4) and the (b) (4) DP consistency lots ((b) (4)) were shown to meet the acceptance specifications and thus the batches were considered comparable.

Commercial-scale DP process: The DS manufacturing process was transferred from (b) (4) to BN-K. In addition, the filling process of the MVA-BN DP was transferred to a new filling line at (b) (4) (filling line (b) (4)) to provide higher capacity, reduce processing time, and to increase flexibility for a larger batch size. The pre-filling target titer for formulation was increased from (b) (4) TCID₅₀/mL to (b) (4) TCID₅₀/mL to increase the shelf life of DP. The formulation step was modified by (b) (4) due to the increased batch size. The BLA indicates that the DP process was validated on filling line (b) (4) and a comparability study was performed with (b) (4) consistency validation lots ((b) (4)).

Reviewer's comments:

The comparability of commercial-scale lots ((b) (4)) were evaluated against the industrial-scale validation lots ((b) (4)) based on analytical release test results. The comparability evaluation showed that the changes to the DP manufacturing process had no impact on DP quality. Preclinical

studies in mice showed comparability based on immunogenicity and efficacy for different DP lots ((b) (4)) using the lethal intranasal vv-WR challenge model in mice.

Optimized commercial-scale DP process (Section 5.0):

The commercial-scale DP process was subjected to initial optimization involving the preparation of (b) (4) DP qualification lots, including lots # (b) (4) in AUG-2008. The process optimizations were implemented to (b) (4)

(b) (4) in the (b) (4) product. The changes introduced to the DP process were subsequently validated in a process validation in DEC-2008, which resulted in the production of (b) (4) PV DP lots. The process optimizations relevant for the DP process were:

(b) (4)

Reviewer's comments:

Comparability of the optimized commercial-scale DP process (AUG-2008), the revalidated DP process (DEC-2008) and the initial validated DP process (JUL-2007) were evaluated using quality testing data. Based on the release test results, the consistency of DP lots produced in 2007 and 2008 was demonstrated.

Evaluation of visible particles in DP lots: The commercial-scale DP manufacturing during DEC-2008 resulted in (b) (4) DP lots at (b) (4). (Only (b) (4) DP lots were manufactured in JUL-2007 and AUG-2008, respectively). The BLA indicated that

(b) (4) of the (b) (4) manufactured DP lots (DEC-2008) had high rejection rates of (b) (4) due to (b) (4) during the visual inspection. Higher rejection rates ((b) (4)) were also observed in (b) (4) out of the (b) (4) DP lots manufactured in the AUG-2008 filling campaign. This occurrence prompted a failure investigation to compare lots from DEC-2008 with high and acceptably low rejection rates as well as other lots manufactured in AUG-2008 and JUL-2007. In this investigation, particle size, distribution and impurities (i.e., *Total protein* and *Host cell DNA*) were determined by (b) (4) analyses.

Reviewer's comments:

The investigation showed that both rejected DP vials and released DP vials contained (b) (4) and were composed of (b) (4). The difference between the rejected and released DP vials was based on the number of (b) (4). The high rejection rates initiated another formal investigation (FI) due to the enhanced presence of (b) (4) in the affected DP lots where it was determined that these observations were related to a combination of factors. Thus, the FI suggested that the potential root cause of the (b) (4) was a combination of (b) (4) during the manufacture of the DS and/or in the final DS batch. To reduce (b) (4) in the product, a number of changes were made to optimize the DS manufacturing process (e.g., (b) (4)).

The visual inspection results from (b) (4) filling campaigns in 2011 (APR, MAY/JUN, AUG, SEP/OCT and DEC) revealed rejection rates below (b) (4), in which most rejections were due to capping issues and scratches of vials.

Process improvements implemented in a DP campaign 2011 (Section 6.0):

Change in target titer for formulation:

In 2011, the target formulation was reduced from (b) (4) TCID₅₀/mL to 3.8×10^8 TCID₅₀/mL based on data from recent DP lots. The average release titer for DP lots after the change of the target titer was $8.71 \log_{10}$ TCID₅₀/mL (95% CI: $8.68 - 8.74 \log_{10}$ TCID₅₀/mL) [corresponding to 5.1×10^8 TCID₅₀/mL (95% CI: $4.8 \times 10^8 - 5.5 \times 10^8$)]. This average was considered comparable with the predicted average level of (b) (4) TCID₅₀/mL [corresponding to (b) (4) TCID₅₀/mL].

Reviewer's comments:

DP lots manufactured in AUG-2011 (DP lots (b) (4)) were considered comparable to the 2008 PV DP lots manufactured in DEC-2008 based on quality data.

Manufacturing of clinical trial lots in 2012 and 2013 (Section 7.0):

The commercial-scale manufacture process at BN-K and (b) (4) produced (b) (4) DP lots (F00100, F00101, F00102 and F00103) that were formulated and filled in MAY-2012 from (b) (4) DS lots (M00322, M00355, M00394, M00410, M00411, M00414, M00421 and M00426). A part of these DP lots was released as Phase 3 clinical trial lots as indicated:

- DP lot C00001 (F00100), C00002 (F00101) and C00003 (F00102) were used for the Phase 3 lot-consistency study (POX-MVA-013); and DP lot C00004 (F00103) was used for clinical study POX-MVA-027.

In 2013, another DP lots (F00238) was manufactured and used for the Phase 3 pivotal clinical efficacy study (POX-MVA-006). The comparability study evaluated the clinical trial DP lots against the 2008 PV DP lots as shown in **Table 30**.

Table 30: Comparability of test results for clinical trial lots and 2008 PV DP lots

Test	Specification	Results of 2008 PV DP lots	Results of Clinical trial lots, 2012	Results of Clinical trial lots, 2013
Sterility	No bacterial or fungal growth detected	complied	complied	complied
Virus titer	Nominal titer: $8.3 \log_{10}$ $\text{TCID}_{50}/\text{mL}$ Release Specification: $(b) (4) - 8.9 \log_{10}$ $\text{TCID}_{50}/\text{mL}$ Shelf life Specification: $8.0 - 8.9 \log_{10}$ $\text{TCID}_{50}/\text{mL}$	Lot <u>0101208</u> : $8.68 \log_{10}$ $\text{TCID}_{50}/\text{mL}$ Lot <u>0111208</u> : $8.73 \log_{10}$ $\text{TCID}_{50}/\text{mL}$ Lot <u>0121208</u> : $8.69 \log_{10}$ $\text{TCID}_{50}/\text{mL}$ Lot <u>0131208</u> : $8.69 \log_{10}$ $\text{TCID}_{50}/\text{mL}$	Lot <u>C00001</u> : $8.8 \log_{10}$ $\text{TCID}_{50}/\text{mL}$ Lot <u>C00002</u> : $8.7 \log_{10}$ $\text{TCID}_{50}/\text{mL}$ Lot <u>C00003</u> : $8.7 \log_{10}$ $\text{TCID}_{50}/\text{mL}$ Lot <u>C00004</u> : $8.8 \log_{10}$ $\text{TCID}_{50}/\text{mL}$	Lot <u>F00238</u> : $8.5 \log_{10}$ $\text{TCID}_{50}/\text{mL}$
Bacterial endotoxins	(b) (4)	Lot <u>0101208</u> : (b) (4) Lot <u>0111208</u> : (b) (4) Lot <u>0121208</u> : (b) (4) Lot <u>0131208</u> : (b) (4)	Lot <u>C00001</u> : (b) (4) Lot <u>C00002</u> : (b) (4) Lot <u>C00003</u> : (b) (4) Lot <u>C00004</u> : (b) (4)	Lot <u>F00238</u> : (b) (4)
Abnormal toxicity/ General safety	No toxicity observed in guinea pigs and mice	Complied	Complied	Complied
Identity	Identity shown by (b) (4)	Complied	Complied	Complied
Appearance	Milky white to light-yellow colored suspension	Complied	Complied	Complied
pH	7.7 (b) (4)	Lot <u>0101208</u> : 7.7 Lot <u>0111208</u> : 7.7 Lot <u>0121208</u> : 7.7	Lot <u>C00001</u> : 7.7 Lot <u>C00002</u> : 7.7 Lot <u>C00003</u> : (b) (4)	Lot <u>F00238</u> : 7.7

		Lot 0131208: 7.7	Lot C00004: (b) (4)	
Extractable volume	≥ 0.50 mL	Lot 0101208: (b) (4) mL Lot 0111208: (b) (4) mL Lot 0121208: (b) (4) mL Lot 0131208: 0.5 mL	Lot C00001: (b) (4) mL Lot C00002: (b) (4) mL Lot C00003: (b) (4) mL Lot C00004: (b) (4) mL	Lot F00238: (b) (4) mL

Process improvement implemented in 2014 (Section 8.0):

Stability data for DS material revealed a decline in titer over time. Thus, a (b) (4) model was investigated to assess if an additional (b) (4) testing performed (b) (4) would be more appropriate to use in the DP formulation calculation instead of using the release titer of the DS material, which is determined (b) (4) after DS production.

(b) (4)

Reviewer's comments:

Based on the results of the (b) (4) study and stability data for DS batches stored at (b) (4), BN implemented the (b) (4) model. An additional (b) (4) is determined (approx. (b) (4)) prior to the filling time of the DP and used for the formulation calculation, when DS batches are older than (b) (4).

Process validation of the DP process in 2014: The (b) (4) model was implemented in the MAY-2014 filling campaign whereby (b) (4) DP lots were manufactured. Of the (b) (4) DP lots, (b) (4) DP lots ((b) (4)) were used in a process validation exercise. Virus titer results of all DP lot filled in the MAY-2014 filling campaign were shown to be within the virus titer specification, supporting the use of the (b) (4) approach.

Changes to be implemented in the next DP manufacture campaign: No further manufacturing of MVA-BN DP for the US supply has been performed since 2014. The process validation status of the DP process since 2014 is still considered valid since no major changes were implemented to date. All changes are related to the

implementation of the current DS (b) (4). Information regarding the new (b) (4) is covered in Section 3.2.S.2.6 of the BLA.

3.2.P.2.4 Container Closure System

The container closure system components (e.g., glass vials, stoppers, and caps) used in formulated DP filling process is the following:

- 2 mL (b) (4) borosilicate glass injection vial
 - Sourced by: (b) (4); Complies with (b) (4) for (b) (4) glass
 - Sourced by: (b) (4); Complies with (b) (4) for (b) (4) glass
- 13 mm grey rubber stopper
 - Sourced by: (b) (4); Complies with (b) (4) requirements.
- 13 mm aluminum cap with polypropylene seal
 - Sourced by: (b) (4); Complies with (b) (4) requirements.

Extractables and leachables (E/L) studies (Section 2.0):

Extractables information from the supplier (Section 2.2):

The test methods suitable for the testing of leachables in the DP are based on the extractables data of the primary packaging material that comes in direct contact with the DP (i.e., vials and rubber stopper). This BLA Section provides extractables study data (from the supplier) for the rubber stopper. However, the original extractables data were not considered sufficient for a leachables study plan design, since the rubber stoppers are pre-washed and sterilized prior to the DP packaging process. Thus, an additional extractables study on the washed/sterilized stoppers was performed at an external test facility, (b) (4).

Additional extractable study: The extractables study was performed on the washed/sterilized stoppers (from (b) (4)) using different solvents, extraction techniques and analytical methods as outlined:

- Solvent and extraction techniques used to prepare samples (i.e., rubber stopper material) for analysis include:
(b) (4)
- The analytical techniques used for detection and identification of extractables (types: (b) (4))
(b) (4)

Reviewer's comments:

This BLA Section (in Table 3) presents the extractables test results of the washed/sterilized rubber stoppers obtained from (b) (4). This study detected and identified a total of (b) (4) chemical entities. The concentrations of the extractable chemical entities were expressed in "mg/kg" units. The following chemical entities showed the highest concentration (mg/kg): (b) (4).

Leachables study of DP – test methods (Section 2.3):

The testing of leachables released into the DP over time was outsourced to a contract laboratory, (b) (4), which developed analytical test methods as outlined:

(b) (4)

The re-validation/verification of test methods for quantitation of leachables at the AET (*Analytical Evaluation Threshold*) defined for the DP was performed at (b) (4) in JAN-2015. (As noted, (b) (4) was omitted from the leachables study for the DP because the target extractable compounds were specific to a disposable no longer used in the manufacturing process).

For the leachables study of the DP, the AET value was set to (b) (4). The leachables study is assessed for safety according to the critical threshold criteria established by the Product Quality Research Institute (PQRI) for extractables and leachables for Parenteral and Ophthalmic Drug Products (PODP). The AET value is based on dosing, filling volume and a Safety Concerning Threshold (SCT) of (b) (4) for Class 3 compounds.

The test study plan for leachables for the DP involved (b) (4) different storage temperatures, storage times, and time points. The study parameters are intended to simulate the DP manufacturing process conditions based on processing time and temperatures and to include worst-case scenarios. The leachables study uses actual DP lot samples obtained from the validated commercial-scale process at (b) (4). The test plan for the leachables study was performed as outlined:

- Recommended storage conditions of DP test sample at -20°C ± 5°C:
 - (i) Storage period: (b) (4); and (ii) Contact time points: 0, 6, 9, 12, 24, 36, (b) (4) months.
- Accelerated conditions of DP test samples at (b) (4):
 - (b) (4)
- Container type: (i) (b) (4) - clear 2 mL (b) (4) borosilicate injection vials; (ii) (b) (4) - (b) (4) rubber stopper.

(Note: The leachables study on DP was initiated in MAR-2015, and testing is planned for up to (b) (4). An AET value of (b) (4) was defined for this study).

Results from the leachables study of the DP (Section 2.3.1):

This BLA Section presents the results of the DP leachables study at the accelerated temperature (finalized) and at the -20°C storage temperature in tabular format. Currently, 36 months of DP leachables data are available at the -20°C temperature.

Reviewer's comments:

For the DP accelerated leachables study performed at (b) (4) of test results are provided for (b) (4) target leachables (in Table 6 of this BLA Section). At time point 0 months, all leachables were shown to be below the AET (set to (b) (4)). However, the concentration of (b) (4) exceeded the AET after the 1st month and levels of (b) (4) increased over time (the highest level was ~11.5 µg/mL at TP = 6 months). The levels of (b) (4) appeared to increase near and slightly above the AET limit (from (b) (4)). Among the (b) (4) target leachables analyzed (not including (b) (4)), all chemical entities were well below the AET limit or not detected, over time. The DP accelerated leachable study revealed (b) (4) unknown peaks/compounds with the (b) (4) analytical methods. (b) (4) of the unknown compounds were observed to have levels in the DP above the AET. (b) (4) of these (b) (4) unknown compounds were subsequently identified (based on spiking experiments) as (b) (4).

For the real-time DP leachables study performed at -20°C, 36 months of data for (b) (4) target leachable chemicals are provided (in Table 7 of this BLA Section). At every time points, all target leachable chemicals were well below the AET limit or not detected, except for (b) (4) which varied over time ((b) (4)). (b) (4) unknown compounds were detected in the DP with concentrations occurring near or above the AET limit, over time. (b) (4) of the unknown compounds were subsequently identified as (b) (4).

While (b) (4) was shown at concentrations over the AET in the DP accelerated leachables study, (b) (4) is classified to have a low order toxicity and the levels are not considered to be a health risk. The concentration of (b) (4) exceeded the AET in the accelerated leachables study, whereas (b) (4) levels were slightly below the AET in the real-time study. The BLA states that the (b) (4) most probably originated from the glass vials and from the siliconized rubber stoppers. However, the levels of (b) (4) are not of concern because (b) (4) are non-toxic.

Container closure integrity testing (Section 3.0):

(b) (4) test:

The container closure integrity (CCI) of the assembled 2 mL container (vials, stoppers and caps) was initially examined at (b) (4) using a (b) (4) method in 2008. This CCI study involved (b) (4)

Based on the following testing, container closure integrity was demonstrated.

The sensitivity of the container closure study was investigated in further CCI studies performed in 2009. The following two (2) studies used a (b) (4) method on different sets of (b) (4) vials. (b) (4) vials were prepared by (b) (4)

(b) (4) was intended to simulate leakage into the vials. The following two test conditions were applied:

(b) (4)

Reviewer's comments:

The visual inspection of the test "vials (b) (4)" showed no (b) (4), which provided further confirmation to the initial (b) (4) test performed in 2008. However, the BLA indicated that the sensitivity of (b) (4) test methods was significantly lower than expected according to published results. As indicated, the studies done with (b) (4) vials showed no correlation between the (b) (4) and the (b) (4). Therefore, BN concluded that neither test method approaches were suitable for quantitatively detecting leaks in the vial containers. No further investigations were performed to address this discrepancy. However, the CCI of the vials were further evaluated by the (b) (4) rate and (b) (4) methods described below.

(b) (4) rate testing:

The (b) (4) rate study was outsourced to a contract laboratory, (b) (4), in 2009 to further examine the integrity of assembled vial containers (vials, stoppers and caps) at (b) (4) at -25°C. The following testing was performed on vials (b) (4). The test measured (b) (4) from the vials (N=(b) (4) per set) and the actual (b) (4). The BLA indicated that tested vials showed no (b) (4) at (b) (4) temperature conditions. Thus, it was concluded that the container closures assembled on the (b) (4) filling line (b) (4) were properly closed and can resist (b) (4) at (b) (4) and the specified storage temperature (-20°C ± 5°C).

The BLA indicates that a process change of the crimping pressure was introduced, in which the crimping pressure was changed from (b) (4). This change was implemented to enable storage of the vial-DP at lower temperatures. The closure process was revalidated in 2014 using the (b) (4) rate test. Vials crimped at both (b) (4) were tested in a validation study at room temperature and at (b) (4), with additional testing performed at -25°C (b) (4) to provide supportive data. Based on the following testing, all vials crimped at both (b) (4) passed the

(b) (4) rate at all temperature conditions. Thus, it was concluded that the risk of (b) (4) in all testing temperatures is minimal.

(b) (4) measurements of vials- supportive data:

The effect of cold temperature conditions on the ability of the rubber stoppers to maintain a seal (with the vials) was examined in the following (b) (4) measurement analysis (by (b) (4)). Briefly, (b) (4)

Reviewer's comments:

The BLA indicates that (b) (4) for the rubber stopper used for the container closure system is (b) (4). Thus, the temporary loss of CCI for the assembled vial containers is considered unlikely at the storage temperatures of -20°C (b) (4). The enclosed results from the (b) (4) measurements indicate that the DP container closure systems can maintain CCI over time at the storage temperature of (b) (4).

3.2.P.2.5 Microbiological Attributes

The commercial-scale MVA-BN DP is supplied as sterile product and the formulation does not include any preservative. As part of the release testing, the following microbiological tests are performed:

- Sterility was tested for on the (b) (4) DP.
- Bacterial endotoxins were tested for on the (b) (4) DP.

Container-closure system integrity (CCI) was verified using the (b) (4) method and the (b) (4) test method. The CCI of the vial-DP was also determined during stability testing. A CCI test is performed (b) (4) and at the end of the stability study, and a sterility test is performed at the (b) (4) of the stability study. The BLA indicates that all results from CCI testing and sterility testing have met their corresponding acceptance specification.

3.2.P.2.6 Compatibility

As indicated, the MVA-BN drug product (DP) is supplied as suspension for injection. No reconstitution diluents are required for the DP. Extractables and leachables study information is described in this memo under Section 3.2.P.2.4.

Adsorption to container:

The infectious virus titer trends observed during stability studies do not indicate a potential adsorption of DP to the container. Present infectious virus titer results do not reveal an initial drop in titer, which would be indicative of adsorption to the container.

3.2.P.3 Manufacture

3.2.P.3.1 Manufacturer(s) and Contract Laboratories

List of Manufacturer(s) and facilities:

The drug substance (DS) is manufactured by Bavarian Nordic A/S, Denmark.

Drug product is manufactured, labelled and packaged by:

(b) (4)

Drug product storage and release is conducted by:

Bavarian Nordic A/S

Hejreskovvej 10A

DK-3490 Kvistgård, Denmark

The testing of the DP and intermediates is performed either in-house or by approved subcontractors. A tabulated overview of the names, address and responsibility of each testing site is provided in **Table 31**:

Table 31: Test sites

Name and Address	Responsibility
(b) (4)	Drug product, (b) (4) and stability testing: Sterility, Bacterial endotoxins, (b) (4), pH
Bavarian Nordic A/S Hejreskovvej 10A 3490 Kvistgaard, Denmark	Drug product and stability testing: Appearance, pH, Extractable volume, Total protein
Bavarian Nordic (b) (4)	Drug product and stability testing: Identity Infectious virus titer
(b) (4)	Stability testing: Sterility

3.2.P.3.2 Batch Formula

The validated batch size range for the commercial-scale drug product (DP) is (b) (4), which corresponds to a theoretical amount of (b) (4) vials, respectively. To prepare the DP, the DS batches are (b) (4) with the formulation buffer (10 mM Tris, 140 mM NaCl, pH 7.7) to dilute them to the target formulation titer (*i.e.*, $T_t = 3.8 \times 10^8$ Inf. U/mL). The BLA provides the manufacturing formula used for the DP filling process based on the information shown in **Table 32**. Depending on the virus titer and the (b) (4) of individual DS batches, the manufacture of a DP lot may require (b) (4) to be (b) (4) with formulation buffer to achieve the final bulk volume.

(b) (4)

A tabulated overview of the composition of the DP lot is shown in **Table 33** below.

Table 33: Composition of a DP lot

Component	Quantity per 0.5 mL Dose	Reference
Active substance: MVA-BN	At least 0.5×10^8 Inf. U	In-house
Excipients: Tris (Tris-hydroxymethyl-aminomethane, Trometamol)	0.61 mg	(b) (4)
Sodium chloride (NaCl)	4.1 mg	(b) (4)
Water for injection (WFI)	q.s.to pH 7.7	(b) (4)

The BLA provides a combination plan used to determine the number of doses per DS (b) (4), and the formula to determine the number of doses per DP lot. There is a fill overage per DP dose set to (b) (4) mL to ensure an extractable volume of > 0.5 mL of DP. The current DP formulation also incorporated a (b) (4) analysis step for DS batches determined to be over (b) (4). The use of input virus titers of the DS for the calculation in the formulation step is assessed under the following considerations:

- If DS is (b) (4), then the original release titer is used.
- If DS is (b) (4) (and until expiry date), then a (b) (4) will be performed to update the input virus titer.

3.2.P.3.3 Description of Manufacturing Process


The DP manufacturing process consists of (b) (4), formulation (b) (4) of DS with the formulation buffer to produce the bulk DP and the filling of single dose vials with the bulk DP followed by inspection, labeling and packaging of vials. The main manufacturing process steps of the DP and release tests are illustrated in **Appendix F** (flowchart diagram) of this memo.

Preparation of the formulation buffer:

The (b) (4) formulation buffer (FB) is prepared in (b) (4) quantities of (b) (4) of FB comprised of the following components:


- Tris(hydroxymethyl)aminomethane (Trometamol): (b) (4)
- Sodium chloride: (b) (4)
- Water for Injection (WFI) q.s. to (b) (4)
- (b) (4) : q.s. to adjust pH to 7.7

(b) (4)



Filling: The DP is filled on Line (b) (4) in Building (b) (4). The final bulk suspension is (b) (4) during the entire filling procedure.

(b) (4)



(b) (4)

During the filling process, vials are conveyed automatically under the filling needles, (b) (4) vials at a time. The filling volume is set to (b) (4) per vial. The filled vials enter the stoppering unit, are closed with sterile rubber stoppers and then vials enter the crimping machine where they are sealed. (The filling, closing and crimping speed is approximately (b) (4) vials per minute).

Visual inspection, labeling and packaging of the vials: Assembled vials are transferred in regular intervals to a packing line located in building (b) (4) (vials are kept between (b) (4) during the transfer). Vials are inspected semi-automatically prior to labelling. Samples are taken for sterility testing. (b) (4) vials are placed in plastic blister trays, and (b) (4) of these trays are packed into a folding box. A shipping box contains (b) (4) folding boxes. The labelling and packaging is done at (b) (4). The packaged DP is transported to the cold room ((b) (4)) and placed on pallets.

Freezing and storage: The entire DP lot is placed in a freezing chamber and frozen under controlled conditions at (b) (4). The DP lot is transported to a storage freezer (-20°C). After (b) (4) storage at -20°C, the release samples are tested for *bacterial endotoxins, appearance, extractable volume, identity, infectious virus titer, pH* and *total protein*. The DP lot is kept in the storage freezer until shipment.

Hold times (Section 5.0):

The formulation buffer is stored at (b) (4) for a maximum of (b) (4). The storage and processing times are defined for the manufacturing process of the DP (shown in Table 34).

Table 34: Holding times during DP manufacture.

Manufacturing step	Allowable time
(b) (4)	(b) (4)
(b) (4)	(b) (4)
(b) (4)	(b) (4)
(b) (4)	(b) (4)
(b) (4)	(b) (4)
Storage before sampling (-20°C)	(b) (4)

Reviewer's comments:

Section 3.2.P.3.3 of the BLA describes the DP manufacturing process and the enclosed information was found to be acceptable as submitted.

3.2.P.3.4 Controls of Critical Steps and Intermediates

The formulation buffer and the bulk drug product (DP) are considered process intermediates. All process steps, operation limits and ranges of the process parameters are summarized in tabular form in **Appendix G** of this memo. Among the (b) (4) process steps and (b) (4) process parameters listed in Appendix G, the following process steps and parameters were assessed to be potentially critical in the DP manufacturing process:

- DS Storage and shipment:
 - Storage time for DS: (b) (4) .
 - Labeling and packaging:
 - Placement of blisters in secondary boxes: (b) (4) .
- (**Note:** acceptance specifications shown in italics).

The release tests performed during the DP manufacturing process and corresponding acceptance criteria/specification limits are summarized in **Table 35** of this memo. The indicated release tests represent output parameters which are checked to monitor the DP process intermediates as part of the overall control strategy. Justification for the individual release tests are provided in **Appendix H** of this memo.

Table 35: Summary of release tests for process parameter intermediates.

Process step	Test	Acceptance criteria
(b) (4)		
After filling, before packaging	Visual inspection	No visible particles, correct closure, no glass defects, fill volume correct, milky suspension
	AQL testing of visual inspection	AQL action limits AQL: Critical defects (b) (4)% Major defects (b) (4)% Minor defects (b) (4)% Product-specific particulate (b) (4)%
Drug product	Release testing specifications are outlined in Module 3.2.P.5.1	

(b) (4)

(b) (4)

As indicated by the validation data, the formulation buffer does not harbor a bactericidal or fungicidal effect. (*Acceptance criteria:* (b) (4); and *Justification:* No final sterilization is performed during filling of the vaccine).

3.2.P.3.5 Process Validation and/or Evaluation

General process validation approach:

An overview of the process validation (PV) used to evaluate and demonstrate control of the manufacturing process and its ability to adequately and consistently produce DP is described. The PV evaluation is performed according to predetermined acceptance criteria for in-process control (IPC) tests, established critical quality attributes (CQAs), and other product-related characteristics. Thus, each PV study involves the manufacture of a minimum of (b) (4) DP lots, which are planned and executed according to validation master plans (VMPs) or process validation protocols (PVPs). The PV studies are intended to demonstrate that the manufacture process meets the pre-defined specifications and quality attributes as assessed by the following types of predetermined acceptance criteria:

- The ability to meet acceptance criteria for all IPCs is covered in Section 3.2.P.3.4.
- The ability to meet specifications for all routine release tests that control for CQAs are covered in Section 3.2.P.5.1.
- The ability to meet additional acceptance criteria for qualified, non-routine tests. (The additional testing during the PV is not routinely performed as part of batch release to characterize the manufacturing process).

Process Validation (PV) of the DP process in 2005 (Section 1.3):

The industrial-scale DS and DP manufacturing process were initially developed at (b) (4). The validation of process scheduled the manufacture of (b) (4) DS batches in OCT/NOV-2005 (as covered under Module 3.2.S.2.5 of this memo). The (b) (4) DS batches were subsequently used for validation of the drug product (DP) process in NOV/DEC-2005.

Process validation lots (Section 1.3.1):

This validation study involved the manufacturing of (b) (4) consecutive DP lots shown in **Table 36** of this memo.

Table 36: Information of DP lots in the 2005 Process Validation

DP lot Number	DS batch used	Date	Bulk volume (L)	No of Vials
(b) (4)				

The PV was performed according to a process validation protocol (Validation Master Plan [VMP]: (b) (4) doc no. I-VP-002). The validation study was performed at “set point”

and according to acceptance criteria established based on data from all process test runs, which were performed during the development, establishment and characterization stage of the process. The production data from the PV study consisted of process parameters and results for the (b) (4) PV DP lots according to the main three process steps: (i) (b) (4) , (ii) Formulation/ Filling and (iii) Freezing.

Reviewer's comments:

The enclosed 2005 PV study information indicated that the industrial-scale process can consistently produce the DP material. The filled PV lots were tested for sterility, bacterial endotoxins, general toxicity, identity, appearance, pH, and extractable volume. The packaged DP lots were tested for infectious virus titer. All PV lots fulfilled the release acceptance specifications.

Process Validation of the DP process in 2007 (Section 1.4):

In 2007, the commercial-scale DP process was revalidated. While the DP process was unchanged, the filling process was transferred to a different filling line (Line (b) (4)) at (b) (4). Thus, PV evaluation consisted of the production and filling of (b) (4) DP lots on Line (b) (4) using DS batches manufactured from BN-K. (This PV study was performed according to a Validation Master Plan [VMP]: I-VB-034 at (b) (4)).

Process validation lots (Section 1.4.1):

The process validation in JUL-2007 resulted in the manufacturing of (b) (4) DP lots shown in **Table 37** of this memo.

Table 37: Information of DP lots in the 2007 Process Validation

DP lot Number	DS batch used	Date	Bulk volume (b) (4)	No of Vials
(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)

The following PV study evaluated the formulation, filling, labelling, packaging and storage of the MVA-BN DP. This PV was performed at set point according to pre-defined acceptance criteria. The DP process in 2007 was split into preparation of the formulation buffer and manufacture of the DP. The formulation buffer was manufactured to (b) (4) and immediately used in the process. The production data from this PV study consisted of process parameters and results for the (b) (4) PV DP lots according to the main three process steps: (i) (b) (4) , (ii) Formulation/Filling and (iii) Freezing.

Reviewer's comments:

The enclosed 2007 PV study information indicated that the DP process can consistently produce DP. The DP filling process was shown to meet the acceptance

specifications for the following parameters: (b) (4). Homogeneity control testing at different stages of the filling process and packaging process were evaluated using two analytical tests: (1) Virus titer and (2) Total protein content. All filled PV lots met their corresponding acceptance specifications for sterility, bacterial endotoxins, general toxicity, identity, appearance, pH, extractable volume and virus titer.

Process Validation of the DP process in 2008 (Section 1.5):

In 2008, the DP manufacturing process was revalidated, following the optimization of the commercial-scale process, according to pre-defined set points and acceptance criteria defined in a process validation protocol. (This PV study was performed according to a Validation Master Plan [VMP]: I-VB-034 at (b) (4)).

Process validation lots (Section 1.5.1):

The process validation in DEC-2008 resulted in the manufacturing of (b) (4) DP lots shown in **Table 38** of this memo. The (b) (4) DP lots were produced within a ten-day filling campaign and were produced using a (b) (4) DS (b) (4) from (b) (4) different DS batches.

Table 38: Information of DP lots in the 2008 Process Validation

DP lot Number	BN_DS batch used	Date	Bulk volume (b) (4)	No of Vials
(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)

The following PV study plan evaluated formulation, filling, labeling, packaging and storage of the DP process. This PV was performed at set points according to pre-defined acceptance criteria.

Overview of changes implemented to the DP process (Section 1.5.3):

The following changes were implemented to optimize the DP process:

(b) (4)

(b) (4)

Reviewer's comments:

The original acceptance specification for the visual inspection was set to a rejection rate of (b) (4) % for the vial-PV lots. However, the number of vials observed with visible particles was above the original acceptance specification for all DP lots, as high as (b) (4) % for vials with visible particles. In this case, a rejection rate increased to (b) (4) % was deemed acceptable.

The filling homogeneity of the PV lots was evaluated based on the following analytical tests: MVA-BN DNA titer (by (b) (4) analysis), total protein and infectious virus titer. The results from this homogeneity evaluation confirmed the consistency of the filling process.

All 2008 PV DP lots conformed to acceptance specifications for identity, appearance, pH, extractable volume, sterility, bacterial endotoxins, virus titer and abnormal toxicity/ general safety. Thus, the DP manufacturing process was considered validated.

Validation of the DP process in 2014 (Section 1.6):

The revalidation of the DP manufacturing process in 2014 resulted in the manufacture of (b) (4) DP lots at set points according to pre-defined acceptance specifications. An overview of the PV DP lots manufactured in MAY-2014 is shown in **Table 39** of this memo. (This PV study was performed according to a Process Validation Protocol, Doc. No. 820001314).

[The 2014 DP PV study was submitted to Amendment 290 of IND 11596 (submission date: 23-OCT-2014) and a full review memo was prepared].

Table 39: Information of DP lots in the 2014 Process Validation

DP lot No.	BN_DS batch used	Production Date	Total Bulk (b) (4)	Expected Vials
(b) (4)				

Process validation results (Section 1.6.3):

The formulation of the (b) (4) PV lots was performed according to a combination plan. The (b) (4) during formulation was performed using (b) (4) for batches (b) (4) at the start of manufacturing campaign. The (b) (4) was implemented in this validation. The target titer for the final bulk was set to (b) (4) TCID₅₀/mL.

The production of (b) (4) PV lots was executed according to routine process parameters (outlined in this BLA Section). The enclosed process parameter data are shown to be within their corresponding acceptance specifications/ranges.

Release test results of the 2014 PV lots: The final bulk material was tested for (b) (4). The final DP lots were tested for *sterility, bacterial endotoxins, abnormal toxicity/ general safety, identity, appearance, pH, extractable volume, virus titer, total protein* and *packaging control*. All PV lots met their corresponding acceptance specifications.

Homogeneity during formulation and filling: The homogeneity of the formulation and filling process was evaluated by collecting vial samples at (b) (4) different time points (TP) across the process (i.e., at TP: (b) (4)). The samples were analyzed for (b) (4). The results were assessed by statistical analysis. The enclosed linear data for the three analytical test parameters showed that the formulation and filling process resulted in homogeneous product.

Validation of the formulation buffer hold time (Section 1.8):

The hold time of the formulation buffer (FB) was evaluated by performing a stability study. This study analyzed (b) (4) FB batches (**Table 40**), which were stored at (b) (4) and tested at regular intervals (i.e., at TP: (b) (4)) for (b) (4). All test results from the stability study were satisfactory and thus supported a holding time of up to (b) (4).

(b) (4)

Reviewer's comments:

The enclosed 2014 PV DP validation study information indicated that current MVA-BN DP process is considered validated.

3.2.P.4 Control of Excipients

3.2.P.4.1 Specifications

The following excipients used to manufacture the MVA-BN drug product (DP) correspond to the ingredients of the Formulation Buffer:

- Tris/Trometamol [Tris(hydroxymethyl)aminomethane; alternative name: *Tromethamine*]
- Sodium Chloride
- Water for Injection (WFI)

Analytical test methods and specifications for the indicated excipients comply with (b) (4). The analytical test methods used for the excipients are (b) (4) methods and thus validation is not required.

Representative Certificate of Analysis (CoA) documents for *Tris/Trometamol* and *sodium chloride* are provided by the drug product manufacturer (b) (4) and are enclosed in 3.2.P.4.1 – Appendix 1 and Appendix 2, respectively. The CoA documents provide adequate information certifying that the excipients comply with release testing specifications for identity, quality and microbiology.

3.2.P.4.2 and 3.2.P.4.3 Analytical Procedures and Validation of Analytical Procedures

All analytical methods are (b) (4) methods. Thus, a formal validation is not required.

3.2.P.4.4 Justification of Specifications

The specifications for the excipients correspond to (b) (4) requirements.

3.2.P.4.5 Excipients of Human or Animal Origin

Excipients used in the DP formulation are not of human or animal origin.

3.2.P.4.6 Novel Excipient

No novel excipients were used to manufacture process of the drug product.

3.2.P.5 Control of Drug Product

3.2.P.5.1 and 3.2.P.5.6 Specification(s) and Justification of Specification(s) Specification(s):

Test parameters, analytical procedures and acceptance criteria used for the routine release testing of the MVA-BN DP are summarize below in **Table 41** of this memo. In addition, test parameters, analytical procedures and acceptance criterial for the release testing for the DP stability testing are summarized in **Table 42**.

Table 41: Release specification for Drug Product (DP)

Test parameter	Analytical procedure	Acceptance criteria
Appearance	Visual inspection (b) (4)	<u>Transparency/Turbidity</u> : Milky. <u>Color</u> : Light yellow to pale white. <u>State</u> : Suspension. <u>Particles</u> : Free from visible extraneous particles. <u>Closure</u> : Completely closed vial. Caps firmly and evenly attached.
pH	(b) (4)	(b) (4)
Extractable volume	(b) (4)	≥ 0.50 mL
Identity	(b) (4)	Identity confirmed
Sterility	(b) (4)	No growth of bacterial and fungi
Bacterial endotoxins	(b) (4) method	(b) (4)

	(b) (4)	
Infectious virus titer	(b) (4)	(b) (4) – 8.9 log ₁₀ Inf. U/mL ((b) (4) - 7.9×10 ⁸ Inf. U/mL)
Total protein	(b) (4)	(b) (4)

Table 42: Shelf life specifications for Drug Product

Test parameter	Analytical procedure	Acceptance criteria
Appearance	Visual inspection (b) (4)	<u>Transparency/Turbidity</u> : Milky. <u>Color</u> : Light yellow to pale white. <u>State</u> : Suspension. <u>Particles</u> : Free from visible extraneous particles. <u>Closure</u> : Completely closed vial. Caps firmly and evenly attached.
pH	(b) (4)	7.5 – 7.9
Identity	(b) (4)	Identity confirmed
Sterility	(b) (4)	No growth of bacterial and fungi
Bacterial endotoxins	(b) (4)	(b) (4)
Infectious virus titer	(b) (4)	8.0 – 8.9 log ₁₀ Inf. U/mL (1.0×10 ⁸ - 7.9×10 ⁸ Inf. U/mL)
Container closure integrity	(b) (4)	(b) (4)

Reviewer's comments (Sections 3.2.P.5.1 and 3.2.P.5.6):

Enclosed information pertaining to the release test parameters, acceptance criteria and justification of specification(s) for the DP is acceptable as presented. The release tests and acceptance criteria are appropriate aspects of the DP control strategy to ensure product quality, safety and efficacy. No deficiencies were identified in these BLA Sections.

3.2.P.5.2 and 3.2.P.5.3 Analytical Procedures and Validation of Analytical Procedures

An overview of analytical procedures for release and/or stability test programs of the DP is shown in **Table 43**. Appearance and Infectious virus titer were reviewed under Section 3.2.S.4.2 in this memo. Analytical assay procedures including *sterility*, *bacterial endotoxins* content, *total protein* content, *extractable volume* and *pH* were reviewed separately by DBSQC reviewers.

Table 43: Overview of the analytical procedures for release and stability testing of the DP

Analytical procedure	Method	Reference	Method SOP
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Sterility	(b) (4)	(b) (4)	(b) (4) QOB-PM-001
Bacterial endotoxin	(b) (4)	(b) (4)	(b) (4) QOB-PM-011
Identity	(b) (4)	N/A; in-house procedure	SOP BN0002649 Identity Testing of MVA-BN by (b) (4)
Total Protein	(b) (4) assay	(b) (4)	BN0002583 (b) (4) Assay for Quantitation of Protein
Extractable volume	(b) (4)	(b) (4)	BN0002574 Extractable Volume Test
Appearance	(b) (4) : Visual inspection	(b) (4)	SOP BN0002580 Appearance Test
pH	(b) (4)	(b) (4)	BN0002637 pH measurement
Infectious virus titer	(b) (4)	N/A; in-house procedure	BN (b) (4)

(b) (4) test (Section 2.5):

This test is performed as part of the stability testing program of the DP, in accordance with (b) (4). The (b) (4) test involves the testing of (b) (4) DP vials. Briefly, (b) (4)

The sample vials conform to the CCI acceptance criterion if (b) (4).

3.2.P.5.4 Batch Analyses

The BLA provides the DP batch analyses data obtained during the development and validation of the large-scale DS manufacturing process (*i.e.*, the industrial-scale process at (b) (4) and the commercial-scale process at BN-K). The DP batch analyses results correspond to all key DP lots manufactured (as liquid frozen formulation) starting during the period of 2005 to 2014, which represent important development batches, process validation batches, relevant stability indicating batches, and any DP lots that were used in non-clinical and clinical studies.

DP batch analyses results from process test runs (PRT) 2005: DP lot 170505 lot (used for Phase I/II clinical studies: POX-MVA-011, -008 and -005) was manufactured as part of the PTR characterization runs. This DP batch met the release specifications for *identity, appearance, pH, extractable volume, sterility, bacterial endotoxins, virus titer and abnormal toxicity/general safety test.*

DP batch analyses results from process validation in 2005: PV DP lots 0031105, (b) (4) were manufactured as part of the process validation (PV) activities of the industrial-scale DS process in (b) (4) (during OCT/DEC-2005). (DP lot 0031105 was used as a clinical lot). The following DP lots met the release specifications for *identity, appearance, pH, extractable volume, sterility, bacterial endotoxins, virus titer* and *abnormal toxicity/general safety test*.

DP Batch analyses results from 2005/2006: DP lots (b) (4) were manufactured, using industrial-scale DS lots, to support clinical and non-clinical studies. The following DP lots met the release specifications for *identity, appearance, pH, extractable volume, sterility, bacterial endotoxins, virus titer* and *abnormal toxicity/general safety test*.

DP Batch analyses results from process validation in 2007: PV DP lots (b) (4) were manufactured as part of the process validation of the commercial-scale DS process (of FEB/MAR-2007) in BN-K. MVA-BN DP lot (b) (4) were used for non-clinical and clinical studies. The PV DP lots met the release specifications *identity, appearance, pH, extractable volume, sterility, bacterial endotoxins, virus titer* and *abnormal toxicity/general safety test*.

DP Batch analyses results from qualification lots 2008: Qualification DP lots 0050808 (b) (4) and 0070808 were manufactured using DS batches produced from the DS process optimization study. DP lots 0050808 and 0070808 were used in several clinical studies (POX-MVA-024, -028, -03x and -30). These DP lots met the release specifications for *identity, appearance, pH, extractable volume, sterility, bacterial endotoxins, virus titer* and *abnormal toxicity/general safety test*.

DP Batch analyses results from process validation 2008: DP lots 0101208, 0111208, 0121208 and 0131208 were manufactured during the process validation campaign during 2008 in (b) (4). These PV DP lots were used in three (3) clinical studies (POX-MVA-028, -029 and -030). (In addition, these DP lots were placed in the stability program as primary stability lots, and relevant stability data are presented in Module 3.2.P.8.3). The PV DP lots met the release specifications for *identity, appearance, pH, extractable volume, sterility, bacterial endotoxins, virus titer* and *abnormal toxicity/general safety test*.

Batch analyses results for routine DP lots produced in 2010 / 2011: DP lot (b) (4) were obtained from routine manufacturing campaigns during the period between 2010 and 2011. The indicated DP lots were used in several nonclinical studies and were placed into the stability study program. These DP lots met the release specification for *identity, appearance, pH, extractable volume, sterility, bacterial endotoxins, virus titer* and *abnormal toxicity/general safety test*.

Batch analyses results for Clinical trial DP lots 2012: DP lots C00001, C00002, C00003, C00004 (b) (4) were manufactured using (b) (4) DS batches produced from routine manufacturing campaigns during JUN-2011 and FEB-2012. DP lot

C00001, C00002, and C00003 were used to support the Phase 3 clinical trial for the lot-to-lot consistency pivotal study (POX-MVA-013); DP lot C00004 was for clinical study POX-MVA-027; and DP lot C00005 was for a non-clinical study. The (b) (4) DP lots were shown to meet the release test acceptance specifications as summarized in **Table 44** of this memo.

Table 44: Batch analyses results of clinical trial lots 2012

Test	Acceptance criteria	C00001 (b) (4)	C00002 (b) (4)	C00003 (b) (4)	C00004 (b) (4)	(b) (4)
Identity	Identity confirmed (PCR)	complied	complied	complied	complied	complied
Appearance	Pale milky colored homogeneous suspension	complied	complied	complied	complied	complied
pH	(b) (4)	7.7	7.7	(b) (4)	(b) (4)	(b) (4)
Extractable volume	≥ 0.50 mL	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)
Sterility	No growth of bacterial and fungi	complied	complied	complied	complied	complied
Bacterial endotoxins	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)
Virus titer	(b) (4) – 8.9 log ₁₀ TCID ₅₀ /mL	8.8 log ₁₀ TCID ₅₀ /mL	8.7 log ₁₀ TCID ₅₀ /mL	8.7 log ₁₀ TCID ₅₀ /mL	8.8 log ₁₀ TCID ₅₀ /mL	8.7 log ₁₀ TCID ₅₀ /mL
Abnormal toxicity/ General safety test	No toxicity observed in guinea pigs and mice.	complied	complied	complied	complied	complied

Batch analyses results of DP lots filled with 2013 PV DS batches: DP lots (b) (4) were manufactured using DS batches produced in the process validation campaign (at BN-K) during MAY/JUN-2013. (As indicated in Section 3.2.S.4.4 of this memo, DP lots (b) (4) were manufactured by combining the PV DS batches and routine DS batches, which were produced during JUN/SEP-2013). The DP lots were shown to meet the release specifications for *identity, appearance, pH, extractable volume, sterility, bacterial endotoxins, virus titer, abnormal toxicity/general safety test* and *total protein*.

Batch analyses results of clinical trial DP lot (b) (4): DP lot F00238 was produced using DS batches obtained from a routine manufacture campaign of JUN/SEP-2013. DP lot F00238 was used to support the Phase 3 pivotal efficacy study POX-MVA-006. This DP lot was shown to meet the release test specifications as summarized in **Table 45** of this memo.

Table 45: Batch analyses results of clinical trial lot 2013: DP lot F00238

Test	Acceptance criteria	F00238
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Identity	Identity confirmed by PCR	complied
Appearance	Pale milky colored homogeneous suspension	complied
pH	(b) (4)	7.7
Extractable volume	≥ 0.50 mL	(b) (4)
Sterility	No growth of bacterial and fungi	complied
Bacterial endotoxins	(b) (4)	(b) (4)
Virus titer	(b) (4) – 8.9 log ₁₀ TCID ₅₀ /mL	8.5 log ₁₀ TCID ₅₀ /mL
Abnormal toxicity/ General safety test	No toxicity observed in guinea pigs and mice.	complied
Total protein	(b) (4)	(b) (4)

DP Batch analyses results from process validation 2014: DP lot (b) (4) were manufactured in a process validation study (in (b) (4)) using DS batches produced during the routine manufacturing campaign in NOV-2013 to JAN-2014. These DP lots were placed in the stability program as primary stability lots and stability results are presented in Module 3.2.P.8.3 support storage at the SNS. The PV DP lots met the release specifications for *identity, appearance, pH, extractable volume, sterility, bacterial endotoxins, virus titer* and *abnormal toxicity/general safety test*.

3.2.P.5.5 Characterization of Impurities

Information on the characterization of impurities is covered in Section 3.2.S.3.2 (Impurities) of this memo since impurities are predominantly introduced from the drug substance (DS) process. The formulation of the DS with Formulation Buffer does not introduce additional process-related impurities.

3.2.P.6 Reference Standards or Materials

Analytical test methods for the DP that use reference standards or material are identical to the test methods used for analysis of the DS. Information on reference standards or materials used for analysis of the DP is covered in Section 3.2.S.5 of this memo.

3.2.P.7 Container Closure System

The assembled container closure system (CCS) for storage of the formulated DP consists of a glass vial, rubber stopper and an aluminum cap with a polypropylene closure (with flip tear up). The primary container components are reiterated below:

- **Vial:** 2 mL
 - Identity of material: (b) (4) borosilicate glass injection vial
 - Supplied by: (b) (4); Complies with (b) (4) for (b) (4) glass
 - Second supplier by: (b) (4); Complies with (b) (4) for (b) (4) glass
- **Stopper:** 13 mm grey rubber stopper ((b) (4))
 - Identity of material: Bromobutyl compound filled with silicate filler

- Supplied by: (b) (4) ; Complies with (b) (4) requirements.
- **Cap:** 13 mm silver/yellow cap
 - Identity of material: aluminum cap with polypropylene seal (with flip tear up).
 - Supplied by: (b) (4) ; Complies with (b) (4) requirements.

Vial (Section 1.2): Incoming vials are tested for external aspects, dimensional attributes, functionality and for physiochemical characteristics ((b) (4)) content in compliance with (b) (4). The BLA provides a technical drawing of the vial with detailed dimensional information. (Vials are pre-washed and sterilized).

Stopper (Section 1.3): Incoming stoppers are tested for external aspects, dimensional attributes, and functionality. Chemical and physical attributes are verified according to the supplier's certificate of analysis in compliance with (b) (4). The BLA provides a technical drawing of the stopper with detailed dimensional information. (Stoppers are pre-washed and autoclaved).

Cap (Section 1.4): Incoming caps are tested for external aspects, dimensional attributes and functionality. The BLA provides a technical drawing of the cap with detailed dimensional information. (Stoppers are pre-washed and autoclaved).

For information related to Extractables/Leachables and (b) (4) (based on CCI testing) of the CCS, refer to Section 3.2.P.2.4 of this memo. Adsorption of DP to the container is briefly addressed in Section 3.2.P.2.6.

3.2.P.8 Stability

3.2.P.8.1 Stability Summary and Conclusion and 3.2.P.8.3 Stability Data

The MVA-BN DP is intended for storage at -20°C (b) (4). Based on the results from real-time long-term stability studies of several primary stability DP lots stored at -20°C, a shelf life of 36 months was assigned to the DP. For storage of the DP at (b) (4), a shelf life of (b) (4) has been proposed based on available long-term stability data from (b) (4) primary stability lots.

Stability study data of DP in -20°C storage:

Primary stability DP lots: The assigned shelf life of 36 months for the DP stored at -20°C is based on the stability results generated from the following primary stability DP lots:

- **2008 PV DP lots:** (b) (4) primary stability lots ((b) (4)) were manufactured in the process validation campaign in 2008.
 - (b) (4) months of real-time stability data are available for the 2008 PV DP lots.
 - Use: Clinical trials POX-MVA-028, POX-MVA-029 and POX-MVA-030.
 - Stability study status: study completed.
- **2012 Clinical Trial Material:** (b) (4) clinical trial DP lots ((b) (4)) were manufactured in 2012 for use in the Phase 3 lot-consistency clinical study (POX-MVA-013) as well as for other clinical and nonclinical studies.

- (b) (4) months of stability data available for DP lot (b) (4); and 36 months of stability data are available for all other DP lots.
- **Use:** Clinical trial POX-MVA-013 and POX-MVA-027.
- Stability study status: study completed.
- **2013 DP lots (from DS PV 2013):** (b) (4) primary stability lots ((b) (4)) were filled with DS batches manufactured in the 2013 process validation campaign.
 - (b) (4) months of real-time stability data are available for the 2013 DP lots.
 - **Use:** US Strategic National Stockpile (SNS)
 - Stability study status: study completed.
- **2013 Clinical Trial Material:** A single clinical lot ((b) (4)) was manufactured for use in the Phase 3 pivotal efficacy study (POX-MVA-006).
 - (b) (4) months of real-time stability data are available for the 2013 DP clinical lot.
 - **Use:** Clinical trial POX-MVA-006
 - Stability study status: study completed.
- **2014 PV DP lots:** (b) (4) primary stability lots ((b) (4)) were manufactured in process validation campaign in 2014.
 - (b) (4) months of real-time stability data are available for the 2014 PV DP lots.
 - **Use:** SNS
 - Stability study status: study is ongoing.
- **Annual stability lots:** (b) (4) DP lots manufactured in 2010, 2011, 2014, 2015, 2016 and 2017 were included in the annual stability study program done at -20°C.
 - 2010 and 2011 DP lots: (b) (4) months of real-time stability data are available.
 - 2014 DP lots: (b) (4) months of real-time stability data are available
 - 2015 DP lots: 36 months of real-time stability data are available
 - 2016 DP lots: 24 months of real-time stability data are available
 - 2017 DP lots: 18 months of real-time stability data are available

Results: *All stability results in the annual stability studies are shown to be within specification.*

Stability study test plan for DP stored at -20°C ± 5°C:

Real-time stability data were generated from a testing frequency (TP) of up to (b) (4) months (b) (4) years) as follows: TP = 0, 3, 6, 9, **12**, 18, **24**, 30 and **36**; with optional time points: TP = (b) (4)). The following stability-indicating test parameters were used as outlined below (acceptance specifications shown in italics):

- **Appearance:**
 - Transparency/Turbidity: *Milky*;
 - Color: *Light yellow to pale white*;
 - State: *Suspension*;
 - Particles: *Free from visible extraneous particles*.
- Infectious virus titer: *8.0 – 8.9 log₁₀ Inf. U/mL (1.0×10⁸ – 7.9×10⁸ Inf. U/mL).*
- pH: 7.7 (b) (4)

(pH specification was initially 7.7^{(b) (4)}).

- Identity: *Identity confirmed*.
- (Identity testing: TP = 0, 12, 24, 36, (b) (4)).
- Sterility: *No growth of bacteria and fungi*.
- (Sterility testing: TP = 0, 36 (b) (4)).
- Container closure integrity (CCI): (b) (4).
- (CCI testing: TP = 12, 24, 36, (b) (4)).

(The analytical procedures used for stability test parameters are described under Section 3.2.S.4.2 of this memo).

Stability data assessment and shelf life estimation for DP stored at -20°C:

The stability data from the above (b) (4) primary stability DP lots were combined to assess the shelf life of the DP stored at -20°C. A statistical linear regression analysis approach, that was determined to be acceptable, was applied to analyze the available virus titer data (up to (b) (4) months) generated from these DP lots. Thus, pooled virus titer data were analyzed using a common slope and individual intercepts model. The output of the Inverse Prediction analysis performed on all DP lots determined the earliest time at which the lower one-sided 95% CI (*confidence interval*) intersected the minimum clinical limit (*i.e.*, 8.0 log₁₀ Inf. U/mL). DP lot # (b) (4) was estimated to have the lowest predicted days for the lower one-sided 95% CI at (b) (4) days (corresponding to (b) (4) months).

While the statistical evaluation supports a shelf life of (b) (4) months with regards to virus titer, a shelf life of 36 months was assigned to the DP stored at -20°C.


Reviewer's comments and Information Request:

*Based on the submitted real-time stability data generated from the primary stability DP lot samples, I agree with the shelf life claim of 36 months at the intended storage temperature of -20°C ± 5°C. All virus titer results at TP = 36 months are above specification and thus adequately supports the shelf life claim of 36 month. However, after TP = 36 months, OOS virus titer results (*i.e.*, due virus titer results below the specification limit) were observed at TP = (b) (4) months (for DP lot (b) (4)) and TP = (b) (4) months (for DP lot (b) (4)). All stability indicating results within the 36-month period met the acceptance criteria except for lot (b) (4), which reported an OOS appearance result at TP = 36 months.*

An Information Request (IR #19) was communicated by DBSQC to BN on 20-MAR-2019 to request the most recent testing of the 2014 PV DP lots (b) (4). BN confirmed that stability results for the 2014 PV DP lots (at -20°C) in BLA Section 3.2.P.8.3 in Table 8 were current.

(b) (4)

(b) (4)



3.2.P.8.2 Post-Approval Stability Protocol and Stability Commitment

The BLA presents the post-approval stability test plan for primary stability batches or new process validation batches that are representative of the current manufacturing process. The stability studies are conducted at the storage temperature of $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$ (b) (4) for up to three (3) years or (b) (4), respectively. The stability-indicating test parameters for the DP lots consist of *infectious virus titer, appearance, pH, sterility, identity, and container closure integrity*. Tabulated overviews of the post-approved stability test plan are shown in **Table 46** and **Table 47**.

Table 46: Stability test plan for DP – primary stability and process validation batches at -20°C ± 5°C.

Time points (Months)	Test Parameter /Acceptance criteria					
	Infectious virus titer	Appearance	pH	Sterility	Identity	Container closure integrity
	8.0 - 8.9 log ₁₀ Inf. U/mL (1.0-7.9×10 ⁸ Inf. U/mL)	Transparency/Turbidity: <i>Milky</i> Color: <i>Light yellow to pale white</i> State: <i>Suspension</i> Particles: <i>Free from visible extraneous particles</i>	(b) (4)	No growth of bacteria and fungi detected	Identity confirmed	No traces of (b) (4) solution
0	X	X	X	X	X	--
3	X	X	X	--	--	--
6	X	X	X	--	--	--
9	X	X	X	--	--	--
12	X	X	X	--	--	X
18	X	X	X	--	--	--
24	X	X	X	--	--	X
30	X	X	X	--	--	--
36	X	X	X	X	X	X

Table 47: Stability test plan for DP – primary stability and process validation batches at (b) (4).

Time points (Months)	Test Parameter /Acceptance criteria					
	Infectious virus titer	Appearance	pH	Sterility	Identity	Container closure integrity
	8.0 - 8.9 log ₁₀ Inf. U/mL (1.0-7.9×10 ⁸ Inf. U/mL)	Transparency/Turbidity: <i>Milky</i> Color: <i>Light yellow to pale white</i> State: <i>Suspension</i> Particles: <i>Free from visible extraneous particles</i>	(b) (4)	No growth of bacteria and fungi detected	Identity confirmed	No traces of (b) (4) solution
0	X	X	X	X	X	--
3	X	X	X	--	--	--
6	X	X	X	--	--	--
9	X	X	X	--	--	--
12	X	X	X	--	--	X
18	X	X	X	--	--	--
24	X	X	X	--	--	X
30	X	X	X	--	--	--
36	X	X	X	--	--	X
(b) (4)	X	X	X	--	--	X
	X	X	X	--	--	X
	X	X	X	--	--	X
	X	X	X	--	--	X
	X	X	X	--	--	X

(b) (4)	X	X	X	--	--	X
	X	X	X	X	X	X

(*) Optional time points dependent on final shelf life assignment.

[Note: Regular stability testing intervals are used as follows: every (b) (4) months in the first year, every (b) (4) months in the second and third year of testing, and (b) (4) a year afterwards (*i.e.*, **TP** = 0, 3, 6, 9, **12**, 18, **24**, 30, **36**, (b) (4) months). (*) Optional stability testing is at **TP** = (b) (4) months].

(b) (4) stability batches:

BN is committed to include (b) (4) batch per (b) (4) in the (b) (4) stability study program if the market production occurs during the calendar year. Future post-approval (b) (4) stability studies will be conducted at the recommended storage temperature at $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$ for a minimum of 3 years and at (b) (4) for up to (b) (4) years. The stability-indicating test parameters for (b) (4) DP lots consist of *infectious virus titer*, *appearance*, *pH*, *sterility* and *identity*. Tabulated overviews of the post-approval stability test plans for DP at $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$ and at (b) (4) are shown in **Table 48** and **Table 49** of this review memo.

Table 48: Post-approval stability test plan for DP – (b) (4) stability lot batches at $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$.

Time points (Months)	Test Parameter /Acceptance criteria				
	Infectious virus titer	Appearance	pH	Sterility	Identity
	8.0 - 8.9 log ₁₀ Inf. U/mL (1.0-7.9×10 ⁸ Inf. U/mL)	Transparency/Turbidity: Milky Color: Light yellow to pale white State: Suspension Particles: Free from visible extraneous particles	(b) (4)	No growth of bacteria and fungi detected	Identity confirmed
0	X	X	X	X	X
3	X	X	X	--	--
6	X	X	X	--	--
9	X	X	X	--	--
12	X	X	X	--	--
18	X	X	X	--	--
24	X	X	X	--	--
30	X	X	X	--	--
36	X	X	X	X	X

Table 49: Post-approval stability test plan for DP – Annual stability lot batches at (b) (4)

Time points (Months)	Test Parameter /Acceptance criteria				
	Infectious virus titer	Appearance	pH	Sterility	Identity
	8.0 - 8.9 log ₁₀ Inf. U/mL	Transparency/Turbidity: Milky	(b) (4)	No growth of bacteria and	Identity confirmed

	(1.0-7.9×10 ⁸ Inf. U/mL)	Color: Light yellow to pale white State: Suspension Particles: Free from visible extraneous particles		fungi detected	
0	X	X	X	X	X
3	X	X	X	--	--
6	X	X	X	--	--
9	X	X	X	--	--
12	X	X	X	--	--
18	X	X	X	--	--
24	X	X	X	--	--
30	X	X	X	--	--
36	X	X	X	--	--
(b) (4)	X	X	X	--	--
	X	X	X	--	--
	X	X	X	--	--
	X	X	X	--	--
	X	X	X	--	--
	X	X	X	--	--
	X	X	X	X	X

(*) Optional time points dependent on final shelf life assignment.

[Note: Regular stability testing intervals are used as follows: every (b) (4) months in the first year, every (b) (4) months in the second and third year of testing, and (b) (4) a year afterwards (*i.e.*, **TP** = 0, 3, 6, 9, **12**, 18, **24**, 30, **36**, (b) (4) months). (*) Optional stability testing is at **TP** = (b) (4) months].

Reviewer's comments and Information Request (IR):

The shelf life for the DP at the intended storage temperatures of -20°C ± 5°C and (b) (4) is up to 36 months and (b) (4), respectively. While the BLA indicated that the DP is intended for long-term storage at either -20°C or (b) (4), there was no indication whether DP lots should remain at their initial storage temperature during shipment and long-term storage until administration. To gain further information regarding the intended storage temperatures, an Information Request (enclosed in IR # 8; dated, 21-DEC-2018) was sent to BN to request a clarification on whether DP lots placed in either -20°C or (b) (4) storage is intended to remain at their original storage temperatures during shipment and long-term storage until administration. In Amendment 10 (dated, 09-JAN-2019), BN confirmed that DP lots are intended to remain at the original storage temperatures however, that shipment of the vialled-DP lots is performed at ≤ -15°C. Therefore, BN's response to IR # 8 (Request # 5) was found to be acceptable.

3.2.A APPENDICES

3.2.A.2 Adventitious Agents Safety Evaluation and Viral Clearance Studies

Raw materials of biological origin and corresponding certificate of analyses (CoA) are described in Section 3.2.S.2.3 of this memo. Microbiological purity and adventitious

agent test programs used for the (b) (4) are described in Section 3.2.S.2.4 of this memo. Although (b) (4)

Therefore, a virus clearance procedure is not necessary for the MVA-BN DP.

3.2.A.3 Novel Excipients

Not applicable to this BLA submission.

3.2.R Regional Information (USA)

3.2.R Executed Batch Records

Executed batch records were not submitted to this BLA submission. However, executed batch records were reviewed during the Establishment Inspection of the Bavarian Nordic DS manufacturing facility (BN-K) located in Kvistgaard, Denmark in February 25 – March 1, 2019. The following batch records were reviewed during the inspection: Working Seed virus (WSV) lot (b) (4) manufactured on 11-APR-2012; Drug Substance (DS) lot (b) (4) manufactured in JUN-2011; and Drug Product (DP) lot (b) (4) manufactured on 09-MAY-2012. All sections of each respective batch records were observed to be properly filled out, and the Critical-process parameters and in-process sampling were captured in the records.

3.2 R Lot Release Protocol

The BLA provided a lot release protocol. Enclosed in the BLA are a lot release certificate protocol template for MVA-BN DP, Certificate of Analysis (CoA) template for MVA-BN and templates entitled: “Appendix 1: Details on Sterility Testing” and “Appendix 2: Details on Endotoxin Testing”.

Reviewer’s comments and Information Request:

The review of the Lot Release Protocol (LPR) template by DBSQC found the LPR template provided insufficient information pertaining to (b) (4) DP lot release testing associated with the Identity assay and Infectious virus titer assay. Thus, an Information Request was communicated (IR #31) to BN on 14-AUG-2019 to request the inclusion of detailed information for Identity and Virus titer testing. A response from BN to IR #31 was requested by 23-AUG-2019.

3.2.R Control Strategy

This BLA Module contains a section describing the development of the control strategy to enable the consistent manufacture of the DS with the intended quality. This section provides the following information:

- Identification of critical quality attributes (CQAs) associated with the DS and drug product (DP).
- Identification of Critical-process parameters (CPPs) that can impact the DS CQAs and respective justification.
- Summary of in-process controls (IPC).
- Description of control elements integrated to assure conformance to the DS specifications. Control elements are CPP controls, in-process controls, release

testing, stability testing, process characterization, process validation and procedural controls.

Reviewer's comments:

Aspects of the control strategy (e.g., CQAs, CPPS, IPCs, PMTs and the release test program) for the manufacture of DS and DP are described in the review of BLA Sections 3.2.S.2.4, 3.2.S.2.5, 3.2.P.3.5 and 3.2.P.5.1 and elsewhere.

Method Validation Package

All method validation reports and analytical procedures were reviewed in Section 3.2.S.4.2 and 3.2.S.4.3 of this memo.

Combination Products

Not applicable to this BLA submission.

Comparability Protocols

Comparability protocols (as defined in 21 CFR 601.12 990(e)) were not submitted to this BLA.

Other eCTD Modules

Module 1

B. Labeling Review

Full Prescribing Information (PI):

The Prescribing Information (PI) for JYNNEOS is currently under revision. Nonetheless, draft PI contains the following CMC/product-related information:

- The information contained under Dosage Forms and Strengths (3) is consistent with the BLA.
- The Description (11) in draft PI is consistent with the BLA. This section describes MVA-BN product attributes, manufacturing process, formulation, and product-related and process-related impurities.
- Clinical Pharmacology (12): not applicable.
- The information contained under How Supplied/Storage and Handling (16) is consistent with the BLA and appropriate.

Carton and Container Label:

From a CMC/product perspective, the storage temperature displayed on the secondary container label is consistent with BLA. The initial draft carton label (20 single-dose 0.5 mL vials) and final container label displayed two alternative freezing temperatures at either -25°C to -15°C (-13°F to +5°F) or (b) (4). The expiry date at -25°C to -15°C is 36 months (3 years), and the expiry date at (b) (4) is (b) (4).

Reviewer's comment and Information Request:

The draft labels for package and final vial container displayed dual storage temperatures and shelf-lives which were viewed potentially confusing and may lead to

deviations in the intended storage temperature. Therefore, an Information Request (IR #25; dated 25-MAY-2019) was communicated to advise BN to create two different versions of package and final container for each intended temperature for long-term storage of the DP. In the response to IR #25 submitted to Amendment 38 (submission date: 06-JUN-2019), BN elected to use the same carton package for both stockpiling and non-stockpiling purposes including the two targeted storage conditions and shelf-lives. The basis of this decision considered the anticipated limited demand for JYNNEOS for non-stockpiling purposes (likely to be less than one production run) and to allow the flexibility in the production run to supply two different clients for storage at either -20°C or (b) (4). To mitigate the potential for confusion of dual storage conditions indicated on the package, BN has proposed to add a bolded statement indicating that “the expiration date depends on the storage condition.” In a follow-up communication to provide feedback to BN’s response to IR #24 (dated, 14-AUG-2019), CBER’s advice was the following: To reduce the possibility of medication errors due to deviations in storage condition, CBER recommended listing only the -20°C (-25°C to -15°C) storage condition in package insert and on the package label. For stockpiled product to be stored at (b) (4), CBER recommended that BN include a separate memorandum with the product detailing the expiration date for that storage condition.

The final container label for JYNNEOS was revised to display dose volume (0.5 mL), proper name and Lot No. identification. Information related to storage condition and expiration date was removed from the draft label. The revised label design was submitted to Amendment 36 (submission date: 29-MAY-2019).

Modules 4 and 5

Analytical Procedures and Validation of Analytical Procedures for Assessment of Clinical and Animal Study Endpoints

4.2.1.1 Primary Pharmacodynamics (CMC-related nonclinical studies):

The BLA describes the development and application of murine and non-human primate (NHP) animal models to provide relevant animal efficacy and immunogenicity data to support clinical efficacy of MVA-BN. Two murine intranasal (i.n.) challenge models based on vaccinia virus strain WR (vv-WR) and mousepox virus (Ectromelia virus [ECTV]) and three (3) NHP models, using an intravenous, intratracheal and aerosol challenges with monkeypox virus (MPXV), were developed to support an approach for licensure of MVA-BN. The use of vaccinia (vv-WR), ECTV and MPXV are clinically relevant and appropriate since these viral agents are closely related to variola virus, the causative agent of smallpox. The three NHP challenge models with MPXV were evaluated in a separate review by another CMC/product reviewer (Dr. Clement Meseda). The development, characterization and application of the two murine challenge models are covered in this review memo section. Several nonclinical studies (described below) were conducted with the murine challenge models to investigate the following:

- The ability of a single vaccination with MVA-BN to induce equivalent protection as traditional smallpox vaccines (e.g., Dryvax and ACAM2000) in mice challenged (i.n.) with either vv-WR (50x MLD₅₀) or ECTV (58x MLD₅₀).

- The protection afforded by a single or prime-boost vaccination of MVA-BN in large GLP compliant studies with the vv-WR (i.n.) mouse model and the ECTV (i.n.) mouse model.
- The onset of protection afforded by a single vaccination with MVA-BN compared with ACAM2000 in mice challenged (i.n.) with vv-WR (50x MLD₅₀) and in mice challenge with ECTV (58x MLD₅₀).
- The onset of protection afforded by single dose of MVA-BN in immune deficient mice challenged with a lethal dose of ECTV.

Intranasal (i.n.) murine challenge model with a lethal dose of vaccinia virus Western Reserve (vv-WR)

Study number: BN-PRE-2003.019: Protection against the highest applicable intranasal challenge dose of vv-WR following vaccination with MVA-BN

The objective of non-clinical study BN-PRE-2003.019 was to test the protective efficacy of MVA-BN against different lethal (i.n.) doses of vv-WR (vaccinia virus Western Reserve) of ~50x, ~100x, ~250x, ~400x and ~500x MLD₅₀ (*mouse lethal dose 50%*; Note: 1x MLD₅₀ = 8x10⁵ TCID₅₀/mL of vv-WR). This study investigated the ability of MVA-BN to provide protection against extremely high (i.n.) doses of vv-WR of up to ~500x MLD₅₀ (4x10⁸ TCID₅₀/mL). For this study, (b) (4) mice (Female, 6 -7 weeks; N = 100) were set up according to treatment groups: **Group 1 – 5** (Placebo [PBS]; N = 10 per group); and **Group 6 – 10 (MVA-BN)**, 2x of 1x10⁸ TCID₅₀; N = 10 per group).

Treatment schedule: Mice received two (2x) subcutaneous (s.c.) treatments of PBS (Group 1 -5) or MVA-BN (1x10⁸ TCID₅₀), on Day 0 and 28. Afterwards, all groups were challenged (on Day 42) with the various lethal (i.n.) doses of vv-WR (*i.e.*, ~50x, ~100x, ~250x, ~400x or ~500x MLD₅₀). (Each challenge dose in 0.05 mL of vv-WR corresponded to a working dilution of vv-WR).

Mice were monitored daily for clinical parameters: body weight, body temperature, clinical status (*e.g.*, healthy, sick or dead), and survival (% protection). The primary endpoint of the study was death. Pre-vaccination bleed and post vaccination bleed were collected at various time points (*i.e.*, Day 0, week 6 and two (2) days prior to challenge) for analyses by ELISA.

In addition, lung vv-WR titers were determined in mice that succumbed to infection and mice sacrificed at the end of the study. Animals were considered protected when lung virus titers were below the assay detection limit of 5x10³ pfu (or 3.69 log₁₀ pfu).

Results:

Protective efficacy: All immunized mice (in Group 6 – 10) vaccinated with the optimal dose of 1x10⁸ TCID₅₀ of MVA-BN were protected against high challenge doses of up to ~500x MLD₅₀ of vv-WR, whereas all unvaccinated placebo groups (Group 1 – 5) were not protected (0% survival) and succumbed to the infection between Day 4 and Day 6 post challenge. However, immunized mice in groups challenged with 400x and 500x MLD₅₀ had 90% and 70% survival rate, respectively.

Clinical parameters: Body weight (BW), Body temperature (BT) and clinical status were monitored during post challenge time. Placebo groups showed severe disease as displayed by consistent BW loss (max. of ~ -24%) and loss of BT during challenge time. Immunized mice (Group 5 – 10) showed different extents of disease course related to the different lethal doses of vv-WR, as displayed by varying BW loss (with maximal mean BW loss ranging from -18.8%, to -29.2%). However, immunized mice showed a sharp drop in BT (-8.5%) within day 1 - 2 (post challenge) followed by recovery of BT near to baseline levels. Based on these data, the BT parameter was observed to be better predictor of distinguishing survivors and non-survivors.

Lung virus titers: Placebo treated mice showed similar levels of lung vv-WR titers (*i.e.*, titers > 8.0 log₁₀ pfu). Immunized mice (in Group 5 – 8) challenged with lethal doses of up to 250x MLD₅₀ of vv-WR revealed no detectable lung virus titers. Among the immunized mice in Groups 9 and 10 that were challenged with lethal doses of 400x and 500x MLD₅₀, three mice had detectable lung virus titers.

Total MVA-specific antibody response: The average vaccinia-specific antibody titer as measured by ELISA for all immunized mice was determined to be 13,094 (or 4.02 log₁₀). Vaccinia-specific antibody titer levels were not observed to be predictive for protection in this study. (Neutralizing antibody response against vv-WR was not tested in this study).

Study Number: BN-PRE-07-006: Comparability of the immunogenicity and efficacy of different development lots of IMVAMUNE (MVA-BN) in a mouse vaccinia virus challenge model.

The objective of non-clinical study BN-PRE-07-006 was to compare different lots of MVA-BN DP (IMVAMUNE) representing changes in the manufacturing process and manufacturing sites. MVA-BN DP lot 130303 ((b) (4) formulation), DP lot 0120606 (**LF** formulation), and DP lot 0020707 (**LF** formulation) were compared, which respectively represent the early pilot-scale process (DS/DP by (b) (4)), industrial-scale process (DS/DP by (b) (4)) and commercial-scale process (DS by BN-K / DP by (b) (4)). Thus, the lethal intranasal (i.n.) mouse challenge model was used to evaluate and compare protective efficacy and immunogenicity of the different DP lots. (b) (4) mice (female, 6 -7 weeks; N = 205) were set up according to four (4) treatment groups: **Group 1** (Placebo/TBS; N = 10); **Group 2** (lot 130303, 2x of 1x10⁸ TCID₅₀; N = 65); **Group 3** (lot 0120606, 2x of 1x10⁸ TCID₅₀; N = 65); and **Group 4** (lot 0020707, 2x of 1x10⁸ TCID₅₀; N = 65).

Treatment schedule: Mice received two (2x) (s.c.) treatments of TBS (Group 1), lot 130303 (Group 2), lot 0120606 (Group 3), or lot 0020707 (Group 4), on Day 0 and 28. Afterwards, all groups were challenged (on Day 42), using a lethal (i.n.) dose of 50x MLD₅₀ of vv-WR. Mice were monitored daily for clinical parameters: change in body weight (**BW**), body temperature (**BT**), and health status (appearance were scored as: *healthy, sick or dead*).

The study endpoints were the following:

- **Primary endpoint:** Demonstrate equivalent efficacy among the three (3) MVA-BN DP lots, defined as the complete clearance of the challenge virus from lung tissue by Day 6 after i.n. challenge with 50x MLD₅₀ of vv-WR.
- **Secondary endpoint:** Demonstrate equivalent immunogenicity among the three (3) MVA-BN lots. Seroconversion measured by ELISA at 12 days after the second vaccination (on Day 40). Determine vaccinia-virus specific antibody titers by ELISA and by PRNT using serum samples collected from post-challenge terminal bleeds (on Day 46 or 48). Demonstrate a significant correlation between the ELISA and PRNT titers as an additional secondary endpoint.
- **Tertiary endpoint:** GMT and seroconversion rates by ELISA from serum samples collected at all time points (week 0, 4, 6, and final bleed post challenge). Body weight (BW) and body temperature (BT) were monitored post challenged and compared between groups.

Results:

Survival and lung virus titer analysis: The prime-boost vaccination with different MVA-BN lots (*i.e.*, lot 130303, 0120606; and 0020707) provided protective immunity to the lethal vv-WR challenge. Virus titers in lung tissue were determined to be clear of vv-WR, except for a single mouse in Group 2 that had a low virus titer (of 4.398 log₁₀) at Day 6 above assay detection limit (3.398 log₁₀). By contrast, the unvaccinated control group (Group 1: TBS) had high lung viral titers (7.581 to 7.976 log₁₀). Based on the efficacy results, the primary endpoint was fulfilled.

Immunogenicity: All vaccinated mice (in Group 2, -3 and - 4) reached 100% seroconverted (by ELISA) after the first dose of MVA-BN by Day 26. The ELISA GMTs at Day 26 for the three different MVA-BN lots did not appear comparable (*e.g.*, GMTs of 10590, 1887 and 1306 were measured for lot 130303, 0120606 and 0020707, respectively). The second MVA-BN vaccinations elevated GMTs to 54494, 55878, and 27705 (by Day 40), respectively. Post-challenge antibody GMTs (at Day 48) showed a minor change from GMTs obtained on Day 40 in the same vaccination groups. Comparable neutralizing antibody titers (PRNT) were measured in post-challenge time (on Day 48; *e.g.*, GMTs of 3637, 3240, and 2509 were measured lots 130303, 0120606 and 0020707, respectively).

Lastly, statistical analysis between ELISA log₁₀ titers and PRNT log₁₀ titer results, using serum samples obtained from post-challenge terminal bleeds (on Day 48), showed a correlation of $R = 0.364$ ($p = 2 \times 10^{-7}$). Therefore, this analysis claimed a significant correlation of the ELISA log₁₀ titers with the PRNT log₁₀ titers.

Clinical status assessment after challenge: Both unvaccinated and vaccinated groups showed signs of disease up to Day 2 post challenge with vv-WR, as expressed in the loss of BW. Unvaccinated mice showed a pronounced loss of BT on Day 2 (of -18%). Most vaccinated mice (in Group 3 and 4) started to show signs of recovery on Day 4, whereas unvaccinated mice (placebo) remained sick. In contrast, the majority of mice in Group 2 showed signs of sickness on Day 4, as displayed by the different clinical parameters (*i.e.*, appearance, BW loss and BT loss).

Study Number: BN-PRE-07-015: Immunogenicity and putative protective efficacy of sub-optimal doses of liquid frozen MVA-BN following intranasal vaccinia virus challenge of (b) (4) mice.

The objective of nonclinical study BN-PRE-07-015 was to evaluate the protective efficacy induced by increasing doses of MVA-BN ranging from 1×10^4 to 1×10^8 TCID₅₀ in relation to immunogenicity data determined by ELISA and PRNT methods. For this study, six (6) groups of (b) (4) mice (Female, 6 -7 weeks; N = 53) were set up according to following treatment groups: **Group 1** (1×10^4 TCID₅₀; N = 10); **Group 2** (1×10^5 TCID₅₀; N = 10); **Group 3** (1×10^6 TCID₅₀; N = 10); **Group 4** (1×10^7 TCID₅₀; N = 9); **Group 5** (1×10^8 TCID₅₀; N = 9) and **Group 6** (Placebo/TBS; N = 5).

Treatment schedule: All mice received two (s.c.) doses of either TBS or various dose levels of MVA-BN, four (4) weeks apart (on Days 0 and 28). On Day 42, all treatment groups were challenged with a lethal (i.n.) dose of 50x MLD₅₀ of vv-WR. Mice were monitored daily for clinical parameters: change in body weight (**BW**), body temperature (**BT**), and health status (scored as *healthy*, *sick* or *dead*).

Blood sampling were scheduled as follows: pre-vaccination bleed (Day -2), post-vaccination bleed (i.e., Day 7, 14, 27, 35 and 40) and post-challenge bleed (i.e., Day 46 / 52, respectively) were used for ELISA and PRNT analysis.

Results:

Protective efficacy and clinical parameters: All unvaccinated mice (in Group 6) displayed signs of disease from Day 2 to Day 4 post challenge when mice were sacrificed. Further, all vaccinated mice in Group 1 (lowest dose level) died, whereas Group 2 (1×10^5 TCID₅₀) had a 20% survival rate and mice in this group showed a severe disease outcome as expressed by clinical parameters (i.e., pronounced BW loss and BT loss). In contrast, all vaccinated mice in Groups 3, 4, and 5 survived the lethal dose of vv-WR, and the extent of disease course (as expressed by clinical parameters) was reduced in response to the increasing vaccine doses.

Lung vv-WR titers were evaluated when mice either died or when mice were sacrificed. The vaccinations with 1×10^6 to 1×10^8 TCID₅₀ of MVA-BN resulted in undetectable virus titers in lung tissue at Day 14 post challenge. However, the vaccination with 1×10^4 and 1×10^5 TCID₅₀ resulted in high levels of virus titers in lung tissue.

Immunogenicity: Mice vaccinated 1×10^7 or 1×10^8 TCID₅₀ reached 100% seroconversion on Day 27 and Day 7, respectively. Low to middle vaccine dose levels in Groups 1, 2 and 3 resulted in seroconversion rates (on Day 40, 2 days prior challenge) of 20%, 20% and 90%, respectively. The two highest dose levels of MVA-BN (i.e., 1×10^7 and 1×10^8 TCID₅₀) resulted in high ELISA titers at Day 40 (e.g., GMTs of 17028 and 59844, respectively), whereas the low to middle dose levels of MVA-BN resulted in lower ELISA titers at Day 40 (e.g., GMTs of 3, 2, and 114, respectively).

In the PRNT analysis, the two highest dose levels of MVA-BN (i.e., 1×10^7 and 1×10^8 TCID₅₀) induced the highest titers at Day 40 (e.g., GMT of 446 and 2095, respectively). Only, the middle dose level in Group 3 resulted in a PRNT GMT of 1.3 at Day 40.

This study (BN-PRE-07-015) concluded that vaccinia antibody titers measured by ELISA are correlated to protective efficacy, whereas there was no correlation between survival and PRNT titers.

Study Number: BN-PRE-08-022: Immunogenicity and protective efficacy of MVA-BN, Dryvax and ACAM2000 in the vv-WR challenge model in (b) (4) mice.

The objective of nonclinical study BN-PRE-08-022 was to compare the immunogenicity and protective efficacy of MVA-BN with ACAM2000 and Dryvax. A single percutaneous (p.c.) dose of ACAM2000 and Dryvax was compared with a single subcutaneous (s.c.) dose of MVA-BN as well as with a prime-boost vaccination with MVA-BN. For this study, (b) (4) mice (Female, 6 -7 weeks; N = 60) were set up according to the following treatment groups: **Group 1** (Placebo/TBS; N = 10); **Group 2** (Dryvax, 1x dose of 2.5×10^5 TCID₅₀; N = 15); **Group 3** (ACAM2000, 1x dose of $\sim 7.5 \times 10^5$ pfu; N = 15); **Group 4** (1x MVA-BN, 1x dose of 1.0×10^8 TCID₅₀; N = 10); and **Group 5** (2x MVA-BN, 2x doses of 1.0×10^8 TCID₅₀; N = 10).

Treatment schedule: Mice received two (2x) s.c. treatment of TBS (Group 1) or MVA-BN (Group 5), on Day 0 and 28. Mice in **Group 2** (Dryvax), **Group 3** (ACAM2000) and **Group 4** (1x MVA-BN) received a single vaccination at Day 0. All treatment groups were challenged with a lethal (i.n.) dose of vv-WR of 50x MLD₅₀ on Day 42.

Mice were monitored daily for clinical parameters: Body weight (**BW**), Body temperature (**BT**), and health status (scored as *healthy*, *sick* or *dead*). Pre-vaccination bleeds (i.e., Day -2), pre-challenge bleeds (i.e., on Day 14, 26, 35 and 40) and post-challenge bleeds (i.e., Day 46/52) were collected for ELISA and PRNT analysis.

Results:

Immunogenicity: Seroconversion and vaccinia-specific antibody GMT responses were measured by ELISA and PRNT, using serum samples collected at different time points: Day -2 (pre-bleed), Day 7, 14, 26, 35, 40 and post challenge time points (on Day 46 or 52).

- ELISA data: Mice immunized with MVA-BN (Group 4 and 5) reached 100% seroconversion (ELISA) by Day 14, whereas **Group 2** (Dryvax) and **Group 3** (ACAM2000) reached 100% seroconversion on Day 26 and Day 40, respectively. Dryvax and ACAM2000 vaccinations resulted in peak antibody titers (GMTs) of 1130 (on Day 35) and 731 (on Day 40), respectively. MVA-BN induced antibody titers that plateaued between Day 35 and 40. The single (1x) MVA-BN dose resulted in peak ELISA GMTs of 2836 and 2244 (on Day 35 and 40, respectively), and the prime-boost vaccination with MVA-BN resulted in ELISA GMTs of 12,536 and 12,175 (on Day 35 and 40, respectively).
- PRNT data: Vaccinations with MVA-BN (Group 4 and 5) resulted in 100% seroconversion by Day 35. However, vaccinations with Dryvax and ACAM2000 resulted in seroconversion rates of > 80% (by Day 26 - 40) and 80% (by Day 40), respectively. (100% seroconversion rate by PRNT was recorded for all immunized mice at post challenge time point (Day 56). Dryvax and (1x) MVA-BN vaccination both resulted in similar neutralizing antibody response kinetics with

peak PRNT GMTs of 289 (on Day 40) and 258 (on Day 35), respectively. The ACAM2000 vaccination resulted in lower PRNT titers with a peak GMT of 63 (on Day 40). The prime-boost vaccination with MVA-BN elevated the PRNT GMTs by > 4-fold, compared with the single (1x) MVA-BN vaccination, with a peak GMT of 1089 (on Day 40).

Clinical parameters and lung virus titer analysis: The vaccinations with MVA-BN, Dryvax and ACAM2000 conferred protection against a lethal (i.n.) dose of vv-WR, except for a single mouse in the ACAM2000 group which succumbed to the infection. In contrast, unvaccinated mice (in Group 1) succumbed to the infection by Day 4 post challenge. However, all mice immunized with a single vaccine dose (Group 2, -3 and -4) showed similar a disease course, irrespective of the vaccine they received, as expressed by health status observations, BW loss and relative BT loss. However, the prime-boost vaccination with MVA-BN (Group 5) resulted in a milder disease outcome compared with Group 2 and 3, based on the clinical parameters.

Lastly, the lung vv-WR titers were determined from mice that succumbed to the infection or mice that were sacrificed at the end of the study (on Day 46 / 52). Unvaccinated mice (TBS group) showed high lung virus titers (of ~8.16 log₁₀ pfu), whereas mice immunized with 1x MVA-BN or 2x MVA-BN showed undetectable levels of virus titers in lung tissues at Day 10 post challenge. However, a few mice in the Dryvax and ACAM2000 groups were shown to have detectable levels of vv-WR titers.

Study Number: BN-PRE-10-003: Onset of protection of IMVAMUNE (MVA-BN) in a mouse vaccinia virus challenge model.

The objective of nonclinical study BN-PRE-10-003 was to investigate the onset of protection conferred by a single vaccination with MVA-BN and ACAM2000 against a lethal i.n. vv-WR challenge in (b) (4) mice. For this study, (b) (4) mice (Female, 6 -7 weeks; N = 200) were set up according to three (3) main treatment groups: **Group 1** (Placebo/TBS; N = 24); **Group 2** (1x MVA-BN, 1x dose of 1.0×10^8 TCID₅₀; N = 80); and **Group 3** (ACAM2000, 1x dose of $\sim 7.5 \times 10^5$ pfu; N = 96).

Treatment schedule: On Day 0, mice received a single subcutaneous (s.c.) treatment of TBS (Group 1), a single s.c. dose of MVA-BN (Group 2), and a single percutaneous dose of ACAM2000 (Group 3). The study was staggered and treatment groups were subdivided into eight (8) parts (i.e., Part I, -II, -III, -IV, -V, -VI, -VII and -VIII), each part corresponded to a challenge time point (i.e., challenge on Day 0, 1, 2, 3, 4, 10, 14 or 42 post vaccination, respectively). Mice of in each Study Part of Group was challenged with a lethal i.n. dose of vv-WR at 50x MLD₅₀ on the specified time points. (**Note:** Group 1 [N = 3 per Part]; Group 2 [N = 10 per Part]; and Group 3 [N = 12 per Part]).

Blood sampling were scheduled as follows: (i) Pre-vaccination bleeds (on Day -3 for Part I, II, III and IV; and on Day -2 for Part V, VI, VII and VIII); (ii) Pre-challenge bleeds (on Day 3, 9, 13, and 41 for Part V, VI, VII and VIII, respectively); and (iii) Post challenge terminal bleeds were collected on day of sacrifice (i.e., 4 days post challenge for Group 1 or 10 days post challenge for Group 2 and 3).

Results:

Immunogenicity: All mice in **Part I**, **-II** and **-III** (challenged on Day 0, 1 and 2 post vaccination) succumbed to the infection, and serum samples in those parts were not collected or analyzed. All pre-vaccination sera and sera from unvaccinated mice (TBS) had no detectable antibody titers.

- **Part IV** (challenged on Day 3): For study **Part IV**, no pre-challenge serum was collected. Surviving mice (N = 10) in Group 2 (1x MVA-BN) had post challenge antibody titers (GMT) of 3208 (on Day 13)], whereas mice in Group 3 (ACAM2000) did not survive.
- **Part V** (challenged on Day 4): For study **Part V**, no detectable onset of pre-challenge antibody titers were measured (on Day 3). However, post challenge antibody titers were generated in both vaccinated mice (Group 2 and -3; on Day 14).
- **Part VI** (challenged on Day 10), **Part VII** (challenged on Day 14) and **Part VIII** (challenged on Day 42): For study **Part VI**, all MVA-BN vaccinated mice (N = 10) seroconverted with a GMT of 753 prior to challenge (on Day 9); whereas, 9 out of 11 mice in the ACAM2000 group seroconverted with an overall GMT of 35. Pre-challenge antibody titers were determined for immunized mice in study **Part VII** and **Part VIII**. The MVA-BN vaccination in **Part VII** and **-VIII** induced GMTs of 1336 and 5906, respectively; and, the ACAM2000 vaccination in **Part VII** and **-VIII** also induced GMTs of 147 and 1647, respectively.

Protective efficacy and clinical parameters: Survival (protection), disease score (scoring values: 0 - 5), body weight (**BW**) and body temperature (**BT**) were monitored for 4 days for the placebo TBS group (in Group 1) and for 10 days for immunized mice (in Group 2 and -3) beginning on the day of challenge.

- **Part I**, **Part II**, and **Part III**: Up to 2 Days post vaccination, all vaccinated mice of **Part I**, **II** and **III** were not protected against the vv-WR challenge. (Death was recorded on Day 5 to Day 8 for all treatment groups). Otherwise, little differences were observed in terms of disease score (~3.5 – 4.0) as well as the other clinical parameters (i.e., BW and BT).
- **Part IV**: The MVA-BN vaccination conferred protection, whereas the ACAM2000 vaccination did not confer protection to the lethal vv-WR challenge. MVA-BN vaccinated mice of **Part IV** recorded a severe disease outcome as reflected by the high disease scores (~4.0 – 5.0) and substantial loss of BW and BT. However, mice immunized with MVA-BN recovered BT near baseline levels by Day 10.
- **Part V**: All vaccinated mice in **Part V** survived the vv-WR challenge over the 10-day period. Both vaccinated groups displayed similar disease trends up to Day 3. However, the MVA-BN vaccination in **Part V** resulted in a more reduced disease than the ACAM2000 vaccination as displayed by the disease score and other clinical parameters (i.e., BW and BT).
- **Part VI**, **Part VII**, and **Part VIII**: All vaccinated mice (Group 2 and 3) of **Part VI**, **VII** and **VIII** survived the vv-WR challenge over the 10-Day period. Both vaccination groups of **Part VI** and **VII** showed milder disease outcome than the earlier parts of this study. However, the MVA-BN and ACAM2000 vaccinations in **Part VIII** resulted in an extended disease course and delayed recovery than **Part**

VI and VII as shown by the disease scores and other clinical parameters (*i.e.*, BW and BT).

Lung virus load: Lung vv-WR titers were measured from mice that succumbed to infection or mice sacrificed at the end of the study. Unvaccinated mice (Group 1) in all study Parts contained high lung virus titers (8.20 and 8.56 log₁₀). All immunized mice (Group 2 and 3) of Part I, II, III, and IV did not clear the lung virus titers. However, the MVA-BN vaccination of Part IV showed reduced lung virus titers (4.31 log₁₀) and, similarly, the ACAM2000 vaccination of Part V showed reduced lung virus titers (3.02 log₁₀). In the later parts of the study (*i.e.*, Part V – VIII), MVA-BN and ACAM2000 vaccinations resulted in clearance of viral lung titers. This clearance of viral lung titers for MVA-BN was recorded in Part V-VIII of the study, while clearance of viral lung titers for ACAM2000 was recorded in Part VI-VIII.

Study Number: BN-PRE-011-024: Non-inferiority of efficacy and immunogenicity of (b) (4) IMVAMUNE compared to liquid-frozen IMVAMUNE in the mouse vaccinia virus challenge model.

The objective of nonclinical study BN-PRE-11-024 was to demonstrate the non-inferiority of the (b) (4) MVA-BN to the liquid frozen (LF) MVA-BN, given as a single immunization or prime-boost regimen, in terms of protective efficacy and immunogenicity. For this study, (b) (4) mice (Female, 6 -7 weeks; N = 510) were set up according to five (5) main treatment groups: **Group 1** (TBS; N = 10); **Group 2** (2x MVA-BN [(b) (4)]); 2x of 1×10⁸ TCID₅₀; N = 125); **Group 3** (1x MVA-BN [(b) (4)]); 1x dose of 1×10⁸ TCID₅₀; N = 125); **Group 4** (2x MVA-BN [LF]; 2x doses of 1×10⁸ TCID₅₀; N = 125); and **Group 5** (1x MVA-BN [LF]; 1x dose of 1×10⁸ TCID₅₀; N = 125). This study was staggered such that each treatment group was subdivided into Part I and Part II (*e.g.*, **Group 1** [N = 5 per part]; and **Group 2** of Part I [N = 65] and Part II [N = 60] and so forth for Group 3, 4 and 5).

Treatment schedule: On Day 0, mice groups receive first subcutaneous (s.c.) treatment of TBS (Group 1), (b) (4) MVA-BN (Group 2 and 3) or (LF) MVA-BN (Group 4 and 5). On Day 28, mice in Group 1, 2 and 4 received second s.c. treatment of either TBS or MVA-BN. On Day 42, all mice Groups were challenged with a lethal i.n. dose of 50x MLD₅₀ of vv-WR. Mice were monitored for clinical parameters: Body weight (**BW**), Body temperature (**BT**), and Disease score (based on disease-related symptoms).

Blood sampling was scheduled as follows: Pre-bleeds (*i.e.*, Day -2), pre-challenge bleeds (*i.e.*, 26 and 40), and post-challenge terminal bleeds (*i.e.*, 46 / 52), which were collected for ELISA analysis.

The study endpoints were the following:

- **Primary endpoint:** Demonstrate non-inferiority of the (b) (4)-MVA-BN compared with the LF-MVA-BN, when given either as a single immunization or in a prime-boost regimen, based on protective efficacy against an i.n. challenge with 50x MLD₅₀ of vv-WR. .

- **Secondary endpoint:** Demonstrate non-inferiority of peak total antibody response (GMT measured by ELISA) induced by (b) (4) -MVA-BN compared with LF-MVA-BN prior to challenge.
- **Tertiary endpoint:** Compare all other parameters between groups vaccinated with (b) (4) - MVA-BN and LF-MVA-BN. These include seroconversion rates and GMT measure by ELISA at the remaining time points as well as body temperature (**BT**), body weight (**BW**) changes, clinical signs/appearance, and lung vv-WR titers post challenge.

Results:

Protective efficacy: All mice groups vaccinated with MVA-BN, irrespective of formulation presentation, were shown to survive the lethal (i.n.) challenge with vv-WR. Thus the primary study endpoint was fulfilled.

Immunogenicity: All mice groups vaccinated with MVA-BN reached 100% seroconversion on Day 26, irrespective of the formulation presentation of MVA-BN. On Day 26, after the first dose of MVA-BN, all immunized groups had similar antibody titers (GMTs ranged from 4160 to 4549). On Day 40, the boost vaccination with (b) (4) -MVA-BN resulted in a ~22-fold increase of GMT of 96,366, while the boost vaccination with LF-MVA-BN resulted in a ~12-fold increase of GMT of 56,379. Post challenge GMT results for all immunized groups were comparable.

Statistical comparison of total antibody response data showed that the (b) (4) - formulation induced a non-inferior total vaccinia-specific antibody response compared with the LF-formulation. Thus, in this case, non-inferiority was demonstrated for the secondary study endpoint.

Clinical parameters: BW, BT and disease score during the post challenge time were recorded and evaluated. Both formulations of MVA-BN were shown to reduce the severity of the disease course to similar levels. The prime-boost regimen resulted in a milder disease course, whereas the single dose administration of MVA-BN conferred protection but it was accompanied with a more severe disease outcome.

Lastly, all mice groups vaccinated with MVA-BN, irrespective of formulation presentation, either in prime-boost or single vaccinations, were shown to clear virus titers from lung tissue at 10 Days post challenge.

Study Number: BN-PRE-011-028: Non-inferiority of onset of protection of (b) (4) IMVAMUNE compared to liquid-frozen IMVAMUNE in the mouse vaccinia virus challenge model.

The objective of nonclinical study BN-PRE-11-028 was to demonstrate non-inferiority of the (b) (4) MVA-BN to the liquid frozen (LF) MVA-BN, given as a single immunization, in terms of survival after a lethal (i.n.) challenge with vv-WR on Day 4 – 10 post-vaccination. This study was designed to investigate and compare the onset of protection afforded by a single vaccination of the (b) (4) and LF formulations of MVA-BN. For this study, (b) (4) mice (Female, 6 -7 weeks; N = 780) were set up according to three (3) main treatment groups: **Group 1 (TBS, 1x dose; N = 20); Group 2 (MVA-BN [(b) (4)], 1x dose of 1×10⁸ TCID₅₀; N = 380); and Group 3 (MVA-BN [LF], 1x of**

1×10⁸ TCID₅₀; N = 380). This study was staggered such that each treatment group was subdivided into Part I, Part II, Part III and Part IV (e.g., **Group 1**, Part I – IV had N = 5 per Part; **Group 2 / 3**, Part I had N = 5 and Part II - IV had N = 125 per part).

Treatment schedule: On Day 0, all mice received a single (s.c.) treatment of TBS (Group 1), (b) (4) MVA-BN (Group 2) or (LF) MVA-BN (Group 3). A lethal (i.n.) challenge dose of 50x MLD₅₀ of vv-WR was given on different days post vaccination: **Part I** = Day 0; **Part II** = Day 4; **Part III** = Day 7; and **Part IV** = Day 10. All mice were monitored daily for various clinical parameters: body weight (**BW**), body temperature (**BT**), and disease score (based on disease-related symptoms).

Blood sampling were done as follows: Pre-vaccination bleed, pre-challenge bleed and post-challenge bleed samples were collected at various time points (Day) depending on the “Part” of the study: **Part I** (i.e., Day -5, 4); **Part II** (i.e., Day -2, 3, 14); **Part III** (i.e., Day -2, 6, 17); and **Part IV** (i.e., Day -6, 9, 20), respectively. Serum samples were analyzed by ELISA to measure seroconversion rates and vaccinia-specific antibody responses (GMT).

The endpoints for this study were the following:

- **Primary endpoint**: Demonstrate non-inferiority of the (b) (4) -MVA-BN to **LF**-MVA-BN in terms of the survival of (b) (4) mice challenged (i.n.) with a lethal dose of 50x MLD₅₀ of vv-WR on Day 4, 7 or 10 after a single vaccination.
- **Secondary endpoint**: Comparison of vaccinia-specific antibodies induced by (b) (4) - and LF MVA-BN in terms of seroconversion rates and GMTs measured by ELISA, using serum samples collected on the day(s) before challenge. In addition, comparison of lung virus clearance rates and other clinical parameters, as expressed by BW loss, BT changes post-challenge, and disease scores (based on clinical appearance), between the two vaccination groups.

Results:

Protective efficacy: All mice groups vaccinated with MVA-BN, irrespective of formulation presentation, were shown to survive the lethal i.n. challenge with vv-WR at all time points (Day 4, 7 and 10). Thus the primary study endpoint was fulfilled.

Immunogenicity: The seroconversion rates and vaccinia-specific antibody titers (GMTs) are described for challenge time points on Day 4 (Part II), Day 7 (Part III) and Day 10 (Part IV) after vaccination.

- **Part II**: None of the immunized mice of Part II of the study seroconverted by Day 3 (prior to challenge), irrespective of the formulation type. The post challenge GMTs for both vaccine groups appeared comparable (on Day 14).
- **Part III**: Several vaccinated mice in Part III (of the study) seroconverted (e.g., 87% and 93% for (b) (4) - and **LF**-MVA-BN groups, respectively), and ELISA antibody titers (GMTs) were considered comparable (e.g., GMTs values of 146 and 171 were recorded, respectively). Post challenge GMTs (on Day 17) for the (b) (4) - and LF MVA-BN showed a 23-fold and 16-fold increase in titers, respectively.

- **Part IV:** The immunizations in Part IV using both formulations of MVA-BN resulted in 100% seroconversion by Day 9. The antibody titer (GMT) induced by (b) (4) formulation (*i.e.*, GMT of 1649) was determined to be superior to that of the LF formulation (GMT of 970) of MVA-BN. Post challenge GMTs (on Day 20) for the (b) (4) - and LF MVA-BN were elevated by 6-fold and 11-fold, respectively.

Clinical parameters: BW, BT and disease score during post challenge times of the two immunized groups displayed similar trends when mice were challenged on Day 4, 7 or 10 post-vaccination. A reduced disease outcome, as expressed by each clinical parameter, was observed in immunized mice compared with unvaccinated mice (Group 1/TBS).

Lastly, all immunized mice were shown to clear lung vv-WR titers from lung tissue at 10 Days post challenge when challenged on Day 4, Day 7 or Day 10 post single vaccination with MVA-BN, irrespective of formulation presentation. Immunized mice challenged on the same day of vaccination succumbed to infection and harbored high lung virus titers, which were comparable to those of the TBS treated group.

Intranasal (i.n.) murine challenge model with a lethal dose of Ectromelia Virus (ECTV)

Study Number: BN-PRE-05-010: Efficacy of vaccination with MVA-BN following intranasal challenge with different challenge doses of Ectromelia Virus

The objective of non-clinical study BN-PRE-05-010 was to further develop the ECTV challenge model by evaluating the protective efficacy afforded by MVA-BN in terms of survival after a challenge with different lethal doses of ECTV. For this study, (b) (4) mice (7 or 9 weeks; N = 90) were set up according to nine (9) main treatment groups: **Group 1 – 3 (Placebo;** N = 10 per group; 2x doses of **PBS**); **Group 4 -6 (MVA-BN,** 2x doses of 1.0×10^8 TCID₅₀; N = 10 per group); and **Group 7 – 9 (MVA-BN,** 1x dose of 1.0×10^8 TCID₅₀; N = 10 per group).

Treatment schedule: On Day -42 (prior to challenge on Day 0), mice received first subcutaneous (s.c.) treatment of PBS (Group 1 – 3) or MVA-BN (both Group 7 – 9 / Group 4 – 6). On Day -14, mice received second s.c. treatment of either PBS (Group 1 – 3) or MVA-BN (only Group 4 – 6). All treatment groups were challenged with a different lethal dose of ECTV of 42x (1×10^3 TCID₅₀), 420x (1×10^4 TCID₅₀) or 4200x MLD₅₀ (1×10^5 TCID₅₀) on Day 0. Mice were monitored for changes in clinical parameters: Body weight (**BW**), Body temperature (**BT**), health status (scored as *healthy*, *sick* or *dead*), and clinical signs of disease. Pre-vaccination bleed (on Day -44) and post-vaccination bleed (Day -2) samples were collected for ELISA analysis, and lung ECTV titers were determined at the end of the study or when mice died.

Results:

Protective efficacy and onset of disease: Unvaccinated mice in the placebo groups (Group 1 – 3) displayed varying onset of disease (*i.e.*, Day 8, 6 and 4, respectively) in response to the three lethal dose levels of ECTV, and the unvaccinated mice eventually died (or were sacrificed) in a somewhat successive manner, with times of death

occurring between Day 8 and 11, Day 5 and 8, and Day 4 and 6 in response to challenge doses of 42x, 420x and 4200x MLD₅₀ of ECTV, respectively. All immunized mice (in Group 7 – 9 / Group 4 – 6) survived the different lethal doses of ECTV. However, the vaccinated groups of mice displayed temporary or extended signs of illness according to the MVA-BN vaccination regimen (*i.e.*, 1x dose vs. 2x doses of MVA-BN) and the different lethal dose levels of ECTV.

Clinical parameters: The prime-boost vaccination with MVA-BN resulted in a milder disease course following the different (*i.n.*) challenge doses with ECTV as reflected by the modest loss of BW and BT. The single immunization with MVA-BN was unable to reducing the disease course of the lethal (*i.n.*) challenge doses of 420x and 4200x MLD₅₀ of ECTV to a similar extent as that of the prime-boost immunization. All mice vaccinated with a single dose of MVA-BN displayed a higher loss of BW and BT after the (*i.n.*) challenges with ECTV.

ELISA and lung virus titer analysis: The ELISA results showed that all pre-vaccination sera were negative for vaccinia-specific antibodies as well as sera from the placebo treatment groups (Group 1 – 3). The prime-boost vaccination with MVA-BN consistently induced a higher antibody response (GMTs 4.04 – 4.11 log₁₀) compared with the single vaccination of MVA-BN (GMTs 2.92 – 3.17 log₁₀) prior to the lethal challenge.

ECTV titers in lung tissue were examined to assess the titer levels or clearance of ECTV from treated mice. The unvaccinated mice showed high mean lung virus titers after challenge, whereas all immunized mice had no detectable lung titers of ECTV, even at the highest challenge dose of 4200x MLD₅₀ of ECTV.

Thus, it was concluded that the MVA-BN vaccine was capable of fully protecting mice from lethal doses of ECTV. The single dose of MVA-BN conferred 100% survival.

Study Number: BN-PRE-07-023: Protective efficacy of MVA-BN in (b) (4) mice challenged with three different doses of Ectromelia virus.

The objective of non-clinical study BN-PRE-07-023 was to investigate the protective efficacy of MVA-BN (1×10⁸ TCID₅₀) given as a prime-boost vaccination, on Week 0 and 4, to mice challenged (*i.n.*) with different lethal doses of ECTV on Week 6. For this study, (b) (4) mice (Female, 6 - 8 weeks; N = 45) were set up according to six (6) treatment groups: **Group 1 – 3 (Placebo, 2x doses of PBS; N = 5 per group); and Group 4 -6 (MVA-BN, 2x doses of 1×10⁸ TCID₅₀; N = 10 per group).**

Treatment schedule: Mice received subcutaneous (*s.c.*) treatments of PBS (in Group 1 – 3) or MVA-BN (in Group 4 – 6) on Day -42 and Day -14 (prior to challenge on Day 0). Afterwards, all groups of mice were challenged with three (3) different doses of ECTV of 29x, 58x or 580x MLD₅₀ on Day 0. Mice were monitored daily for changes in clinical parameters (for 21 days post challenge): Body weight (**BW**), Body temperature (**BT**), and health status (scored as *healthy*, *sick* or *dead*). Lung ECTV lung titers were determined at the end of the study observation or when mice died.

Results:

Protective efficacy and onset of disease: Unvaccinated mice in the placebo groups (Group 1 – 3) displayed varying onset of disease (*i.e.*, at Day 6 -7 and Day 4 -5) in response to the increased lethal dose levels of ECTV (*i.e.*, 29x, 58x or 580x MLD₅₀), and unvaccinated mice died in a somewhat successive manner with times of death occurring on Day 8 – 9 and Day 7 in response to the increased challenge doses of ECTV, respectively. All mice in the vaccinated groups (Group 4 – 6) survived the different lethal doses of ECTV. The highest challenge dose of ECTV resulted in onset of illness recorded at Day 5 in vaccinated mice and clinical symptoms at Day 7. (One vaccinated mouse succumbed to the infection on Day 8 which high lung virus titers of 8.19 log₁₀ pfu).

Clinical parameters: Change in BW and BT in response to the ECTV infection were recorded. Vaccinated mice (Group 4 -5) challenged with either 29x or 58x MLD₅₀ of ECTV showed a modest loss in BW whereas the BT results showed no change. However, the highest challenge dose of ECTV resulted in a substantial loss of BW (of -25%) in vaccinated mice (Group 6) on Day 10 - 11, and a transient drop in BT (of -6%) was recorded on Day 9 – 11.

Lastly, ECTV titers in lung tissue were examined to assess titer levels or clearance of ECTV in treated mice. Unvaccinated mice in the placebo groups displayed high lung virus titers (6.87 – 7.45 log₁₀ pfu). Vaccinated groups of mice showed either a substantially reduction in lung virus titers or clearance of ECTV from lung tissue. Vaccinated mice (Group 6) challenged with the highest dose of ECTV showed a 60% clearance of virus from lung tissue, whereas the remaining 40% of these mice contained detectable lung titers (ranging from 3.40 to 8.19 log₁₀ pfu).

Study Number: BN-PRE-08-019: Comparison of the immunogenicity and protective efficacy of IMVAMUNE and Dryvax in a lethal ECTV challenge model in (b) (4) mice.

The objective of non-clinical study BN-PRE-08-019 was to verify that the lethal dose of 58x MLD₅₀ of ECTV (used in study BN-PRE-07-023) was a suitable challenge dose and to compare the immunogenicity and protective efficacy of MVA-BN and Dryvax in the murine ECTV challenge model. For this study, (b) (4) mice (Female, ~7 weeks; N = 40) were set up according to four (4) treatment groups: **Group 1 (Placebo)**, 2x doses of TBS; N = 5); **Group 2 (Dryvax)**, 1x doses of 2.5×10⁵ TCID₅₀; N = 15); **Group 3 (MVA-BN)**, 1x dose of 1×10⁸ TCID₅₀; N = 10); and **Group 4 (MVA-BN)**, 2x doses of 1×10⁸ TCID₅₀; N = 10).

Treatment schedule: Mice received (s.c.) treatments of PBS (in Group 1) or MVA-BN (in Group 4) on Day 0 and Day 28 (prior to viral challenge on Day 42); or, mice received a single (s.c.) dose of MVA-BN or a single (p.c.) dose of Dryvax on Day 0. Afterwards, all mice groups were challenged with a lethal (i.n.) dose of ECTV of 58x MLD₅₀ on Day 42. Mice were monitored daily for changes in clinical parameters (for 21 days post challenge): Body weight (**BW**), health status (scored as: *healthy*, *sick* and *dead*), and clinical signs of disease. Pre-vaccination bleed (on Day -2) and post vaccination bleed samples were collect (*i.e.*, on Day 7, 14, 26, 35, and 40) for ELISA and PRNT analysis.

Lung ECTV titers were determined at the end of the study observation or when mice died.

Note: The Dryvax group (N = 15) had two mice died prior to the (i.n.) challenge and three mice did not seroconvert and these mice were subsequently excluded from the study. Thus, the Dryvax group consisted of 10 mice.

Results:

Protective efficacy and clinical parameters: All unvaccinated mice succumbed to the infection after the challenge dose of 58x MLD₅₀ of ECTV (as revealed by elevated sickness on Day 6 and subsequent death recorded on Day 7 – 8 (post challenge)). All immunized mice were shown to survive the lethal (i.n.) challenge with ECTV. The prime-boost vaccination with MVA-BN protected mice from onset of disease and transient loss of BW. In contrast, the single vaccination with MVA-BN and Dryvax showed onset of disease course as displayed by the extended loss of BW, which gradually recovered towards the end of the study (Day 21 post challenge). Therefore, this study concluded that the dose of 58x MLD₅₀ of ECTV was a suitable challenge dose based on all unvaccinated succumbing to the infection and vaccinated mice surviving the challenge.

ELISA, PRNT and lung virus titer analyses: 100% seroconversion (by ELISA titers) was reached in mice vaccinated once with MVA-BN (Group 3 and 4) at Day 7. The immunization with Dryvax (Group 2) resulted in 100% seroconversion at Day 35 post-vaccination. The single vaccination with MVA-BN (Group 3) induced ELISA GMTs from 1301 to 22,786 on Day 7 and 40, respectively. The vaccinia-specific antibody response was elevated with a second MVA-BN vaccination (on Day 28) and GMTs reached a plateau from 17,473 to 98,240 (on Day 26 to Day 35). The Dryvax vaccination resulted in a peak GMT of 1111 on Day 40.

A single vaccination with MVA-BN was capable of conferring 100% seroconversion of mice, as measured by PRNT, on Day 14. This single vaccination with MVA-BN resulted in a peak PRNT GMT of ~1000 by Day 26 – 35. The prime-boost vaccination with MVA-BN elevated the PRNT GMT to ~4700 at Day 40. The vaccination with Dryvax resulted in 80% seroconversion prior to challenge of mice, and a peak PRNT GMT of ~293 was measured at Day 40.

ECTV titers in lung tissue were examined to assess the titer levels or clearance of ECTV in treated mice. The unvaccinated mice treated with TBS showed high mean lung virus titers (8.14 log₁₀ pfu) after challenge, whereas mice vaccinated with a single or prime-boost vaccination of MVA-BN had undetectable lung ECTV titers. In contrast, this analysis showed that the Dryvax vaccination resulted in 80% of mice clearing ECTV from lung tissue.

Study Number: BN-PRE-10-004: Onset of protection of IMVAMUNE in a mouse Ectromelia Virus challenge model.

The objective of non-clinical study BN-PRE-10-004 was to investigate the onset of protection induced by MVA-BN compared with ACAM2000 in the ECTV challenge model in (b) (4) mice. The survival rate conferred by MVA-BN was compared with that

of ACAM-2000. For this study, (b) (4) mice (Female, 6-7 weeks; N = 174) were set up according to three (3) main treatment groups: Group 1 (Placebo/TBS; N = 30); Group 2 (1x MVA-BN, one dose of 1.0×10^8 TCID₅₀; N = 72); and Group 3 (1x ACAM2000, one dose of $\sim 7.5 \times 10^5$ pfu; N = 72).

Treatment schedule: On Day 0, mice received a single subcutaneous (s.c.) treatment of TBS (Group 1) or MVA-BN (Group 2) or a single percutaneous (p.c.) dose of ACAM2000 (Group 3). The study was staggered such that treatment groups were subdivided into six (6) parts (*i.e.*, **Part I**, **-II**, **-III**, **-IV**, **-V** and **-VI**) with each “Part” corresponding to a challenge time point: *i.e.*, Day -1, 0, 4, 7, 10 or 42, respectively. Thus, mice were challenged with a lethal (i.n.) dose of 58x MLD₅₀ of ECTV one day prior to vaccination (*i.e.*, Day -1), on the day of vaccination (*i.e.*, Day 0), and after vaccination (*i.e.*, Day 4, 7, 10 and 42). Mice were monitored daily for changes in health status and body weight (BW). (**Note**: Group 1 [N = 5 per **Part**]; Group 2 [N = 12 per **Part**]; and Group 3 [N = 12 per **Part**]).

Blood sampling were done as follows: (i) Pre-vaccination bleed samples (on Day -4 for Part I, -II, -III and -IV; and on Day -2 for Part IV and -VI); (ii) Pre-challenge bleed samples (on Day 3, 6, 9, and 41 for Part III, IV, V and VI, respectively); and (iii) Post challenge terminal bleeds were collected at end of study (*i.e.*, Day 21 post challenge).

Results:

Immunogenicity: All mice immunized with ACAM2000 in **Part I** and **-II** (*challenged on Day -1 and Day 0*) succumbed to infection and, as result, no post challenge sera were collected in these parts of the study (pre-vaccination only). As expected, all pre-vaccination sera and sera from unvaccinated mice (TBS group) had no detectable antibody titers (in all study parts). For Part I and -II of the study, only antibody titers were analyzed in surviving mice.

The MVA-BN vaccination induced antibody responses in mice before the ACAM2000 vaccination. An 83% seroconversion rate was recorded in MVA-BN immunized mice in Part IV (on Day 6) of the study, whereas a single mouse seroconverted in the ACAM2000 group of Part IV. A 100% seroconversion was recorded in the ACAM2000 group of Part VI (at Day 41 post vaccination). At this same time point (*i.e.*, Day 41), the ACAM2000 vaccination resulted in an ELISA GMT of 1254. In comparison, the MVA-BN vaccination resulted in ELISA GMTs of 85, 1434 and 7435 on Day 6, 9 and 41 post-vaccination.

Protective efficacy and clinical parameters: Survival (protection), disease score (disease level ranged from 0 - 5) and BW were monitored for 4 days for the TBS group (Group 1) and for 10 days for the vaccinated mice (in Group 2 and 3) starting on the day of challenge.

- **Part I** and **Part II**: All treatment groups developed moderate to severe signs of disease following the ECTV challenge. Unvaccinated mice and mice vaccinated with ACAM2000, in Part I of the study, died at similar rates (from 7 – 9 days), whereas ACAM2000 group in Part II showed a slower rate of death (9 – 12 days). All except one mouse vaccinated with MVA-BN in Part I died (from 8 - 21 days), and MVA-BN conferred partial protection in Part II of the study (with 4 out

of 12 mice surviving). All treatment groups of Part I and II exhibited high loss of BW (e.g., BW loss in Part II ranged from -33.3% to -31.2%).

- **Part III:** All immunized mice survived the (i.n.) challenge with ECTV, whereas all unvaccinated mice (TBS) died. Mice in the MVA-BN group did not develop signs of disease other than transient mild disease recorded on one day after the challenge. The majority of mice in the ACAM2000 group displayed mild sign of disease that lasted a few days. Otherwise, all immunized groups of mice displayed mild loss of BW after 1 - 2 days post challenge.
- **Part IV, Part V and Part VI:** Both vaccinated groups of mice in the last three parts of the study survived the (i.n.) challenge with ECTV, whereas all unvaccinated mice died. In addition, the MVA-BN vaccinated group of Part IV and V showed no signs of disease, whereas the ACAM2000 group of Part V developed transient mild signs of disease and showed a mild loss of BW. In contrast, both vaccinated groups of mice in Part VI recorded mild signs of disease as well as pronounced loss of BW (e.g., peak BW loss was -18.8% and -21.3% for ACAM2000 and MVA-BN, respectively).

Reviewer's comments:

Taken together, the study showed the onset of protection was conferred by a single dose of MVA-BN compared with a single dose of ACAM2000 in mice challenged with ECTV. However, MVA-BN provided partial protection when administered on the same day of challenge with ECTV, whereas ACAM2000 did not confer protection when administered on the same day of virus challenge. Also, MVA-BN was observed to elicit a more rapid antibody response than ACAM2000.

Study Number: BN-PRE-11-027: Non-inferiority of the efficacy and immunogenicity of (b) (4) IMVAMUNE compared to liquid-frozen IMVAMUNE in a mouse Ectromelia Virus challenge model.

The objective of nonclinical study BN-PRE-11-027 was to demonstrate the non-inferiority of the (b) (4) MVA-BN to the liquid frozen (LF) MVA-BN, given as a single immunization or prime-boost regimen, in terms of protective efficacy and immunogenicity in the murine ECTV challenge model. For this study, (b) (4) mice (Female, 6 -8 weeks; N = 510) were set up according to five (5) main treatment groups: **Group 1** (TBS, 2x doses; N = 10); **Group 2** (1x MVA-BN (b) (4), 1x dose of 1×10^8 TCID₅₀; N = 125); **Group 3** (2x MVA-BN (b) (4), 2x doses of 1×10^8 TCID₅₀; N = 125); **Group 4** (1x MVA-BN [LF], 1x dose of 1×10^8 TCID₅₀; N = 125); and **Group 5** (2x MVA-BN [LF], 2x doses of 1×10^8 TCID₅₀; N = 125). This study was staggered such that each treatment group was subdivided into **Part I** and **Part II** (e.g., **Group 1** of **Part I** [N = 5 per]; and **Group 2** of **Part I** [N = 60] and **Part II** [N = 65] and so forth for Group 3, -4 and -5).

Treatment schedule: On Day 0, mice received first (s.c.) treatment of TBS (in Group 1), (b) (4) MVA-BN (in Group 2 and -3) or (LF) MVA-BN (in Group 4 and -5). On Day 28, mice in Group 1, -3 and -5 received a second s.c. treatment of either TBS or MVA-BN. Afterwards, all groups of mice were challenged (on Day 42) with a lethal (i.n.) dose of

58x MLD₅₀ of ECTV. Mice were monitored daily for changes in clinical parameters: body weight (**BW**) and disease score (based on disease-related symptoms).

Blood sampling was scheduled as follows: Pre-vaccination bleed (*i.e.*, Day -2), pre-challenge bleed (*i.e.*, Day 26 and 40), and post-challenge bleed (*i.e.*, Day 63) samples were collected for ELISA analysis.

The endpoints for this study were as follows:

- **Primary endpoint:** Demonstrate non-inferiority of the (b) (4) -MVA-BN compared with the **LF**-MVA-BN, when given either as a single immunization or in a prime-boost regimen, in terms protective efficacy against an i.n. challenge with 58x MLD₅₀ of ECTV. .
- **Secondary endpoint:** Demonstrate non-inferiority of the peak total antibody response (GMT measured by ELISA) induced by (b) (4) -MVA-BN compared with **LF**-MVA-BN prior to challenge.
- **Tertiary endpoint:** Compare all other parameters between groups vaccinated with (b) (4) - MVA-BN and **LF**-MVA-BN. These included seroconversion rates and GMT measure by ELISA at the remaining time points as well as body weight (BW) changes and clinical signs/appearance post challenge.

Results:

Protective efficacy: All MVA-BN vaccinated mice were shown to survive the lethal (i.n.) dose of 58x MLD₅₀ of ECTV, irrespective of vaccine formulation. Thus the primary study endpoint was fulfilled.

Immunogenicity: The vaccinations with both the (b) (4) - and LF formulations of MVA-BN induced statistically comparable peak vaccinia-specific antibody responses (as shown by ELISA GMT results). Thus, the peak total antibody response induced by the (b) (4) - MVA-BN was assessed to be non-inferior to the LF-MVA-BN prior to challenge.

Clinical parameters: Disease scores and BW during post challenge time points were recorded and assessed. The vaccinations with (b) (4) - or LF formulations of MVA-BN were able to reduce the severity of the disease course. The prime-boost vaccinations with MVA-BN protected against the onset of disease as well as against any noticeable loss of BW. However, the single dose of MVA-BN resulted in a mild disease course, which was associated with a pronounced loss of BW at ~ Day 9 post-challenge (*e.g.*, peak BW loss of -21.3% and -14.9% for 1x MVA-BN (b) (4)] and 1x MVA-BN [LF] were recorded, respectively,).

Study Number: BN-PRE-12-029: Non-inferiority of onset of protection of (b) (4) IMVAMUNE compared to liquid-frozen IMVAMUNE in a mouse Ectromelia Virus challenge model.

The objective of non-clinical study BN-PRE-12-029 was demonstrate non-inferiority of the (b) (4) MVA-BN to the liquid frozen (**LF**) MVA-BN, when given as a single immunization, in terms of survival after a lethal (i.n.) challenge with ECTV. This study was designed to investigate and compare the onset of protection conferred by a single vaccination of the (b) (4) - and LF formulations of MVA-BN. For this study, (b) (4)

mice (Female, 6 -7 weeks; N = 1040) were set up according to three (3) main treatment groups: **Group 1** (TBS, 1x dose; N = 40); **Group 2** (MVA-BN [(b) (4)], 1x dose of 1×10^8 TCID₅₀; N = 500); and **Group 3** (MVA-BN [LF], 1x dose of 1×10^8 TCID₅₀; N = 500). This study was staggered such that each treatment group was subdivided into Part I, Part II, Part III and Part IV (e.g., **Group 1**, Part I – IV had N = 10 per Part; and **Group 2** and **3**, Part I – IV had N = 125 per Part).

Treatment schedule: On Day 0, all mice received a single (s.c.) treatment of TBS (in Group 1), [(b) (4)] MVA-BN (in Group 2) or (LF) MVA-BN (in Group 3). A lethal (i.n.) challenge dose of 58x MLD₅₀ of ECTV was given on different days post vaccination: **Part I** = on Day 3; **Part II** = on Day 4; **Part III** = on Day 7; and **Part IV** = on Day 10. All mice were monitored daily for various clinical parameters after challenge (i.e., change of body weight (**BW**) and disease score (based on disease-related symptoms).

Blood sampling was scheduled as follows: Pre-vaccination bleed, pre-challenge bleed and post-challenge bleed samples were collected on various time points (Day) depending on the “Part” of the study: **Part I** (i.e., Day -1, 24); **Part II** (i.e., Day -4, 3, 25); **Part III** (i.e., Day -6, 6, 28); and **Part IV** (i.e., Day -1, 9, 31), respectively. Serum samples were analyzed by ELISA to measure seroconversion rates and vaccinia-specific antibody response (GMT).

The endpoints for this study were the following:

- **Primary endpoint**: Demonstrate non-inferiority of the [(b) (4)]-MVA-BN to LF-MVA-BN in terms of the survival of [(b) (4)] mice challenged with a lethal (i.n.) dose of 58x MLD₅₀ of ECTV on Day 3, 4, 7 or 10 after a single vaccination.
- **Secondary endpoint**: Comparison of vaccinia-specific antibodies induced by [(b) (4)] - and LF MVA-BN in terms of seroconversion rates and GMTs measured by ELISA, using serum samples collected the day before challenge. In addition, clinical parameters, as expressed by BW loss and disease scores (clinical appearance), were compared between the two immunized groups.

Results:

Protective efficacy: All mice groups vaccinated with MVA-BN, irrespective of formulation presentation, were shown to survive the lethal (i.n.) challenge with ECTV at all time points (i.e., Day 3, 4, 7 and 10). Thus the primary study endpoint was fulfilled. (Unvaccinated mice in the TBS group succumbed to the infection and died between Day 7 and 10 post challenge).

Immunogenicity: The vaccinations in Part II (of the study) did not result in seroconversion at Day 3 prior to challenge (on Day 4). The post challenge GMTs for both vaccinated groups of Part II appeared similar (on Day 25). Several immunized mice in study Part III seroconverted by Day 6 (e.g., seroconversion rates of 90% and 87% were recorded for [(b) (4)] - and LF-MVA-BN groups, respectively), and ELISA titers (GMT) were considered comparable (e.g., GMT values of 209 and 156 were recorded, respectively). Last, the vaccinations in study Part IV were shown to confer 100% seroconversion at Day 9 prior to challenge (on Day 10). ELISA GMTs at Day 9 were 1880 and 929 for [(b) (4)]-MVA-BN and LF-MVA-BN, respectively, and post-challenge GMTs

on Day 31 in Part IV were further elevated (e.g., GMTs values for (b) (4) - and LF-MVA-BN groups were 29778 and 26031, respectively).

Clinical parameters: The BW and disease score (determined by clinical appearance) of the two immunized groups exhibited similar trends after challenge on Day 3, 4, 7 or 10. Mild or minimal signs of disease were recorded for each part of the challenge study, as displayed by a minor decrease in BW after challenge.

Reviewer's comments:

This study claimed that all study endpoints were fulfilled as supported by the study results. Thus, this study considered the (b) (4) - formulation of MVA-BN to be non-inferior to the LF-formulation of MVA-BN.

Summary Research Report: Immediate and therapeutic protection against lethal poxvirus infection in severely immune compromised animals.

The objective of this study was to investigate the onset of protection by a single vaccination of MVA-BN against lethal (i.n.) challenges with ECTV. This study used (b) (4) mice and two different immune deficient mice (i.e., (b) (4)), which are related to the wild type (b) (4) genetic background, that are known to be more susceptible to viral infection including poxviruses.

For this study, mice (Male and Female) were challenged with different (i.n.) doses of ECTV (i.e., 10, 30, 1×10^2 , 1×10^3 , 1×10^4 and 1×10^5 TCID₅₀). MVA-BN (1×10^8 TCID₅₀) was administered via the i.n. route either in combination with the ECTV challenge dose or up to 72 hours after ECTV challenge. The health status of infected mice were monitored daily and mice with grave signs of illness and body weight loss of > -25% were sacrificed.

Results:

Protective efficacy: Protection of wild type (wt) and (b) (4)-deficient mice were evaluated in ECTV challenge experiments. The MVA-BN treatment administered simultaneously with challenge or after challenge with a high (i.n.) dose of 1×10^5 TCID₅₀ of ECTV resulted in protection of wt mice ((b) (4)), whereas all untreated mice (control) succumbed to the infection when challenged with 1×10^4 or 1×10^5 TCID₅₀ of ECTV. The (b) (4)-deficient mice are indicated to be 40-times more susceptible to ECTV infection than the immune competent wt mice. Thus, the MVA-BN treatment protected (b) (4)-deficient mice from a high lethal dose of ECTV (1×10^2 TCID₅₀), whereas all untreated mice (control) succumbed to infection. In addition, a single dose of MVA-BN administered up to 48 hour after ECTV challenge was shown to confer protection of the (b) (4)-deficient mice, whereas MVA-BN given at 72 hour post challenge resulted in a 40% survival rate of these same mice.

The ability of MVA-BN to confer protection in (b) (4) deficient mice was evaluated in ECTV challenge experiments. The MVA-BN treatment was shown to provide protection when mice were challenged with the ECTV (1×10^2 or 1×10^3 TCID₅₀) on the same day, whereas all untreated (b) (4) deficient mice (control) died within 10 days post challenge with ECTV (1×10^2 TCID₅₀). Moreover, vaccination of (b) (4) deficient mice with Dryvax resulted in death of these mice, even in the absence of ECTV challenge.

Reviewer's comments:

This study indicated that a single dose of MVA-BN (treatment given via the intranasal route) can confer protection against a lethal ECTV infection occurring on the same day of MVA-BN treatment. In addition, MVA-BN conferred protection at 48 hours post challenge with ECTV, which indicated that MVA-BN can be used in a post-exposure setting.

4.2.2.3 (Pharmacokinetics) – Biodistribution**SRI Study No.: G182-03 – Pilot biodistribution study with MVA-BN in male and female rabbits.**

The objective of the following distribution study was to develop a (b) (4) assay method for detecting vaccinia-specific (b) (4), to determine the time of peak tissue concentration of vaccinia-specific transcripts in rabbits after a single injection, use this information in the design of a definitive distribution study. This study was conducted to provide preliminary data on the distribution of MVA-BN based on the (b) (4) of MVA (b) (4) in rabbits at a dose that exceeds the highest dose level anticipated for clinical use. The information from this study determined sampling time points and tissues to be applied to a definitive distribution study plan.

For this study, (b) (4) rabbits (Male [M] and Female [F]; N = 18M+18F) were set up according to six (6) treatment groups (with N = 3M+3F rabbits per group). The rabbit groups received either a single intramuscular (IM) or subcutaneous (SC) injection (0.2 mL) of vehicle control (e.g., TBS; in Group 1 or Group 2), Dryvax (2×10^7 pfu) as positive control (in Group 3 or Group 4), and MVA-BN (1×10^8 TCID₅₀; in Group 5 or Group 6). Tissues were harvested at 24 and 48 hours and 6 days post injection (2 animals [N = 1M+1F] per time point per group). Sixteen (16) different tissue samples were analyzed for vaccinia (b) (4) per animal. (These tissues included: injection site *skin* and *muscle*, *whole blood*, the right and left *kidneys* and *lungs*, *heart*, *brain*, *thymus*, *spleen*, *liver*, pooled draining *lymph nodes* (popliteal, inguinal, and iliac nodes), *mesenteric lymph node*, *bone marrow* from a hind leg femur, and either the *ovaries* or *testes*). For each sample, (b) (4)

(b) (4)

Results:

Vaccinia virus distribution: The enclosed distribution study showed that the injection site skin was the predominant tissue positive for vaccinia (b) (4) following IM or SC injections with MVA-BN (9 out of 12 rabbits were tissue positive) and Dryvax (11 out of 12 rabbits were tissue positive). No or very weak MVA-BN (b) (4) was observed at Day 6 post injection, whereas Dryvax (b) (4) were detected up to Day 6 in the injection site. Other than the injection skin tissue, there were single instances of tissues positive for MVA-BN (b) (4) detected in mesenteric lymph node, spleen, pooled lymph nodes and injection site muscle, which were weakly detected. This study showed that the vaccinia (b) (4) was broader in Dryvax treated rabbits than for MVA-BN. For Dryvax, vaccinia (b) (4) was detected in lungs, liver, spleen, pooled lymph

nodes, and injection site muscle, in addition to the injection site skin. However, for the vehicle control group (IM and SC administrations), five (5) muscle samples and a single lung sample tested weakly positive for vaccinia (b) (4) in the (b) (4) assay. The explanation for these results may be related to contamination during (b) (4) or necropsy. Nevertheless, (b) (4) of these putative positive tissues were not repeated due to insufficient amounts of saved tissue.

Clinical observations: The study indicated that the SC injections of MVA-BN and Dryvax vaccines resulted in swelling and greater prevalence of red discoloration at the injection site than when the vaccines were administered using the IM route.

Reviewer's comments:

The following MVA-BN distribution study detected weak vaccinia (b) (4) in a few tissues (i.e., mesenteric lymph node, spleen and pooled lymph nodes) other than the injection site skin. The earliest time point used in this study was 24 hours post injection. The study considered the possibility that peak vaccinia (b) (4) in outlying tissue may occur at an earlier time point than those examined in this study (i.e., 24 hours post injection). Thus, based on these results and conclusions of this study, it was proposed that the definitive MVA-BN distribution study include an earlier analysis time point (e.g., 5 – 15 hours post injection) and analysis of fewer tissues, especially at the later time points. This study report provides detailed procedures for molecular biology and (b) (4) methods.

SRI Study No.: G216-04 – Definitive biodistribution study with MVA-BN in male and female rabbits.

The objective of SRI Study No. G216-04 was to provide confirmatory data on the distribution of MVA-BN after a single 0.5 mL intramuscular (IM) or subcutaneous (SC) injection of 1×10^8 TCID₅₀ of MVA-BN in rabbits. An (b) (4) method was used to detect (b) (4) in various tissues from rabbits injected with MVA-BN compared with TBS (vehicle control). For this study, (b) (4) rabbits (N = 42 Male+42 Female [= 84]) were set up according to four (4) treatment groups. The rabbit groups received either a single IM or SC injection (0.5 mL) of vehicle control (in Group 1 [N = 6M+6F; **IM**] or Group 2 [N = 6M+6F; **SC**]) or 1×10^8 TCID₅₀ of MVA-BN (in Group 3 [N = 15M+15F; **IM**] or Group 4 [N = 15M+15F; **SC**]). Tissues were harvested at 15 and 48 hours and 7 days post injection: (i) vehicle control groups (N = 2M+2F per time point per group) or (ii) MVA groups (N = 5M+5F per time point per group). Eleven (11) different tissue samples and whole blood were analyzed for (b) (4) vaccinia (b) (4) per animal. (These tissues included: injection site *skin* and *muscle*, the right *kidneys*, the right *lungs*, *heart*, *brain*, *spleen*, *liver*, pooled draining *lymph nodes* [popliteal, inguinal, and iliac nodes], *mesenteric lymph node*, and either the *ovaries* or *testes*). (b) (4) from the different tissues and whole blood from each rabbit was analyzed at the specified time points after treatment. Only tissues that tested positive for vaccinia (b) (4) at 48 hours were further analyzed at Day 7 post injection in the two different dose administration groups. For each tissue sample, (b) (4)

(b) (4) . Samples that were determined to be positive for vaccinia virus (b) (4) were analyzed by (b) (4) in (b) (4)).

Results:

The enclosed MVA-BN distribution study revealed that vaccinia (b) (4) (by (b) (4) analysis) was in general comparable between the MVA-BN SC and IM dose groups (as well as between Male and Female rabbits). Vaccinia (b) (4) was predominantly detected in the injection skin site and muscle tissue at 15 and 48 hours. However, at Day 7, one skin sample in the IM group and five (5) skin samples in the SC group tested weakly positive for vaccinia (b) (4) . Some of the following tissues and samples tested positive for vaccinia (b) (4) at 15 hours post injection: (i) blood samples and lung samples; (ii) one heart sample and two mesenteric lymph node samples; (iii) liver samples; and (iv) spleen samples (both IM and SC treatments). At 48 hours post injection, the number of vaccinia (b) (4) positive samples and tissues decreased. Among the different tissues analyzed, all kidney, brain, ovary, and testes samples were negative for detectable vaccinia transcripts.

Clinical observations: The study indicated that injection of MVA-BN did not produce any clinical findings of body weight changes.

Reviewer's comments:

The following distribution study in rabbits confirmed that the highest occurrence of vaccinia positive tissues following SC or IM injection of MVA-BN was predominately limited to the injection site. The positives with (b) (4) method ranged from (b) (4) (as determined by (b) (4) software).

5.3.1.4 Reports of Bioanalytical and Analytical Method for Human Studies (Clinical Assays):

Both vaccinia-specific ELISA (*enzyme-linked immunosorbent assay*) and vaccinia-specific PRNT (*plaque reduction neutralizing test*) were predominantly used in all phases of the clinical studies, whereas the ELISPOT (*enzyme-linked immunospot*) assay was employed for exploratory testing in Phase I and II clinical studies. Development of ELISA and PRNT methods, for measurement of vaccinia-specific antibody responses, were subsequently validated to support Phase I/II and Phase III clinical trials. In the course of developing the ELISA and PRNT methods, a number of re-optimizations (or re-developments) were performed in response to changes or replacement of critical reagents, controls and/or replacement of input antigen lots, which, in many cases, altered the performance of the assays, prompting revalidation of a new assay version. Consequently, the different assay versions are accompanied with their respective development and validation reports and revised SOP documents, which were submitted to this BLA. Thus, the method validation reports and SOP documents related to PRNT ((b) (4)) and ELISA ((b) (4)) used in the Phase III clinical studies are covered in this review memo. The measurement of serum antibody responses to

vaccinia virus is considered an important indicator in evaluating clinical efficacy of smallpox vaccines. Therefore, both assays used in the clinical evaluation are appropriate for their intended use and they adequately assess the response to the vaccines in clinical trials.

Vaccinia-specific PRNT:

The PRNT, using vaccinia virus WR strain (vv-WR) as the input antigen, was co-developed by Bavarian Nordic (BN) and (b) (4), a contract research organization (CRO), to measure vaccinia-specific neutralizing antibody titers in serum samples collected from nonclinical and clinical studies. The original development (Doc. No.: 50000037, Ed.01), pre-validation (Doc. No.: 82000141, Ed.01) and validation reports (Doc. No. 82000128, Ed.01) for the vv-WR-based PRNT (v.1) were submitted to Amendment 79 of IND 11596 (submission date: 31-OCT-2007), and a full review memo was prepared. The use of vv-WR for the validated PRNT was considered acceptable on the basis that the vv-WR strain was derived from the (b) (4) vaccine strain (as discussed during the EOP2 meeting, 27-JAN-2009).

PRNT method version (b) (4):

The PRNT (assay version (b) (4)) was used in the immunogenicity testing of Phase III lot-consistency clinical study (POX-MVA-013) and a Phase II study POX-MVA-027. The PRNT underwent re-development in response to a number of changes intended to reduce the variability occurring when a different lot of (b) (4) was used in the assay (this was also examined in Amendment 2 to BN-CA-2007.001-DR; Doc. No. 50000037). Therefore, the replacement of (b) (4) for (b) (4), as a component in the (b) (4) medium (i.e., (b) (4)), was investigated. The use of (b) (4) (instead of (b) (4)) in the PRNT resulted in higher GMT and thus altered the validation status of the assay. In addition, the PRNT was re-developed with a new (b) (4) lot ((b) (4)) for cell culture application and a new vv-WR lot ((b) (4)) that was used for preparation of (b) (4) virus storage stocks.

Re-development of PRNT (b) (4):

The development report BN-AD-2014.001-DR (Doc. No. 50000111) has been reviewed in this this memo. This following report was initially submitted to Amendment 311 of IND 11596 (submission date: 13-AUG-2015), and full review memos were prepared (by biostatistician and product reviewers).

The re-development of the PRNT was performed according to SOP/CA/017, Ed.08 (effective date: 08-SEP-2007) following the replacement of key assay reagents, which included the following qualification studies:

- i. The use of (b) (4) (instead of (b) (4)) in the (b) (4) medium was assessed in a comparability study performed by PRNT testing of post-vaccination sera samples (N = (b) (4)). The use of (b) (4) in the assay was shown to increase the GMT by a factor of (b) (4) in comparison to (b) (4).
- ii. A new vv-WR storage stock (i.e., lot (b) (4)) was prepared ((b) (4)) in (b) (4) and qualified in a comparability study with the initial vv-WR

storage stock (i.e., (b) (4)) in experiments using (b) (4) reference samples (i.e., (b) (4)) and post-vaccination serum (N = (b) (4)), as well as comparing the average (b) (4). Both virus storage stocks (i.e., lot (b) (4) and lot (b) (4)) were assessed to be comparable and thus can be used interchangeably in the assay.

- iii. Last, a new (b) (4) lot ((b) (4)) for cell culture was qualified in a comparability study with a previous (b) (4) lot ((b) (4)) involving the assaying of the VIG reference samples (i.e., (b) (4)). This study showed that the (b) (4) lot increased the overall GMT values compared to the (b) (4) lot; however, the differences were within the expected variability range for the assay.

The re-development of PRNT ((b) (4)) led to establishment of the following assay acceptance criteria:

- Positive control acceptance range: A new positive control (i.e., Lot (b) (4)) was developed for inclusion in routine PRNT testing as an acceptance criterion. The new positive control consists of (b) (4). The multiple testing of the positive control (dataset of N = (b) (4) values resulted in a GMT of 170 with a variance [RSD%] of (b) (4)%) were used to establish the acceptance range (tolerance limits with 95% certainty) with lower and upper limits of (b) (4).
- (b) (4)

Method Validation for the PRNT ((b) (4)):

The method validation report MUC/QCD/VAL/BN0003536/MVR (Doc. No. 82000478, Ed.01), method validation protocol (Doc. No. 82000430, Ed.01) and SOP BN0003536 (v. 2.0; effective date: 11-DEC-2014) for the PRNT ((b) (4)) were reviewed in this memo. The following method validation report was initially submitted to Amendment 311 of IND11596, and full review memos were prepared (by the biostatistician and product reviewers). To quantitate vaccinia-specific neutralization antibody titers (PRNT₅₀), (b) (4)

(b) (4)

According to SOP BN0003536 ((b) (4) effective date: 11-DEC-2014), the following are the acceptance criteria for each assay run:

(b) (4)

Assay characterization, pre-validation and validation:

The quantitative performance of the PRNT ((b) (4)) was evaluated according to key validation parameters *specificity, accuracy, precision (repeatability and intermediate precision), dilutional linearity, range, detection and quantitation limit*. The pre-validation experimental results described in the development report (Doc. No. 50000111) were used to establish the validation acceptance criteria for *accuracy and precision*.

Specificity: *Specificity* of the assay was examined by (b) (4)

Based on the enclosed results, the PRNT ((b) (4)) was considered to have acceptable *specificity*.

Accuracy: The BLA states that no acceptable or official reference standards are available for anti-vaccinia antibodies that can be used to evaluate *accuracy* of this

assay. Therefore, the acceptance criteria were set for the (b) (4)

For *accuracy*, GMT results from the testing of the (b) (4) titer samples (*i.e.*, (b) (4)) were generated from a total of (b) (4) batch runs performed by (b) (4) operators on (b) (4) different days (*Day* (b) (4)). (As noted, each batch run consisted of (b) (4) replicates for each titer sample). All GMT results (as non-log titers) were compared with the reference standard GMT values to assess closeness of the two values. Based on the enclosed results, all GMT results for each batch run (with (b) (4)) were shown to be within the acceptance limits, fulfilling the acceptance criterion for *accuracy* set to (b) (4) for the each reference titer.

Reviewer's comments:

See review comments below regarding discrepancies identified in study design for examining absolute accuracy (in PRNT (b) (4))

Precision: The acceptance criteria for *precision* (*i.e.*, *intermediate precision* and *repeatability*) were determined using pre-validation study data (development report, Doc. No. 50000111). The validation acceptance criteria for *precision* were set to %RSD values of (b) (4) % for titer samples (b) (4) and (b) (4) % for the (b) (4) titer sample.

Repeatability (within batch or inter-plate variability) was evaluated by the testing of the (b) (4) titer samples (*i.e.*, (b) (4)) in a set of (b) (4) batch runs by (b) (4) different operators on (b) (4) different days. The %RSD results for (b) (4) samples were shown to range between (b) (4). For the (b) (4) sample, the %RSD results were shown to range between (b) (4). Therefore, the enclosed results for the four titer samples were shown to be within the specified acceptance criteria.

For *intermediate precision* (inter-day, inter-operator, and inter-batch variability), the (b) (4) titer samples ((b) (4)) were tested in a set of (b) (4) batch runs by (b) (4) operators on (b) (4) different days. The %RSD results were shown to be (b) (4) % (for the (b) (4) sample), (b) (4) % (for the (b) (4) sample), (b) (4) % (for the (b) (4) sample), and (b) (4) % (for the (b) (4) sample). Thus, the enclosed results for the (b) (4) titer samples were shown to be within the specified acceptance criteria. Therefore, the overall variability of the assay was deemed acceptable.

Dilutional linearity and range: *Dilutional linearity* was evaluated using a set of (b) (4) dilution samples of (b) (4) (*i.e.*, (b) (4)) along with (b) (4). The results of (b) (4) titer determinations per dilution sample were plotted on a linear regression line. The linearity of the regression line was shown to have a R^2 value (b) (4) and a slope of (b) (4), which was not consider significantly

different from the predicted slope of 1. Based on these results, the assay displayed acceptable dilutional linearity.

The linear range of the assay was assessed with a wide titration range of (b) (4) from (b) (4) and subsequently using (b) (4) fold dilution steps of the (b) (4) dilution samples (i.e., (b) (4)). The titration curves for dilution samples (b) (4) resulted in an average PRNT₅₀ titer (non-log GMT) of (b) (4) with a variance of (%RSD) of (b) (4)% between the five titer values (of the dilution samples).

Reviewer's comments:

For routine testing of sera samples according to the PRNT protocol, (b) (4) twofold dilution steps will be performed, starting at (b) (4). The BLA indicates that the following dilutional linearity study results confirmed that samples within the titer range from (b) (4) can be tested in the PRNT based on using a standard starting dilution. The BLA stipulates that samples with titers greater than (b) (4) must re-tested using a higher starting dilution so that the sample is within the linear range of the assay.

Detection limit and quantitation limit: The estimation of *Detection Limit* (DL) and the *Quantitation Limit* (QL) were obtained based on the ICH guidance document: *Validation Of Analytical Procedures: Text and Methodology Q2(R1)*. This pre-validation study indicated that the DL (*limit of detection* [LOD]) was set to a titer of (b) (4) using the following formula: (b) (4) (i.e., (b) (4)). In a similar way as for the DL, the QL (or *lower limit of quantitation* [LLOQ]) was set to a titer of (b) (4) using the following formula: (b) (4). As proposed, sample titers occurring between (b) (4) cannot be quantitated accurately and precisely. Therefore, such samples will be assigned a titer of (b) (4).

Reviewer's comments:

See review comments regarding discrepancies identified in the estimations of LOD and LLOQ values for the PRNT (b) (4).

PRNT method version (b) (4):

The PRNT version (b) (4) was used in the immunogenicity testing of the Phase III pivotal clinical efficacy study (POX-MAV-006) and a Phase II study (i.e., POX-MVA-037). The PRNT underwent re-development due to exchange of an old input vv-WR antigen lot for a newer viral antigen lot. In addition, a new positive control lot was developed and a lot of FBS was changed. As result, these new changes altered the performance of the assay and thus impacted the validation status of PRNT.

Re-development of the PRNT, assay version 4(v.4):

The development report BN-QCA-2017.003-DR (Doc. No. 50000201, Ed.01) has been reviewed in this this memo. This following report was initially submitted to Amendment 369 of IND 11596 (submission date: 10-AUG-2018), and full review memos were prepared. The re-development of the PRNT method was performed according SOP BN0003536 (b) (4) which evaluated the exchange of the old vv-WR lot (Lot No. (b) (4)) for a new vv-WR lot (i.e., Lot # (b) (4) from (b) (4)), since the titers of two

virus lots were not comparable. In addition, the PRNT was re-developed with a new positive control lot (i.e., lot (b) (4)) that was prepared (by (b) (4)) and a new (b) (4) lot (i.e., Lot No. (b) (4)) from (b) (4)).

The re-development of the assay included the following qualification studies:

- i. The comparability of the previous PRNT version (b) (4) and the new PRNT version (b) (4) was assessed by testing (b) (4) serum samples (from clinical trial, POX-MVA-013) using the new assay version (along with a new virus dilution: (b) (4)); titers by assay (b) (4) were compared with titers reported for the clinical trial POX-MVA-013 (by assay (b) (4)). Thus, GMTs of (b) (4) (assay (b) (4); N = (b) (4)) and (b) (4) (assay (b) (4); N = (b) (4)) were obtained, indicating that the new assay (b) (4) resulted in titers that were higher by a factor of (b) (4). This factor was determined to be statistically different from 1.
- ii. A new virus (b) (4) stock preparation (i.e., (b) (4)) was prepared (by a (b) (4) of the virus storage stock) and qualified by testing it in comparison with a previous virus (b) (4) stock (i.e., (b) (4)); a (b) (4) of virus storage stock) using clinical samples (from POX-MVA-013). The titer results generated from (b) (4) samples for both virus (b) (4) preparations - # (b) (4) resulted in GMTs of (b) (4), respectively, which were considered comparable. (As indicated, the two virus (b) (4) preparations will not be used interchangeably for the testing in a clinical trial).

The re-development of PRNT (b) (4) led to the establishment of the following assay acceptance criteria:

- Positive control acceptance range: A new positive control (i.e., lot (b) (4)) was developed for inclusion in routine PRNT testing as acceptance criterion. The new positive control consists of (b) (4)

(b) (4)

Method Validation for the PRNT (b) (4):

The method validation report MUC/QCD/VAL/BN0003536/MVR (Doc. No. 82000994, Ed.01), method validation protocol (Doc. No. 82000993, Ed.02) and SOP BN0003536 (b) (4); *effective date: 11-NOV-2017*) for the vaccinia virus-specific PRNT (b) (4) were reviewed in this memo. The following validation report was initially submitted to Amendment 369 of IND 11596, and full review memos were prepared (by biostatistician and product reviewers). The determination of vaccinia-specific neutralizing antibody titers is performed as described for PRNT (b) (4) (above), except for

the preparation of the working stock of vv-WR changed. In SOP BN0003536 (b) (4), the working stock of vv-WR is prepared to a final dilution of (b) (4) of the viral storage stock. Equal volumes of the working stock virus are dispensed to the serially diluted serum mixtures.

According to SOP BN0003536 (b) (4); dated, 12-NOV-2017), the following are the acceptance criteria for each assay run:

(b) (4)

Assay characterization, pre-validation and validation:

The quantitative performance of the PRNT (b) (4) was evaluated according to key validation study parameters *specificity, accuracy, precision (repeatability and intermediate precision), dilutional linearity, range, detection and quantitation limit*. The pre-validation study results contained in the development report (Doc. No. 50000201) were used to establish the validation acceptance criteria for *accuracy* and *precision*.

Specificity: *Specificity* was evaluated as part of the re-development study of the PRNT. Here, (b) (4) pre-vaccination serum samples from subjects enrolled as vaccinia-naïve subjects (in clinical study POX-MVA-013) were tested by performing titration of the samples. The results from (b) (4) values revealed that (b) (4) samples had negative titers (whereas (b) (4) samples had positive titers). Thus, the testing of (b) (4) samples generated (b) (4)% seronegative titers. The seropositive rate of vaccinia negative samples was (b) (4)%. Based on the enclosed assessment, the PRNT (b) (4) was considered to have acceptable *specificity*.

Accuracy: For *accuracy*, the acceptance criteria for the (b) (4) in-house (b) (4) reference standards (b) (4) were set based on averaged titer values (*i.e.*, GMTs (b) (4), respectively), which were previously obtained through multiple testing of individual titer samples. (Note, the nomenclature for all reference titer samples contains the prefix “(b) (4)”). The acceptance criteria for *accuracy* were set to a range of (b) (4) (for all samples), which were based on the previous assay version (*i.e.*, (b) (4)). (Thus, the specified acceptance ranges for the (b) (4) titer samples was as follows: (b) (4))

(b) (4)

For accuracy, GMT results for the (b) (4) reference titer samples (i.e., (b) (4)) were generated from a total of (b) (4) batch runs performed by (b) (4) operators on (b) (4) different days (Day (b) (4)). These GMT (as non-log₁₀ titers) results were compared with the reference standard GMT values to assess closeness of the paired values. All GMT results for (b) (4) samples were shown to be within the acceptance limits, fulfilling the acceptance criterion for accuracy set to (b) (4) for the each reference titer.

Reviewer's comments:

Previously, the biostatistical reviewer advised BN that the study design for accuracy (used for PRNT (b) (4)) was more intended for examining precision. In the absence of a conventional or accepted reference standard (or value), absolute accuracy for the PRNT cannot be determined. Thus, it was recommended that relative accuracy be measured, which can be established through dilutional linearity. Although BN acknowledged that absolute accuracy cannot be demonstrated for the PRNT, the sponsor stated that they have investigated relative accuracy. Nonetheless, the current method validation report (Doc. No. 82000994, Ed.01) does not explicitly show an assessment of relative accuracy.

Precision: The acceptance criteria for precision (i.e., intermediate precision and repeatability) were set to %RSD values of (b) (4) for titer samples (b) (4) and (b) (4) for the (b) (4) titer sample (as described in the development report (Doc. No. 50000201)).

For repeatability (within batch or intra-assay variability), the (b) (4) titer samples (i.e., (b) (4)) were tested in a set of (b) (4) batch runs by (b) (4) different operators on (b) (4) different days (Day (b) (4)). The %RSD results for (b) (4) samples were shown to range between (b) (4). For the (b) (4) sample, the %RSD results were shown to range between (b) (4). Based on the enclosed results, the (b) (4) titer samples were shown to be within the specified acceptance criteria.

For intermediate precision (inter-day, inter-operator, and inter-batch variability), the (b) (4) titer samples ((b) (4)) were tested in a set of (b) (4) batch runs by (b) (4) operators on (b) (4) different days. The %RSD results were shown to be (b) (4).

. Based on the enclosed results, the four titer samples were shown to be within the specified acceptance criteria. The overall PRNT variability (%RSD) was calculated to be (b) (4), which was deemed acceptable.

Reviewer's comments and Information Request:

In the precision analyses, titers resulting below the LLOQ of a titer (b) (4) were excluded from the analyses. Previously, the biostatistical reviewer advised BN that the datasets (comprised of a total N = (b) (4) values) from the testing of (b) (4) sample contained a substantial number test values (i.e., N = (b) (4) results) with a titer of (b) (4), which were excluded from the precision analyses (according to the Validation Protocol). The removal of these low titer values from the precision datasets was found to decrease the estimated variability of the assay around the (b) (4) range and thus potentially resulting in

an erroneous interpretation that the assay is sufficiently precise within the titer range of (b) (4). Therefore, an Information Request (IR #13) was communicate to BN on 16-JAN-2019 to explain the discrepancies associated with excluding the titer values of (b) (4) in the (b) (4) titer sample testing (in comment #1B). In Amendment 21 (submission date: 26-FEB-2019), BN agreed with the Agency's evaluation and acknowledged that it was questionable to ignore test values at the DL in the precision analyses.

Dilutional linearity and range: The *Dilutional linearity* was evaluated using a set of (b) (4) dilution samples of (b) (4) (i.e., (b) (4) along with undiluted (b) (4)). The results of (b) (4) titer determinations per dilution sample were plotted on a linear regression line. The linearity of the plotted data was shown to have a R^2 value (b) (4) and a slope of (b) (4) (with SD of (b) (4)), which was considered not significantly different from the predicted slope of 1). Therefore this study indicated that the assay displayed acceptable *dilutional linearity* over seven dilutions.

Linear range was previously assessed over a wide titration range of (b) (4) from (b) (4) and subsequently using (b) (4) fold dilution steps of the (b) (4) dilution samples (i.e., (b) (4)) in the development and validation of PRNT (b) (4).

Detection limit and quantitation limit: *Detection Limit* (DL) and *Quantitation Limit* (QL) values were obtained in the same manner as described for the previous assay version (b) (4). As before, this study calculated the DL (*limit of detection* [LOD]) at a titer of (b) (4) and the QL (or *lower limit of quantitation* [LLOQ]) set to a titer of (b) (4).

As proposed, samples with titers of (b) (4) 20 can be extrapolated from the curve, even though the (b) (4) does not intersect with the titration curve. In addition, sample with titers between (b) (4) cannot be quantitated accurately and precisely and will be assigned a positive titer of (b) (4), whereas titers of (b) (4) will be assign a negative titer of 1. .

Reviewer's comments and Information Request:

The proposed LOD and LLOQ set at a titer of (b) (4), respectively, (for PRNT (b) (4)) were estimated by using an extrapolation method of the dilutional linearity plot generated from test values of (b) (4) dilutions of (b) (4) (i.e., from (b) (4)). Although the ICH Q2(R1) guidance document states that extrapolation can be used for estimating the LOD (which may be subsequently validated by an independent analysis of samples known to be near or prepared at the DL), this practice does not necessarily apply to estimating the LLOQ. Thus, the extrapolation into the range of the LLOQ was considered unacceptable, and it also did not conform to the ICH Q2(R1) guidance document.. Thus, an Information Request (IR #13, dated 16-JAN-2019) communication was sent to BN to explain the discrepancies associated with setting the LLOQ to a titer of (b) (4) based on extrapolation along with recommendations (covered in comment #1A), whereas the LOD at (b) (4) was considered likely based on the nature of the titer determination of the PRNT. In addition, this communication (IR #13) recommended that the limits should be validated by testing a suitable number of samples prepared at or near the LOD in order to validate the LLOQ. Alternative to this, it was also recommended that the LLOQ could be established based on adequate relative accuracy

and precision data which may be obtained in the range where (b) (4) was diluted down to a titer of (b) (4) in the linearity study. These recommendations sought to reiterate the concern that the relative accuracy and precision of the assay were questionable at a titer of (b) (4) since the PRNT did not appear to be adequately precise near the range of (b) (4) (as indicated above). In Amendment 21 (submission date: 12-FEB-2019), BN provided a more detailed explanation in support of the extrapolation used to estimate the LLOQ (including the LOD) for the PRNT. However, the review of the extrapolation approach for estimating LLOQ and LOD along with BN's rationale did not sufficiently address the discrepancy concerning the approach of setting the proposed LLOQ. Therefore, a separate communication (enclosed in IR #25; dated 23-MAY-2019) was sent to BN to reiterate the discrepancy of setting the LLOQ at a titer of (b) (4), and to recommend that the provisional LLOQ can be established based on confirmatory experimental data, involving the testing of a suitable number of samples with titers (at or near the target range of the LLOQ) to evaluate the relative accuracy and precision at the proposed LLOQ or to adjust the LLOQ accordingly..

In a follow-up response (to IR #25) submitted to Amendment 42 (submission date: 05-JUL-2019), BN indicated that they extended the linearity study for PRNT (assay (b) (4)) down to titers at the LLOQ of (b) (4). The linearity testing was performed with multiple replicates, whereby the log₁₀ titer values from a total of (b) (4) fold dilutions of (b) (4) (from (b) (4)) were plotted against (b) (4) on a regression line. While data points from neat to (b) (4) dilution sample of (b) (4) were plotted on the regression line, the highest sample dilution (i.e., (b) (4)) was not on the regression line but had a median GMT of (b) (4), which was calculated in a range assessed to be less precise. Consequently, a hypothetical change of the LLOQ to 20 was proposed by BN and evaluated for impact on recalculating PRNT (assay (b) (4) and assay (b) (4)) results in the pivotal clinical trials (i.e., POX-MVA-006 and POX-MVA-013). As reported by BN, the shift of the LLOQ to 20 did not lead to a change in the conclusions of the two pivotal clinical studies. The biostatistical review of BN's expanded linearity test results (in Amendment 42) determined that LLOQ at a titer of (b) (4) resulted in substantial assay bias. However, it was noted that the (b) (4) dilution sample (i.e., GMT value of ~20) exhibited lower assay bias (of (b) (4)), which was considered more appropriate to set the LLOQ value at a titer of ~20. In addition, the LLOQ value of 20 was determined to be suitable for PRNT (assay (b) (4)).

To receive concurrence from BN regarding the proposed LLOQ set at a titer of 20 for the PRNT, a communication (enclosed in IR #30; dated, 15-JUL-2019) was sent to BN. In Amendment 47 (submission date: 31-JUL-2019), BN agreed to revising the immunogenicity data enclosed in the Prescribing Information (PI) to reflect the change of the LLOQ.

Vaccinia-specific ELISA method:

The automated vaccinia-specific ELISA was developed and validated in 2003 for detection of MVA specific-antibodies in human sera. A (b) (4) workstation was used to run the ELISA procedure.

ELISA method version (b) (4) :

The automated vaccinia-specific ELISA (version (b) (4)) was used in the immunogenicity testing in the Phase III pivotal clinical studies (i.e., POX-MVA-006 and -013) as well as Phase II studies (i.e., POX-MVA-027 and -037). In 2011, the ELISA method was re-developed following substantial changes associated with replacement of critical reagents (i.e., (b) (4)), and minor changes to (b) (4).

The evaluation of these changes (in 2011) is described in the development report BN-CA-2011.001-DR (Doc. No. 50000083, Ed.01) and the method validation report MUC/CA/VAL/CA029/MVR (Doc. No. 82000235, Ed.01). These reports were initially submitted to Amendment 200 of IND 11596, and a full review memo has been prepared. In 2013, the ELISA (b) (4) underwent further re-development following substantial changes involving replacing critical reagent lots (i.e., (b) (4)).

since older reagent lots were almost depleted or expired. The ELISA re-validation with the replacement of critical reagent lots was performed in preparation for the testing of clinical serum samples from the Phase III pivotal clinical trials of MVA-BN.

Re-development of the ELISA method (assay version (b) (4)):

The development report BN-CA-2013.001-DR (Doc. No. 51000079, Ed.01) has been reviewed in this memo. The following report was initially submitted to Amendment 322 of IND 11596 (submission date: 18-DEC-2015), and full review memo was prepared. The ELISA method (in 2013) was performed according SOP BN0002809 (entitled, "Automated ELISA for Detection of Vaccinia Specific Antibodies in Human Sera") and SOP BN0002501 (entitled, "The ELISA Titer Calculation"). The preparation of (b) (4) is described in SOP BN BN0002821 (entitled, "Preparation of (b) (4) for Vaccinia ELISA"). The ELISA underwent further re-development following the replacement of critical reagent lots of (b) (4).

, and a new positive control (b) (4) reference sample (i.e., Pos Ctrl (b) (4); Lot No. (b) (4)).


The re-development of the ELISA included the following qualification study:

- i. **New reference values for Pos Ctrl (b) (4) :** The Pos Ctrl (b) (4) (Lot # (b) (4)) was assigned a provisional reference value based on a dataset of N = (b) (4) of test values (last qualified in APR-2013). Subsequent testing of the Pos Ctrl (b) (4) (over a 3-month period) was performed to increase the dataset (i.e., N = (b) (4)) for a new reference value set to a GMT of (b) (4) with an acceptable working range of (b) (4) ((b) (4) of the reference GMT). The average OD for the Pos Ctrl (b) (4) sample was set to (b) (4) with an SD of (b) (4) (with a plate acceptance range of (b) (4) (99% tolerance limits)).

Method Validation for the ELISA (b) (4):

The method validation report MUC/CA/VAL/CA029/MVR (Doc. No. 82000345, Ed.01), method validation protocol (Doc. No. 82000234, Ed.03) and SOP BN0002809 for the ELISA method (b) (4) were reviewed in this memo. The following method

validation report was initially submitted to Amendment 322 of IND 11596, and full review memos were prepared (by biostatistician and product reviewers). The ELISA method is performed on a (b) (4). To quantitate vaccinia-specific antibody antibodies in human sera, (b) (4)



According to SOP BN0002809 ((b) (4); *effective date: 15-APR-2015*), the acceptance criteria for each assay run include the following:

(b) (4)



Assay method pre-validation and validation:

The performance of the ELISA (b) (4) was evaluated according to key validation study parameters: *specificity*, *accuracy*, *precision (repeatability and intermediate precision)*, *dilutional linearity*, *range*, and *dilutional linearity*. The pre-validation study in the development report (Doc. No. 51000079) included the evaluation of *robustness*, *matrix effect* and a bridging study of previous ELISA assay versions.

Bridging study of re-developed ELISA to previous assay version: The performance of the re-developed ELISA (assay (b) (4)) was compared with the previous assay versions by assessing *accuracy* of the assay over time. For this study, (b) (4) samples were randomly selected from different clinical trials (*i.e.*, POX-MVA-005, -008 and -011, previously analyzed in 2006 through 2009) and were retested in the present assay version (b) (4). Serum titer results (N = (b) (4)) obtained with assay (b) (4) were compared with historical reported results for each clinical serum sample. However, paired titer results for each sample that exhibited titers (N = (b) (4)) in the quantitative range (*i.e.*, (b) (4)) were used for this analysis. Thus, the test results (N = (b) (4)) of this study appeared comparable with GMTs of (b) (4) (*as reported*) and (b) (4) (*as retested*). For samples with titers of (b) (4), the %Recovery was reported to be (b) (4)%, which was deemed within the acceptance criterion of (b) (4) for *accuracy*.

Reviewer's comments:

The results of this bridging study suggested that the assay performed consistently between 2006 (when POX-MVA-005 was analyzed) through 2009 (when POX-MVA-008 and -011 were analyzed) and up to the most recent titer results obtained in the retesting performed in 2013.

Specificity: *Specificity* was evaluated by testing (b) (4) individual baseline serum samples from vaccinia-naïve subjects randomly selected from 3 clinical trials (*i.e.*, POX-MVA-005, -008, and -011). *Specificity* of the ELISA was demonstrated if (b) (4) of the test results were shown to be negative (*i.e.*, with a titer of 1). The testing of the (b) (4) serum samples resulted in one serum sample with an error message (*Error CV*), whereas the remaining serum samples showed quantal titers (*i.e.*, (b) (4)). However, (b) (4) serum samples showed minimal positive titers of (b) (4), while the remaining (b) (4) sera were seronegative. Thus, a (b) (4) seronegative rate (*i.e.*, (b) (4)) was shown for the serum samples (N = (b) (4)) tested, thereby fulfilling the acceptance criterion (b) (4).

Precision: For evaluation of *precision*, the acceptance criteria were set based on %CV (*Coefficient of Variability*) values obtained through the testing of the (b) (4) QC serum (b) (4)

Intra-assay precision (repeatability): *Intra-assay precision* of the ELISA was investigated by a single operator who tested the (b) (4) QC serum samples (listed above) on each of

the (b) (4) plates. The results (%CV) obtained for each of (b) (4) and Pos Ctrl (b) (4) samples were shown to be within the acceptance criteria (set at a %CV of (b) (4)). The (b) (4) sample yielded quantal titers of 50 and 100, and the Neg Ctrl (b) (4) yielded titers of (b) (4), which were all considered acceptable.

Inter-day precision: *Inter-day precision* was investigated by a single operator who tested the (b) (4) QC serum samples on each of (b) (4) plates with assay runs performed on (b) (4) different days. The results (%CV) obtained for each of the (b) (4) and Pos Ctrl (b) (4) samples were shown to be within the acceptance criteria (set at a %CV of (b) (4)). The (b) (4) sample yielded quantal titers of (b) (4) and the Neg Ctrl (b) (4) yielded titers of (b) (4), which were all considered acceptable.

Inter-operator precision: *Inter-operator precision* of the assay was investigated by (b) (4) operators who independently tested the (b) (4) individual QC serum samples on each of (b) (4) plates run on the same day. (As noted, both operators (b) (4)

The results (%CV) for each of the (b) (4) and Pos Ctrl (b) (4) serum samples met the acceptance criteria (set at %CV of (b) (4) %). The (b) (4) sample yielded quantal titers of (b) (4) on each of (b) (4) plates, and the Neg Ctrl (b) (4) yielded titers of (b) (4).

Intermediate precision: *Intermediate precision* expresses the within-laboratory variation involving a combination of factors: i.e., different days, different operators, etc. Thus, *intermediate precision* of the assay was analyzed by using the datasets obtained from each precision experiment in combination to determine the overall precision. The results from these analyses of each serum sample (i.e., (b) (4) and Pos Ctrl (b) (4)) were shown to be within the acceptance criteria (set at a %CV of (b) (4) %). As expected, the (b) (4) sample yielded quantal titers of (b) (4), and the Neg Ctrl (b) (4) yielded titers of (b) (4), which were all deemed acceptable.

Reviewer's comments:

The overall precision assessment of individual QC serum pools ((b) (4) and Pos Ctrl (b) (4)) showed acceptable variability in response to varying factors that were investigated (i.e., intra-day, intra-operator, and change of critical reagents lots) in the course of performing a (b) (4)-plate run.

Accuracy: For *accuracy*, the acceptance criteria of the QC serum samples ((b) (4), and Pos Ctrl (b) (4)) were based on the GMT reference values (i.e., (b) (4), respectively), which were determined through multiple testing of the QC serum samples in a previous re-development study done in 2011 (detailed in development report BN-CA-2011.001-DR [Doc. No. 50000083, Ed.01] of the BLA). An acceptance range of (b) (4) was set for the reference GMT values of the QC serum samples. Quantile titers of (b) (4) for the (b) (4) serum sample and a titer of (b) (4) for the Neg Ctrl (b) (4) were all considered acceptable. (In addition, (b) (4) % of individual plate titers from the (b) (4) runs combined had to be compliant with the (b) (4) acceptance criteria).

In the *accuracy* evaluation, QC serum samples were tested on (b) (4) plates per run on (b) (4) different days by the same operator testing the (b) (4) QC serum (b) (4) ((b) (4)

(b) (4), Pos Ctrl (b) (4) and Neg Ctrl (b) (4), and GMT values from each of the (b) (4) runs were calculated. The results of the (b) (4) and Pos Ctrl (b) (4) samples were shown to be within the acceptance range of their respective reference values. The results of the (b) (4) serum sample were all acceptable (i.e., quantal titers of (b) (4)), and all Neg Ctrl (b) (4) titers were (b) (4) (except for one which reported an "Error CV" message). Therefore, the accuracy results were shown to be within the acceptance criteria.

In addition, the assessment of titer results on the individual plates revealed that all the QC serum samples tested met the acceptance criterion in that (b) (4) of plate titers from the (b) (4) runs combined had to be within the (b) (4) acceptance range.

Dilutional linearity: *Dilutional linearity* was evaluated over the working range of the assay by performing (b) (4) fold serial dilutions (i.e., (b) (4)) of (b) (4) very high titer samples (selected from clinical trial POX-MVA-024) in vaccinia-negative serum (i.e., Neg Ctrl (b) (4)). The titer results of each serum sample were plotted against its dilution in a linear regression line. The resulting linear curves were drawn with a R^2 value of (b) (4) which were considered acceptable. Data points for these analyses were fitted along the linear regression line (dilution vs. titer) with R^2 values of (b) (4), which fulfilled the acceptance criterion.

Reviewer's comments:

The assay range from a titer of (b) (4) was previously evaluated and supported by an R^2 value of (b) (4) for the dilutions in development report BN-CA-2011.001-DR [Doc. No. 50000083, Ed.01] of the BLA. The quantitation limit (QL) and detection limit (DL) were previously described in the development report (BN-CA-2011.001-DR).

Matrix effect: The matrix effect from different samples was investigated for high titer serum samples that were (b) (4) using a higher than usual dilutions (e.g., (b) (4)) in (b) (4) buffer prior to assaying. For this study, (b) (4) high titer serum samples were (b) (4) in the following manner:

(b) (4)

Matrix effect was examined by assessing whether ELISA GMT results comply with the acceptance range of (b) (4) of the GMT values calculated from the linearity study. Thus, the ratio linearity GMTs and matrix effect GMTs for the high titer serum samples were expressed as %Recovery values.

Reviewer's comments:

No significant matrix effect was observed in the testing of (b) (4) high titer serum samples prepared in (b) (4) buffer compared with the same (b) (4) serum samples used in the dilutional linearity study. Thus, the (b) (4) of serum samples in (b) (4) buffer was assessed to be an acceptable practice.

Robustness: The BLA states that *robustness* for the ELISA has been extensively test in a previous development. However, in the re-development of the ELISA, confirmatory

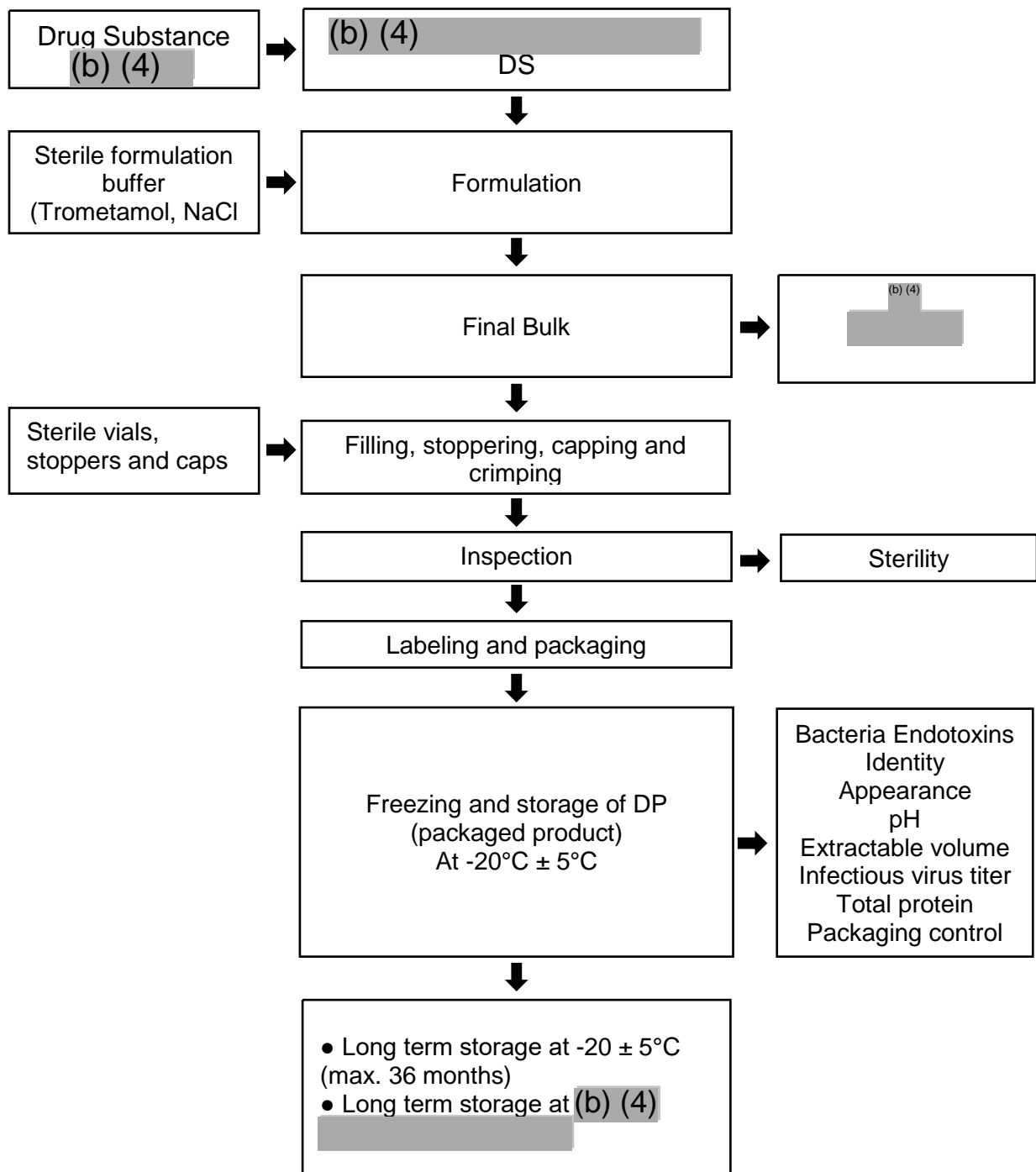
robustness experiments were performed to assess potential effects of using new lots of (b) (4) on assay performance. For robustness, QC serum samples (i.e., (b) (4), Pos Ctrl (b) (4) and Neg Ctrl (b) (4)) were tested on 28 plates each day on (b) (4) different days by the same operator whereby different combinations of (b) (4) lots of (b) (4) and (b) (4) lots of (b) (4) were used. Similarly, the robustness was evaluated with (b) (4) different lots of (b) (4) in the event that (b) (4) lots are changed during assaying.

Reviewer's comments:

Robustness of the assay related to using different lots of (b) (4) in the assay was shown to be acceptable. The combined GMT results from all (b) (4) days for serum samples ((b) (4) and Pos Ctrl (b) (4)) were shown to have %CV values that were within the acceptance limit of (b) (4) (for inter-day precision). In addition, %Recovery results, as expressed by the ratio of "Robust GMTs" and "Reference GMTs" ((b) (4) and Pos Ctrl (b) (4)), were within the acceptance range of (b) (4), except for serum sample (b) (4), which was slight above the acceptance range (i.e., %Recover of (b) (4)). Thus, the different lots of (b) (4) were assessed to be interchangeable in the assay without significantly impacting titer results. Also, robustness was shown for the (b) (4) different lots of (b) (4). Thus, the (b) (4) different (b) (4) lots were assessed to be interchangeable without significant impact to titer results.

Appendix F:

Formulation and filling of Drug Product



(b) (4)

Storage	Time from end of (b) (4) freezing until sampling	(b) (4)	Range was set based on technical expectations and an on the (b) (4) freezer validation.	Non-critical	N/A
	Storage temperature	-20°C ± 5°C	The storage is qualified, alarmed and well controlled.	Non-critical	N/A

	Storage time	≤ 36 months	Shelf life based on real-time stability data.	Non-critical	N/A
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Appendix I

Components Information Table

Components	In the Final Drug Product ¹ (Y/N)	Function / purpose	UNII
Modified Vaccinia Ankara-Bavarian Nordic (MVA-BN) live virus	Y	<u>Active substance immunizing antigen</u> • <u>Active ingredient</u>	(b) (4)
Sodium Chloride (NaCl)	Y	Saline • <u>Inactive ingredient</u>	(b) (4)
Trometamol (Tris-hydroxymethyl-aminomethane)	Y	Buffering agent • <u>Inactive ingredient</u>	(b) (4)
Water for injection (WFI)	Y	Solvent • <u>Inactive ingredient</u>	(b) (4)
(b) (4)			
Gentamicin	N	Antibiotic / cell culture medium component	
(b) (4)			

(b) (4)

Benzonase	N	Nuclease / host cell DNA	
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(b) (4)

