30 May 2013
EMA/369203/2013
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

IMVANEX

Common name: Modified Vaccinia Ankara virus

Procedure No. EMEA/H/C/002596

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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Table of contents

List of abbreviations

ACIP	Advisory Committee on Immunisation Practices
AD	Atopic Dermatitis
AE	Adverse Event
APSV	Aventis Pasteur Smallpox Vaccine
ATC	Anatomical Chemical Code
BN	Bavarian Nordic
CDC	Centers for Disease Control and Prevention
CEF	Chick Embryo Fibroblast
cGMP	current Good Manufacturing Practice
CHMP	Committee for Medicinal Products for Human Use
CoA	Certificate of Analysis
CPMP	Committee for Proprietary Medicinal Products
CTL	Cytotoxic T-Lymphocyte
DMID	Division of Microbiology and Infectious Diseases
DNA	Deoxyribonucleic Acid
DP	Drug Product
DS	Drug substance
DSMB	Data Safety Monitoring Board
EC	Ethics Committee
ECG	Electrocardiogram
ECTV	Ectromelia Virus
ELISA	Enzyme Linked Immunosorbent Assay
EV	Eczema vaccinatum
FAS	Full Analysis Set
FDA	Food and Drug Administration (US)
GCP	Good Clinical Practice
GMT	Geometric Mean Titer
HC	Health Canada
HIV	Human Immunodeficiency Virus
ICH	International Conference on Harmonization
i.m.	intramuscular
IPC	In-Process Control
i.t.	intratracheal
i.v.	intravenous
IND	Investigational New Drug
IRB	Institutional Review Board
MOI	Multiplicity Of Infection
MPXV	Monkeypox Virus
MSV	Master Seed Virus
MVA	Modified Vaccinia Ankara
MVA-BN	Modified Vaccinia Ankara-Bavarian Nordic
NDS	New Drug Submission
NHP	Non-human Primate
NIH	US National Institutes of Health
NIAID	National Institute of Allergy and Infectious Diseases
NYCBH	New York City Board of Health
PCR	Polymerase Chain Reaction
PP	Per Protocol
PPS	Per Protocol Analysis Set
PRNT	Plaque Reduction Neutralisation Test
PT	Preferred Term
RE	Restriction Enzyme
RFP	Request for Proposal
SAE	Serious Adverse Event

SAP	Special Access Programme
s.c.	subcutaneous
SCORAD	Scoring Atopic Dermatitis
SD	Standard Deviation
SFU	Spot Forming Unit
SIAE	Special Interest Adverse Event
SLU	St Louis University
SOC	System Organ Class
SPF	Specified Pathogen Free
TCID	Tissue culture infectious dose
TFF	Tangential Flow Filtration
TVAC	Total Viable Aerobic Counts
ULN	Upper Limit of Normal
US	United States of America
VIG	Vaccinia Immune Globulin
VV	Vaccinia Virus
VV-WR	Vaccinia Virus Western Reserve
WHO	World Health Organization
WSV	Working Seed Virus

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Bavarian Nordic A/S submitted on 29 February 2012 an application for Marketing Authorisation to the European Medicines Agency (EMA) for IMVANEX, through the centralised procedure under Article 3(2)(a) of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 23 June 2011.

The applicant applied for the following indication:

Active immunisation against smallpox infection and disease in persons 18 years of age and older.

The indication includes healthy populations as well as individuals with immune deficiencies and skin disorders such as those who are Human Immunodeficiency Virus (HIV) infected (CD4 ≥ 200 cells/μL) and those who have Atopic Dermatitis (AD) or Allergic Rhinitis (AR).

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application.

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or study(ies).

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision P/0038/2012 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/0038/2012 was not yet completed as some measures were deferred.

Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

Applicant's request(s) for consideration

Marketing Authorisation under exceptional circumstances

The applicant requested consideration of its application for a Marketing Authorisation under

exceptional circumstances in accordance with Article 14(8) of Regulation (EC) No 726/2004. The applicant has stated that they are unable to provide a comprehensive data on the efficacy and safety under normal conditions of use based on the following claim(s):

- Inability to provide comprehensive efficacy and safety data due to rarity of the indication.

IMVANEX is indicated for active immunisation against smallpox infection and disease in individuals 18 years of age and older. Smallpox was officially declared eradicated by the WHO in 1981 and therefore the ability to conduct Phase III field efficacy trials no longer exists. Eradication of the disease for which IMVANEX® is indicated results in an inability of the applicant to provide comprehensive data on the clinical efficacy of the vaccine (i.e. a demonstration of protection from acquiring the disease).

- Inability to provide comprehensive information due to the present state of scientific knowledge.

The immunological mechanisms of protection against smallpox are not fully understood. However, during the global smallpox vaccination campaigns, the development of a major cutaneous reaction following scarification ("take") in response to vaccination with the replication-competent vaccines that were then in use was used as a surrogate parameter of clinical efficacy. However MVA-BN does not replicate in humans and therefore no "take" is formed so that its potential protective efficacy cannot be assessed based on this biomarker. In the past, attempts were made to identify an additional biological efficacy marker based on immunogenicity parameters, since not every vaccinated individual developed a "take" (Mack 1972). Neutralising antibodies were acknowledged as the parameter that most reliably correlated with immunity and protection against smallpox infection and disease, although they may not be solely responsible for protection (Mack 1972; Sarkar 1975). Today, there is growing evidence that antibodies are a key element in protection from both a primary and secondary poxvirus infection (Panchanathan 2008).

Therefore, in the absence of a full understanding of the immunological mechanisms of protection against smallpox there is an inability to evaluate the protective efficacy of MVA-BN based on the humoral response which has been correlated with protection.

- Inability to collect such information because it would be contrary to medical ethics.

The efficacy of IMVANEX cannot be demonstrated in the traditional manner of pivotal human Phase III efficacy trials, since smallpox has been declared eradicated in 1980 (see section 4.1). The WHO as the owner of one of the two last known remaining stocks of VARV would not allow the use of VARV challenge studies in humans since these unethical and unfeasible.

- The CHMP is of the view that the reference to the framework of a Marketing Authorisation under exceptional circumstances in accordance with Article 14(11) of Regulation (EC) No 726/2004 is justified based on the fact that the applicant will most likely not be in a position to provide the

comprehensive clinical data based on the following:

- A comprehensive dossier cannot be provided as it would be contrary to medical ethics. Indeed, the efficacy of IMVANEX cannot be demonstrated in clinical trials since neither human challenge studies nor field efficacy studies are possible.

  A comprehensive dossier cannot be provided due to the present state of scientific knowledge. Indeed, IMVANEX contains a non-replicating vaccinia virus and is not administered by intradermal inoculation. Therefore it is not possible to assess potential protective efficacy based on pock take rates, which is considered to be a clinica l correlate of protection against smallpox for replication-competent vaccines. While the humoral immune response to MVA-BN can be and has been documented, the protective efficacy of MVA-BN cannot be predicted from immunogenicity studies in the absence of an immunological correlate of protection.

Specific obligations have been listed in Annex II of the CHMP opinion.

New active Substance status

The applicant requested the active substance modified Vaccinia Ankara - Bavarian Nordic (MVA-BN) virus contained in the above medicinal product to be considered as a new active substance in itself, as the applicant claims that it is not a constituent of a product previously authorised within the Union.

Licensing status

The medicinal product was not licensed in any country at the time of submission of the application.

1.2. Manufacturers

Manufacturer(s) of the active substance

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

The manufacturing sites had been inspected by EU authorities and are in compliance with the EU Good Manufacturing Practice requirements.

Manufacturer(s) responsible for batch release

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

1.3. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP and the evaluation teams were:

Rapporteur: Ian Hudson Co-Rapporteur: Jan Mueller-Berghaus

- The application was received by the EMA on 29 February 2012.
- The procedure started on 21 March 2012.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 31 May 2012. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 8 June 2012.
- During the meeting on 16-19 July 2012, the CHMP agreed on the consolidated List of Questions to be sent to the applicant. The final consolidated List of Questions was sent to the applicant on 19 July 2013 .
- The applicant submitted the responses to the CHMP consolidated List of Questions on 11 October 2012.
- During a meeting of SAG on 16 November 2012, experts were convened to address questions raised by the CHMP.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 23 November 2012.
- During the CHMP meeting on 10-13 December 2012, the CHMP agreed on a list of outstanding issues to be addressed in writing and in an oral explanation by the applicant.
- The applicant submitted the responses to the CHMP List of Outstanding Issues on 21 March 2013.
- During the CHMP meeting on 22-25 April 2013, outstanding issues were addressed by the applicant during an oral explanation before the CHMP.
- The Rapporteurs circulated the Joint Assessment Report on the responses provided by the applicant, dated 05 April 2013.
- During the meeting on 17-30 May 2013, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a Marketing Authorisation under exceptional circumstances to IMVANEX.

2. Scientific discussion

2.1. Introduction

Imvanex (previously named Imvamune) is a live, non-replicating Modified Vaccinia Ankara (MVA) vaccine that has been developed to provide protection against smallpox in adults. Since the vaccine does not replicate in humans, it was evaluated in healthy subjects and also in HIV-infected subjects and those with active or a history of atopic dermatitis, two groups for which replication-competent smallpox vaccines are contraindicated due to the risk of severe adverse events.

Smallpox eradication
Smallpox virus (Variola virus) is a member of the family Poxviridae belonging to the subfamily Chordopoxviridae and genus Orthopoxvirus. Vaccinia virus contains a large linear double-stranded DNA genome amounting to approximately 190,000 base pairs and encoding more than 200 proteins. Viral particles are typically brick shaped and measure ~300 x 230 nm. Virions released through the cell membrane are enveloped but most virions remain cell-associated and are released by cellular disruption that leaves them without an envelope. Both enveloped and non-enveloped viruses are infectious. The vaccinia viruses used for replication-competent smallpox vaccine production worldwide are laboratory-derived versions of wild-type animal pox viruses, including but probably not limited to horse pox virus. Vaccine strains are genetically distinct from wild type poxviruses and from each other.

Smallpox was eradicated (declaration 1981; last known case in 1977) as a result of the WHO global campaign. There remains concern that stored smallpox virus, with or without genetic engineering, could be deliberately released as a weapon of bioterrorism. For this reason there remains some interest in the development of new smallpox vaccines. Since there is no disease, the likely protective efficacy of new smallpox vaccines has to be inferred from other parameters.

During the global eradication campaign it was recognised that the formation of an appropriately sized pock with subsequent crusting and scarring at the site of primary inoculation was highly correlated with vaccine-induced protection against infection. In particular, the surface area of the scar, as well as the number of scars from previous immunisations, showed an inverse relationship with the case-fatality rate. After successful vaccination, the duration of protection was thought to be at least three years, with at least some degree of protection likely persisting for 10 years or more.

The CHMP Note for Guidance (CPMP/1100/02) states that the likely protective efficacy of a novel smallpox vaccine containing a replication-competent strain directly descended from those in vaccines deployed in the global eradication programme could be inferred from the proportion of vaccinees in whom appropriately sized pocks are achieved at the site of inoculation. Extrapolating from past experience with vaccines that were used in the global eradication programme, it would be expected that a novel smallpox vaccine would induce an adequately sized pock in at least 95% of vaccine-naïve healthy recipients after primary immunisation. The assessment of humoral and cell-mediated immune responses to vaccination is encouraged but mainly with the purpose of exploring any correlation there may be with pock formation.

It is suggested that:
- Assessment of the humoral immune response should include the detection and titration of neutralising antibodies using the intracellular mature virion (IMV) against an international standard (WHO or equivalent). However, it is recognised that there is currently a need for the development of appropriate international standards. If more recent technologies (including ELISA tests) are used, they should be validated against the results of neutralisation tests and should differentiate IgG and IgM responses.

- Assessments of the cell-mediated component of the immune response should include the evaluation of CD8 T-cell activity using sensitive methods, such as cell activation by live virus and interferon (IFNgamma) production (i.e. by ELISPOT and flow-cytometry).

However, the CHMP guideline does not address the assessment of the potential efficacy of other types of smallpox vaccines, including those containing attenuated strains that do not elicit pock formation such as the modified vaccinia Ankara strain (MVA). For such vaccines the evaluation has to take into account non-clinical evidence of protective efficacy and the immune responses observed in human vaccinees.

Immunogenicity of replication competent smallpox vaccines

Following primary vaccination with vaccines used in the global eradication programme a small central lesion (pock or ulcer) of as little as 1-8 mm diameter was reported to be associated with a maximal level of neutralising antibody, although details of study methodologies are not always available and assays have never been standardised. The correlation between lesion size and antibody levels determined by haemagglutination inhibition tests appeared to be much weaker. These findings applied to vaccines containing the New York City Board of Health (NYCB) strain (grown in calf lymph or in eggs) or the Lister-Elstree vaccine strain (grown in sheep flank).

The relative importance of the humoral and cell-mediated immune responses for natural protection against clinically apparent smallpox and for recovery from clinical infection is still debated. However, pre-eradication experience indicated that clinically apparent infection could be prevented by administration of VIG while subjects with hypogammaglobulinaemia could recover well from smallpox provided they had no accompanying T-cell defects. More recently, it has been suggested that circulating antibody and immune memory could be the more important factors for preventing or at least modifying the severity of clinically apparent smallpox, taking into account that the incubation period of ~12 days allows time for clonal expansion of memory B-cells. Once clinical disease is established it seems that the ability to mount an adequate T-cell response is important for recovery.

The evidence supporting a long-lived antibody response to replication-competent smallpox vaccines dates at least from McCarthy et al. (1958), as quoted by Fenner et al. (1988), who found that neutralising antibodies were more persistent than antibodies inhibiting haemagglutination or fixing complement and were sometimes detectable for more than 20 years after vaccination. These findings were confirmed in several other studies (e.g. El Ad et al. 1990; Stienlauf et al. 1999; Crotty et al. 2003; Pütz et al. 2005). However, these data relate to subjects who had received two or more vaccinations over time.

The neutralising antibody titres reported by these and other authors cannot be directly compared across studies because of the differences in the techniques used (e.g. indicator cells, virus inoculum, duration of incubation of the serum/virus mixture, read-out at PRNT50% or 60%; the methods are often not described in detail) and the lack of standards. For example, published neutralising antibody titres measured one month after vaccination of previously naïve subjects with replication-competent NYBH vaccine have ranged from 36 to 1262. In addition, seropositivity rates and seroconversion rates cannot be compared across these studies not only due to the assay variability but also because of variable or unexplained criteria for serological status and response.

There are data to indicate that neutralising antibody begins to wane by the second month after vaccination of previously vaccinia-naïve subjects. For example, in the study of Belshe *et al*. (2004) 103/106 in the group that received undiluted Dryvax developed a major cutaneous reaction and neutralising antibodies increased from <20 prior to vaccination to a GMT 1262 on D28 but had fallen to 796 on D56. Frey *et al.* (2002) followed 20 subjects who received Dryvax, among whom 19 developed a take and 17 had at least a 4-fold increase in antibody titres. The GMTs were 36 at one month and 11 at 12 months post vaccination. Frey *et al* 2003 reported that all 10 vaccinia-naive subjects who received Dryvax developed a take and at least a 4-fold increase in antibody titre with GMTs at 724 and 90 at M1 and M12, respectively.

Ratios of the residual neutralising antibody titres at M12 *vs* the titres at D28 appeared to be are in the same range (12%, 15% and 30%). In fact, these findings are in keeping with those reported in the SmPC for the UK-approved Lister-Elstree strain vaccine (2008; GMP-compliant vaccine including virus grown in cell culture).

This degree of waning of vaccinia-specific antibody by one year after immunisation does not contradict available data on very long-lived persistence of detectable circulating antibody. There are reports of stable titres for 40 to 75 years post-immunisation after more rapid declines during the first three year (El Ad *et al*. 1990; Hammarlund *et al.* 2003; Viner and Isaacs 2005).

Regarding the correlation between neutralising antibody vs. antigen-binding antibody, Galwitz *et al*. (2003) analysed the sera of 165 persons born before 1980 who had received smallpox vaccine once. Antibody assessed by EIA against infected cell lysates gave a positivity rate of 112/165 sera tested (67.9 %) but neutralising antibody was largely absent in that 59/60 sera tested were negative although 44/59 were EIA positive and the only serum positive for neutralising antibody was EIA negative. This study may indicate that the kinetics of antibody determined by virus neutralisation and by EIA are quite different.

Despite these differences in assay results, declines in antibody titres have also been reported with other serological techniques. Pütz *et al*. (2006) used ELISA against vaccinia-infected cell lysates or purified antigens associated with EEV or IMV particles to evaluate the immune response to Lister/Elstree strain vaccine. Using a vaccinia-infected cell lysate as antigen they reported that all vaccinia-naive individuals had mounted a robust antibody response by D21 post vaccination (GMT 3119) but the GMT was 996 at 6 months and 623 (95% CI 115-1,130) at one year post-vaccination. The corresponding percentages with titres at least four fold that on D0 was 100 % on D21, 70 % at M6 and approximately 50% at one year. In addition, similar antibody decline was seen when using five of the six vaccinia (glyco) proteins as specific antigens in the ELISA.

Safety of replication-competent smallpox vaccines

The safety profile of smallpox vaccines that were used up to the time of cessation of routine vaccination following the global eradication of the disease was well described. Serious and life-threatening adverse reactions appeared to occur rarely or very rarely provided that the contraindications and warnings were fully observed. Nevertheless, the current absence of circulating virus carries implications for the benefit-risk relationship regarding vaccination of subjects in clinical studies.

The CHMP Note for Guidance recognised that the number of subjects to be exposed to a new smallpox vaccine in clinical studies should be kept to the minimum necessary to provide adequate assurance regarding its likely protective efficacy and safety. At the time of writing the guideline there was no licensed smallpox vaccine in the EU so it was stated that uncontrolled studies would be acceptable. It was recognised that pre-authorisation clinical studies would not include a sufficient number of subjects to be able to detect rare or very rare adverse reactions, such as encephalitis. It was recommended that the size of the safety database should be sufficient at least to estimate the frequency of uncommon reactions and that the duration of follow-up for assessment of safety should be at least 3 months for all subjects exposed at the time of an initial application for marketing authorisation in case of late development of neurotoxicity.

Subsequently, the US instituted a vaccination campaign of military and civilian personnel in 2002-2003 using Dryvax. During this campaign there emerged reports of myopericarditis in persons with no prior smallpox vaccine history. For example, one report described the first 18 cases of probable myopericarditis among 230,734 vaccine-naïve adults compared to no reported cases among 95,622 re-vaccinated persons. This gave a rate of 7.8 per 100,000 over 30 days which was estimated to be 3.6-fold higher than the background incidence among non-vaccinated military personnel. A later report covered 450,293 military personnel of which 70.5% (317,456) were naïve to smallpox vaccine. There were 37 cases of myopericarditis reported in vaccine-naïve males, giving a rate of 11.6 cases per 100,000 naïve vaccinees.

In 2007 the US FDA approved a new smallpox vaccine ACAM2000, which contains the NYBH strain. In pre-licensure studies 2983 subjects received ACAM2000 and 868 subjects received Dryvax. There were 10 cases of suspected myocarditis - 7/2983 (0.2%) in ACAM2000 subjects and 3/868 (0.3%) in Dryvax subjects. The mean time to onset of suspected myocarditis and/or pericarditis from vaccination was 11 days (range 9 to 20 days). All subjects who experienced these cardiac events were smallpox vaccine-naïve. Two of the 10 subjects were hospitalised while the others were sub-clinical (i.e. were detected by ECG with or without abnormalities of troponin-I) and received no treatment. All cases resolved by 9 months, with the exception of one female subject in the Dryvax group with a persistent borderline abnormal left ventricular ejection fraction on echocardiogram.

Within the Phase 3 ACAM2000 clinical trials there was active monitoring for myocarditis and pericarditis. Among 1,162 vaccine-naïve subjects there were 8 cases of suspected myocarditis and pericarditis identified across both treatment groups (total incidence rate of 6.9 per 1000 vaccinees). The rate for the ACAM2000 treatment group was 5.7 (95% CI: 1.9-13.3) per 1000 vaccinees (5/873) and the rate for Dryvax was 10.4 (95% CI: 2.1-30.0) per 1000 vaccinees (3/289). No cases of myocarditis and/or pericarditis were identified in 1819 previously vaccinated subjects. The long-term outcome of myocarditis and pericarditis following ACAM2000 vaccination is currently unknown.

In the ACAM2000 clinical studies 97% and 92% of vaccine-naïve and previously vaccinated subjects, respectively, experienced one or more AEs. Common events included injection site reactions (erythema, pruritus, pain and swelling) and constitutional symptoms (fatigue, malaise, feeling hot, rigors and exercise tolerance decreased). Overall 10% of vaccine-naïve and 3% of previously vaccinated subjects experienced at least one severe AE. Also, rates for severe AEs were 10% for ACAM2000 and 13% for Dryvax among vaccine-naïve subjects but 4% and 6%, respectively, for previously vaccinated subjects. Headache was reported in 51% and 35% of vaccine-naïve subjects and previously vaccinated subjects, respectively. Severe, vaccine-related myalgia was seen in 1% of vaccine-naïve subjects and <1% of previously vaccinated subjects. Erythema and rash were noted in 18% and 8% of subjects respectively.

Development of the MVA-BN vaccine virus

Vaccinia virus (VV) is a member of the poxvirus family that replicates in the cytoplasm of the host cell without integration of viral DNA into the host cell genome.

Orthopoxviruses have a double-stranded DNA genome that encodes nearly 200 proteins. There are two major infectious forms, the mature virion (MV) and enveloped virion (EV). MVs, which are comprised of a single membrane with more than 20 viral proteins surrounding the virus core, are released by cell lysis and are thought to be responsible for host-to-host spread. EVs derive from MVs by acquiring extra membrane layers containing at least six additional unique viral proteins. EVs are responsible for dissemination of the virus within the host. Antibodies against surface proteins of both infectious particle forms contribute to protection (Moss, 2011).

Modified Vaccinia Virus Ankara (MVA) was derived from the replication-competent dermal vaccinia strain Ankara (Chorioallantois-Vaccinia-Virus Ankara, CVA), which was attenuated after more than 570 continuous passages in primary chicken embryo fibroblasts (CEF) with the aim of reducing the risk of complications associated with the existing replication-competent VV-based vaccines against smallpox.

Since passage 516, the attenuated CVA was called MVA because of the stability of its altered phenotype. During passaging, MVA acquired a multitude of mutations within its genome as well as six major deletions resulting in the loss of 15% (30 kbp) of the original genetic information. The deletions affected a number of virulence and host range genes as well as the gene for the Type A inclusion bodies. As a consequence, MVA exhibits a very restricted host range and replicates only very poorly, if at all, in most mammalian cell types, including primary human cells

and most transformed human cell lines. Non-infectious immature virions and abnormal particles are produced but no infectious MV and EV particles.

The viral genes are transcribed efficiently and the block in viral replication in host cells occurs at the stage of virus assembly and egress. Genetic reconstitution of one of the deletions in the MVA genome affecting, besides other genes, the VV host range gene K1L, restored MVA replication in some mammalian cell lines but did not restore its growth capacity in human cells. In light of these findings and the loss of 15% of its genome, MVA is unlikely to spontaneously regain its replication competency following injection into humans.

Despite these genomic modifications, MVA has retained stable immunogenic properties. MVA is a strong inducer of type I interferon (IFN) in human cells and it expresses a soluble interleukin-1 receptor, which has been implicated as an anti-virulence factor for certain poxviruses. In contrast to VV, MVA does not express soluble receptors for IFN-γ, IFN-α/β, tumour necrosis factor (TNF) and CC chemokines. The virus is described as eliciting a protective immune response against any of the orthopoxviruses.

All MVA strains originate from Prof. Anton Mayr. However, as many passages were used to attenuate MVA there are a number of different strains in existence depending on the passage number in CEF cells. Most are derived from MVA-572 that was used in Germany during a 1978 vaccination program in more than 120,000 human subjects (as part of a two-step vaccination protocol with a conventional VV vaccine against smallpox) or from MVA-575 that was extensively used as a veterinary vaccine.

During the 1978 German campaign intradermal (mostly) and subcutaneous (less often) injections of an MVA vaccine were administered prior to giving a VV smallpox vaccine. Each vial contained 2 x 10^6 freeze-dried infectious units (IU) and was used for two vaccinations after resolution in 0.5 ml saline. Most recipients were vaccinia-naïve children and adults considered to be at risk for adverse reactions to VV smallpox vaccines.

Mild local reactions including reddening and infiltration were observed at the site of injection (0.2 ml intradermal) in ~75% of 5308 individuals but there were no blisters, pustules or ulceration. Among 7098 subjects fever > 38°C occurred in 2.28% and non-specific general symptoms in 4.11%. There were no SAEs. Pre-vaccination with MVA resulted in a reduced number of side effects following a subsequent dose of VV smallpox vaccine and the development of smaller pocks.

A single dose of MVA elicited a weak haemagglutination inhibiting (HI) or virus neutralising antibody (NA) response. Following the subsequent VV smallpox vaccine dose there was a marked immune response, which was interpreted as MVA priming of specific humoral and cellular immune responses. In animal experiments MVA provided protection against variola even in the absence of an antibody response. In some animal studies much higher doses were used vs. that in the campaign vaccine with a clear impact on the magnitude of immune response.

About the product

Imvanex is derived from MVA-584 (i.e. 584th passage in CEF cells) and differs from all other MVA strains in that it has undergone 6-rounds of plaque purification (monoclonal) and is propagated in serum-free conditions. MVA-BN has been developed primarily for use as a viral vector in a variety of vaccine types. Nevertheless, the Company has also pursued its potential use as an alternative to VV smallpox vaccines as a standalone regimen rather than in a sequential regimen of MVA followed by a replication competent VV smallpox vaccine.

Imvanex is a liquid formulation (suspension for injection) for subcutaneous administration which contains Modified Vaccinia Ankara – Bavarian Nordic Live virus no less than 5 x 10^7 $TCID_{50}$ per 0.5 mL dose. The vaccine is presented in single-dose type I glass vials.

2.2. Quality aspects

2.2.1. Introduction

IMVANEX is prepared from Modified Vaccinia Ankara – Bavarian Nordic strain (MVA-BN), an attenuated and host restricted orthopox virus. MVA-BN virus is propagated in CEF, harvested, subjected to ultrasonification, purified by centrifugation, concentrated, subject to an enzymatic digestion step to remove DNA and further ultrafiltration/diafiltration steps. The final bulk vaccine is prepared by mixing drug substance with the formulation buffer to the target $TCID_{50}$ titre.

The vaccine is supplied as a frozen suspension in a 2 ml vial containing a single dose of 0.5 ml. Each 0.5 mL dose should deliver a minimum of 5×10^7 $TCID_{50}$.

2.2.2. Active Substance

The development of the MVA-BN vaccine virus is described in section 2.1. The structure of poxviruses in general has been adequately addressed, and is supported by of the information provided on MVA. The complete nucleotide sequence is provided and open reading frames have been identified and confirmed against the MVA parent strain and other vaccinia virus strains. The Applicant has provided information on the derivation of MVA-BN. The description of deletions, pathogenicity, virulence, and neurovirulence are described in literature concerning experiments conducted with MVA and is considered satisfactory.

Manufacture
The drug substance manufacturing process is conducted under aseptic conditions. CEF cells are prepared from embryonated eggs from SPF chickens after egg incubation. Following trypsinization steps, the primary CEF cells are isolated by centrifugation.

The CEF cells are infected with WSV and incubated in sterile and disposable reaction vessels. Harvests are pooled, homogenized by ultrasonication and clarified by centrifugation. The homogenized harvest is concentrated and purified through several TFF steps. DNA is enzymatically digested with benzonase. A final diafiltration is conducted with the same buffer as is used in drug product formulation.

A description of the drug substance manufacturing process has been provided. The batch size has been defined as supported by in process validation studies of the proposed commercial manufacturing process. An upper range of eggs for CEF production has been specified.

Flowcharts are provided for each process step and key details of the manufacturing process and process parameters, such as cell number and viability, MOI, incubation times and temperatures, are provided in the full description of the manufacturing process.

MVA-BN vaccine virus
Genetic characterization has been conducted on re-derived MVA-BN MSV. Details on the development of MVA-BN are provided in section 2.1.

Preparation of WSV
The crude stock, MVA-BN has been purified through a series of passages in CEF. The re-derived stock is the primary stock (Master Virus Bank, MVB). Certificates of analysis have been provided for each test conducted on MVB and for each of the materials used in MVB production.

Generation of the master seed virus (MSV) and working seed virus (WSV) and the testing have been adequately described. Sufficient data have been provided to indicate that the properties of the seed virus regarding growth in CEF and certain human cell types is the same as the re-derived MVA-BN, although full characterization of MSV, WSV and DS has not been conducted.

During the procedure, the Company has satisfactorily reviewed the testing program for extraneous agents to overcome the difficulties encountered in extraneous agents testing in CEF cells and chicken eggs. MSV and WSV have been tested by PCR testing for potential human virus contaminants and were found to be negative. Details supporting the capacity and specificity of the PCR assays implemented to detect the respective human viruses have been provided. Although release testing confirms the absence of wild-type vaccinia viruses, the MAA will provide additional results of testing of MSV and WSV for the presence of other poxviruses post authorisation.

The SPF flocks used to derive CEF are tested in compliance with Ph. Eur. 2010:50202. An appropriate list of specifications for raw materials used in drug substance production and example CoAs from qualified supplier were provided.

The Applicant has adequately described the In-Process Controls (IPC) applied during drug substance manufacture. Acceptance criteria are based on production of batches produced since 2007 in addition to recent batch data.

The Applicant has provided full descriptions of the methods used for IPC tests. The Company will conduct further testing of virus harvests for human viruses as a validation exercise. The use of TVAC assay is proposed as an IPC to provide an indication of any gross contamination of harvest pools. This is acceptable on the grounds that microbial contamination of DS batches would be detected by sterility testing of drug substance batches. Gentamicin is used in the virus propagation stage of the manufacturing process and data have been provided to demonstrate that key tests for microbial contaminants are not adversely affected by its presence.

Process validation studies have been conducted using the different manufacturing processes. Process test runs were conducted early in development to test selected critical process parameters. The data from these studies indicate that CEF number should be strictly controlled at the inoculation step to ensure process consistency. The Company will narrow the ranges for viable CEF number and MOI and will conduct process validation studies to revise IPC and specifications based on these studies. The process validation studies conducted to date provide an adequate control of the process and related impurities. However it is acknowledged that they have not addressed all impurities or all process steps. The Company is currently in the process of developing and implementing relevant assays and plans to undertake further process validation studies to address additional impurities and process steps. Whether the additional tests for such impurities will need to be included as IPC or in release testing of drug substance will depend on the process validation data provided post-authorization.

The Applicant proposed to allow hold times for CEF, pooled harvest and homogenized harvest during the manufacturing process. Details of the hold period and conditions have been clarified and the proposals are acceptable based on the data provided. Data have been provided to justify a hold period for DS samples before virus titre determination. Consistency of DP lots has been used to support validation of the freezing process applied to DS. The proposed DS sampling strategy ensures that sterility of DS bags is tested.

Manufacturing process development

During development, there have been different versions of the drug substance manufacturing process at different sites. Key changes for drug substance include: change of manufacturing site, increase in egg numbers, change of MOI, change of WSV, change of cell concentration for virus propagation, change in diafiltration and change in the freezing process. Data to demonstrate comparability of batches produced with different manufacturing processes have been provided. Comparison of data from 2007 production and validation batches, with 2008 validation batches demonstrate process consistency following optimization. IPC ranges were revised based on batch data produced in 2011 and 2012. These data have been used to revise IPC ranges. The batches used in pivotal clinical studies appear to contain broadly comparable levels of the impurities that were tested for as those produced by the commercial manufacturing process. The data provided indicate that for batches used in pivotal clinical and non-clinical studies, the actual doses used

were in some cases lower than the nominal titres stated in the clinical reports (10^8 $TCID_{50}$ in 0.5 ml dose). During the procedure, the applicant clarified that the minimum 0.5 ml dose used contained virus of at least 5 x 10^7 $TCID_{50}$.

Data have been provided to show the range of particles sizes in early DS batches which appear reasonably consistent for different batches. The reports indicate a shift over time following storage of drug substance at -20°C. In addition, information provided in responses indicates that visible particles were detected in some DP lots. Whilst the presence of these particles was correlated to concentration at the 2nd TFF step of the DS manufacturing process, a test for aggregation/particle size is requested to be applied to DS and DS intermediates as a characterization exercise and in future stability studies as necessary.

The Applicant has process-related impurities and product-related impurities. Whilst the levels of some of these impurities are determined during release testing of drug substance and have shown to be acceptable, additional tests for impurities are being introduced and data from the planned process validation studies and leachable studies are awaited. Risk assessments have been provided for some impurities based on maximal theoretical concentrations which together with non-clinical data provide reassurance that the levels of these impurities in DP are acceptable from a safety perspective.

Specification

Drug substance release testing includes appearance, pH, endotoxins, sterility, virus titre, identity, total protein, host cell DNA, residual benzonase and residual gentamicin. The acceptance criteria have been revised in line with more recent batches and clinical batch data.
Adequate descriptions of the analytical procedures including validity criteria have been provided.
Determination of Virus titer is performed in a $TCID_{50}$-based ELISA assay on CEF cells. Initially, the MAA proposed the RT-PCR method used for MVA-DNA titration as identity test. During the procedure, the DP identity test based on PCR amplification has also been introduced for DS. This assay cannot distinguish between MVA-BN and other MVA strains, however its use is acceptable as no other MVA strains are used at either DS or DP manufacturing facilities.
To support the proposed end of shelf-life and release specifications for DS and DP the Company refers to clinical data, estimated virus titres at the time of use, and has conducted regression analysis according to the calculation provided in WHO/BS/06.2049-Final, 2006. The proposed lower limit of the drug substance specification for virus titre will have to be sufficient to ensure that DP can be formulated and that DP end-of shelf-life specification (1.0 x 10^8 $TCID_{50}$/mL) can be met.

The Applicant has adequately described and listed the reference standards used in DS release assays and in IPC. The current reference standard for virus titre by $TCID_{50}$ was produced using the commercial scale process and meets specifications.

Details of the DS bags used as container closure system, composition of materials, manufacture and testing conducted by the supplier for leachables and extractables have been provided. A study is on-going to investigate leachables using the drug substance container closure system. One case of leakage of DS bags stored and shipped with this secondary packaging configuration was reported. Details of the investigations and a revised packaging procedure have been provided. Further consideration of alternative DS containers, their storage and secondary packaging for shipping is expected at post authorisation.

Stability
Stability studies have been conducted at the proposed storage conditions, accelerated temperature and ambient temperature.

The stability trend data indicate a significant decrease of virus titre during the proposed storage. A stability study to confirm the effect of cumulative DS and DP storage periods is ongoing Preliminary data support the cumulative shelf-life.

The proposed post-approval stability protocol is acceptable.

2.2.3. Finished Medicinal Product

The vaccine is supplied as a frozen suspension for injection in a 2 ml vial containing a single dose of 0.5 ml.

Table: Composition of the vaccine per vial

Component	Quantity per dose (0.5 mL)	Function	Reference to Standards
Active Substance			
MVA-BN®	Not less than 1.0 x 10^8 $TCID_{50}$/mL	Antigen	In-house specifications
Excipients			
Trometamol (Tris-hydroxymethyl- amino methane)		Buffering agent & stabilizer	Ph. Eur., USP
Sodium Chloride		Isotonic agent	Ph. Eur., USP
Water for Injection	q.s.	Solvent	Ph. Eur., USP

The description of the composition of the vaccine product is generally acceptable and the target formulation titre is stated.

The current target titre for DP is based on evaluation of 15 Drug Product Lots from 2011 but target titre has been changed during development. A study to further investigate whether age of the DS lot at the time of filling has any impact on the release titre for the DP lots will be performed. Depending on the results, a revision of the batch formula may be necessary to take into consideration the age of DS lots at the time of formulation.

Pharmaceutical Development

Two different frozen liquid formulations and a freeze-dried formulation have been used in development. The physicochemical description of drug product is acceptable. The biological properties of the product are described in relation to non-clinical studies and clinical studies.

A total of 8 drug product manufacturing processes have been used during product development. Changes include scale-up, change of site, change in thawing conditions, change in target titre, change in mixing and change in fill volume. Comparability of pivotal clinical and non-clinical batches with those produced by the proposed commercial manufacturing process has been broadly demonstrated.

Manufacture of the product

The drug product manufacturing process consists of thawing drug substance, formulation of drug substance by mixing with the formulation buffer to produce final bulk vaccine. The final bulk vaccine is filled into single dose vials, inspected, labelled and packaged. The Applicant has provided an adequate description of each step of the drug product manufacturing process with details of process parameters and major equipment. The use of multiple bags of DS for formulation of DP was introduced to improve the consistency of DP batches.

The Applicant has provided details of the controls used for formulation buffer, formulation of final bulk vaccine and during filling and packaging. Wide limits for pH have been revised and justified. The range for fill volume is acceptable. Visual testing is conducted on DS bags on receipt at the DP manufacturing site.

Data from a 2007 process validation study conducted using the initial commercial scale process support the stability of the product in terms of virus titre during filling, packaging and storage.
A process validation study conducted in 2008 (re-validated optimized commercial process) demonstrated homogeneity during filling in addition to process consistency. Details of

investigations into visible particles seen during DP process validation have been provided. Investigations showed that that the high levels of visible particles were due to the concentration at TFF steps during manufacture of the DS and that changing the concentration during the final ultrafiltration step resolved the issue. The stability of virus titre during thawing has been adequately validated. Aseptic filling has been adequately validated. The manufacture of batches of formulation buffer has been validated for homogeneity and sterility.

Drug product excipients (trometamol, sodium chloride, WFI and HCl) comply with relevant Ph. Eur. monographs. All analytical methods are compendial methods for which formal validation is not required.

Product specification

Drug product release testing includes identity, appearance, pH, extractable volume, sterility, bacterial endotoxins, virus titre, and abnormal toxicity. The stability specification includes identity, appearance pH, sterility, virus titre and container closure integrity.

The Applicant has provided details of the regression analysis used to determine the lower titre specification for DP at the end of shelf life (24 months). The regression analysis has been conducted using the formula indicated in WHO guideline WHO/BS/06.2049-Final. Data from four DP lots included in stability studies of 36 months have been used to determine the rate of loss of virus titre (slope). The Company has justified the use of the common slope, rather than the worst-case slope in this calculation and the regression analysis can be considered conservative.

The Applicant has provided details of the analytical methods used in drug product testing. The level of detail provided is acceptable. Data from validation studies of analytical methods have been provided. Most of the test methods applied to drug product are the same as are used for drug substance.

Batch data are provided for lots produced in the 2008 re-validation exercise. Specifications are appropriate and acceptably justified. The reference standards for drug product assays are generally the same as for drug substance.

Details of the drug product vials, stoppers and caps, the materials of construction, suppliers, dimensions and tests conducted on receipt of the materials have been provided. The container closure system components comply with relevant Ph. Eur. monographs and where relevant, are tested according to monographs on receipt. Data from leachable studies are awaited.

Stability of the product

The claimed shelf-life for drug product is 2 years at -20°C ± 5°C protected from light. The in-use shelf-life claimed for thawed product is 12 hours at 2°C to 8°C in the dark. Details of the batches used in long term and accelerated stability studies have been provided. Stability indicating parameters included in studies are appearance, virus titre and pH. Summaries of the data have been provided and indicate a significant decrease in virus titre during the shelf life of the product. The Applicant has conducted regression analysis on the combined virus titre data and concludes that these data support a shelf-life of 2 years, however all batches used in this study were above the proposed lower limit for virus titre at release. A stability study to confirm the effect of cumulative DS and DP storage periods is ongoing and preliminary data support the cumulative shelf-life. Improvements to the virus titre assay used at key time points in stability studies are acceptable.

The proposed post-approval stability protocol is acceptable.

Adventitious agents

Viral safety

The potential sources of extraneous viral contamination are biological starting materials and raw materials.

SPF Eggs are produced by chicken flocks free from specified pathogens (SPF) complying with the requirements of Ph. Eur. 5.2.2. and controlled for absence of extraneous agents by in-vivo (adult mice, suckling mice and embryonated eggs) and in-vitro testing (CEF, Vero, MRC-5).

Master and Working Seed Virus (MSV and WSV) are extensively tested during their production or at release for the presence of adventitious viruses to exclude contamination of a panel of human viruses, porcine viruses, extraneous agents in chicks and replication competent reovirus. In order to further ensure viral safety, the applicant will introduce a validated method to test presence of possible contaminating poxviruses (FPV & VV) at post-authorisation. In addition, extraneous agents testing for human viruses by PCR will also be conducted on virus harvests or DS manufactured using the proposed commercial WSV

Although Trypsin is inactivated by UV irradiation and a study evaluating the inactivation of Porcine Parvovirus (PPV) in Trypsin by UV irradiation was provided, it was noted that additional data of PCV testing of MSV and WSV should be provided at post authorisation to further ensure the viral safety.

TSE safety
The materials of animal origin present in the manufacturing process of medium components and reagents are described as complying with the European Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products (EMEA/410/01 Rev. 3). As for other live viral vaccines, no specific steps that might remove or inactivate TSE agents are present in the Imvanex manufacturing process.

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

Details of the drug substance, the manufacturing process, process validation, characterization studies, control of drug substance, reference standards, container closure system and stability studies have been provided. Comparability of the 6 drug substance manufacturing processes and 8 drug product manufacturing processes has been demonstrated. The introduction of stricter process parameters is expected for the inoculation step. Additional tests for impurities are being developed and will be used in further process validation studies. Seed lots have been characterized phenotypically, and confirmatory testing of WSV for human viruses has been conducted. The microbial control is acceptable.

Details of the drug product, the excipients, the formulation development, manufacture, and container closure system for drug product have been provided and were considered satisfactory to ensure the quality of the product

The use of existing stability data and regression calculations conducted to date provide a means of ensuring that DS and DP release titres are suitable to ensure that an appropriate virus titre is present at the end of DP shelf-life.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

The provided documentation is largely considered to cover the quality requirements for a live viral vaccine. As detailed in section 2.2.6, the CHMP recommends the applicant to address the following quality issues in or der to ensure an adequate maintenance of the quality of the product. a stricter control of key process steps, and the introduction of additional tests for impurities expected to be used in further process validation studies. Subsequent tightening of IPC acceptance criteria, and specifications as appropriate, is expected to result from the further process validation data.

2.2.6. Recommendation(s) for future quality development

At the time of the CHMP opinion, there were 16 unresolved quality issues having which were determined not be sufficiently significant to change the Risk-benefit balance of the product. The applicant gave a Letter of Recommendations and committed to implement these recommendations in the ongoing development of the medicinal product.

1. DS and DP specifications for virus titre at release and end-of shelf life should be revised as necessary in case of any modification in the indication, posology and acceptable dose after consideration of updated clinical or non-clinical data
2. Data from DS and DP stability studies at different temperature should be provided to support an improved stability profile.
3. Data from process validation studies should be provided. These data should be used as the basis for IPC acceptance criteria and/or release tests specifications following validation and implementation of additional test methods and to further justify or tighten acceptance criteria and specification for existing test methods. In this respect:

   a) A suitable test for viable cell number should be introduced. Strict controls should be introduced for some manufacturing steps. Data from process validation studies using these strict controls should be provided to support the choice of acceptance criteria. Downstream IPC should be tightened based on the results of these process validation studies.

   b) Data from process validation studies including the results of tests for viable cells, residual impurities should be provided. Appropriate acceptance criteria should be introduced based on the results of these tests and studies, which should be further tightened following additional manufacturing experience.

   c) Additional assays for certain impurities should be applied to DS batches and DS intermediates manufactured in process validation studies to further demonstrate consistency in DS and during DS manufacture.

   d) A suitably validated method for determining MVA-DNA content should be introduced to enable purity of DS and DS intermediates to be evaluated and process consistency with regards to this parameter to be assessed. Data from process validation studies including a suitable test for MVA-DNA content should be provided and appropriate controls and specifications should be introduced based on such data. Controls and specifications for MVA-DNA content should be further tightened following additional manufacturing experience.

   e) A suitably qualified assay should be applied in process validation studies to demonstrate the consistency of a manufacturing step with regard to DNA impurities.

4. A method should be introduced to assess aggregation/particles size of DS process samples, and DS & DP batches if appropriate. Data from DS process samples tested for aggregation/particle size should be provided and appropriate controls or specifications should be introduced based on such data. Inclusion of such a test method in DS and DP stability studies should be considered or the absence of such a test from stability studies should be justified.
5. A suitably qualified test for residual trypsin activity should be applied to CEF produced.
6. A description of the improved method for visual inspection of containers at harvest should be provided and should include reference pictures.
7. Additional data from viral safety testing of MSV and WSV should be provided prior to marketing.
8. Appropriately validated method should be introduced to test MSV and WSV for the presence of contaminating poxviruses . The results from testing of MSV and WSV for FPV and VV should be provided with full details of the analytical procedure(s) and validation data to support the suitability of the analytical procedure(s).
9. To take account of the special populations who may receive Imvanex, extraneous agents testing for human viruses should be conducted on virus harvests or DS manufactured using the proposed commercial. The data from such testing should be provided.
10. Data from revalidation studies of the virus titre assay for pool and harvest samples should be provided to ensure that the assay is valid for all for the complete range of virus titres that may be obtained.
11. The Company should consider the use of additional packing materials to minimize movement of the DS bag within the frame within the transport container.
12. The Company should evaluate alternative container closure systems and/or packaging systems for storage and transport of DS.
13. Data from leachable studies of the DS container closure systems should be provided
14. A study to investigate whether age of the DS lot at the time of filling has any impact on the release titre for the DP lots should be undertaken and the data provided. If necessary, the batch formula should be revised to take into consideration the age of DS lots at the time of formulation.
15. A DP batch that is close to the lower release specification for virus titre should be included in post-approval stability studies.
16. Data from leachables studies of the DP container closure system should be provided.

2.3. Non-clinical aspects

2.3.1. Introduction

Non-clinical pharmacological and toxicology studies were undertaken on Imvanex based on

- CPMP Note for Guidance on preclinical pharmacological and toxicological testing of vaccines (CPMP/SWP/465/95),
- Note for Guidance on Reproductive Toxicology: Detection of Toxicity to Reproduction for Medicinal Products (CPMP/ICH/386/95).

Based on these guidelines secondary pharmacodynamic, pharmacodynamic drug interaction, genotoxicity and carcinogenicity studies were not considered necessary to be performed for Imvanex.

To address the non-clinical pharmacology of Imvanex, the applicant's strategy was to establish test systems where challenges with orthopoxviruses elicited death in animals and to show protection from death when animals were vaccinated with Imvanex and to compare Imvanex in these tests with US-approved smallpox vaccines that contain live replication-competent vaccines (Dryvax, ACAM2000). Studies were done in mice and monkeys. This strategy rests on extrapolating protection of humans infected with variola from data from animal studies showing protection from other pox virus challenges, together with data on human immune responses to vaccination as outlined in the section on clinical aspects further below. As variola has no animal host, challenge studies using live variola virus are not considered informative.

2.3.2. Pharmacology

As direct proof of efficacy cannot be obtained from clinical testing, the applicant's strategy was based on establishing test systems in mice and monkeys in which challenge viruses elicited death in animals. Studies were designed to assess protection from death following challenge in animals that had been vaccinated with Imvanex or with US-approved replication-competent smallpox vaccines (Dryvax, ACAM2000). Immunogenicity was also assessed using ELISA and PRNT. In the ELISA the MVA-derived antigen was used to coat the wells and in the PRNT assay vaccinia virus Western Reserve (VV-WR) was used.

In mice, the applicant characterised testing with the challenge virus VV-WR strain given by the intranasal route. A dose-response for morbidity and mortality was shown and a dose equivalent to 50-fold the median lethal dose was taken forward for use in testing the protective efficacy of Imvanex. When mice previously vaccinated with Imvanex were then challenged with this lethal intranasal dose they survived. In characterising the dose-response for this protective effect of Imvanex, the applicant concluded that the dose of $1x10^8$ $TCID_{50}$ was optimal. Lower doses protected mice from death but there was greater morbidity than with $1x10^8$ $TCID_{50}$; still lower doses of Imvanex resulted in the death of some mice and therefore loss of protection.

There was a clear dose-response for antibody elicited by Imvanex (0.1, 0.4 and 2 $x10^8$ $TCID_{50}$). Titres were higher with a second dose than after a single dose. The amount of challenge virus in the lungs was also quantified: whereas there were very large titres in unvaccinated mice,

challenge virus was not detectable in the lungs of mice vaccinated with Imvanex. Different lots of Imvanex, representing the different production methods, were compared and shown not to be different in such tests. In comparative testing with Dryvax and ACAM2000, 100% seroconversion rates were achieved by day 14 in mice given Imvanex but it took to days 26 to 40 for similar seroconversion rates with Dryvax and ACAM2000. Mice survived a lethal challenge provided they seroconverted.

Subsequently methods were developed to challenge mice with ectromelia, an orthopox virus that naturally infects mice. The applicant showed that mice vaccinated with Imvanex were able to survive a lethal challenge with this virus, whereas unvaccinated mice did not. In comparative testing with Dryvax, all vaccinated mice that seroconverted survived. All mice given Imvanex did seroconvert; however, only 79% of mice given Dryvax seroconverted. Immunogenic responses to vaccination developed more rapidly with Imvanex than with Dryvax.

Lung titres of ectromelia virus were reduced to 0 in mice vaccinated with Imvanex but 2 of 10 mice given Dryvax failed to completely clear the virus, although titres were much lower than in unvaccinated mice.

Data were also provided in challenge studies from monkeys. The CHMP Note for Guidance on the Development of Vaccinia Virus based Vaccines against Smallpox for states that 'the final confirmation of protection of the final product should be investigated in monkeys'. This guidance is limited in scope to replicative viral vaccine that is administered by scarification; however, the principle is established and is generally accepted by the scientific community that for assessment of the potential protective efficacy against variola in humans, studies in primates are appropriate and offer additional evidence as compared to testing in rodents alone. The applicant tested the ability of Imvanex to protect against lethal challenges with monkeypox when this was given by the intravenous or intratracheal routes. With the intravenous challenge, two subcutaneous doses of Imvanex given 28 days apart at $1x10^8$ $TCID_{50}$ were shown to induce protection from death when monkeys were subsequently exposed to monkeypox at $5x10^7$ pfu/ml monkeypox virus. ACAM2000 (US licensed vaccine containing the NYBH strain of vaccinia) was also shown to be effective at eliciting protection.

Exposure of humans to variola is likely to be by inhalation. The applicant tested two different strains of monkeypox virus in methods using intratracheal administration of challenge virus and two different doses of one strain of monkeypox virus, at lethal doses. Again, Imvanex was able to prevent death induced by the viral challenge. For instance, in one of these studies, it was shown that 8 out of 10 monkeys that were given two doses of Imvanex, and 9 out of 10 that were given one dose of Imvanex, survived a challenge that killed 8 out of 8 unvaccinated monkeys.

The immune response was characterised in monkeys. A second dose was shown to result in higher total and neutralising antibody responses compared to a single dose.

In monkeys, lower doses of Imvanex, $1x10^6$ and $1x10^7$ $TCID_{50}$, were compared with $1x10^8$ $TCID_{50}$ Imvanex. At the two higher doses, all monkeys given a lethal challenge survived, whereas 3 out of 5 at the lowest dose died, and 3 of 3 unvaccinated monkeys died. In this study, 6/6 monkeys given ACAM2000 survived. Concerning viraemia, monkeys given Imvanex still showed detectable amounts of challenge virus, whereas in monkeys given ACAM2000, there was complete absence of detectable challenge virus.

Despite reducing mortality and morbidity, vaccination with Imvanex did not show the same degree of suppression of challenge virus replication as was seen with the replication-competent vaccine. Findings in mice that Imvanex did suppress challenge virus replication were, thus, not repeated in monkeys. This finding was considered problematic as it might suggest that Imvanex is not as effective at suppressing viraemia as replication-competent vaccines. If this were to occur in humans there could be transmission from persons who are infected but have no outward signs of disease.

The applicant noted that despite use of the same methods in similar experiments, there appeared to be more severe disease induced by monkeypox challenge in one experiment than in the other. The reason for this remains unexplained but the applicant subsequently showed that a lower challenge dose of monkeypox virus still elicited substantial toxicity. The applicant noted that use of very high challenge doses does not reflect the likely clinical exposure; clearly, humans will not likely be exposed to very large multiples of the human lethal dose of variola. The use of high challenge doses was necessary in order to be able to use reduced numbers of animals in these experiments.

Overall, the data supplied showed protective efficacy in mice of Imvanex in a manner that correlated with clearance of challenge virus from lung tissue and in conjunction with vaccinia-specific immune responses. The applicant concluded that the dose of $1x10^8$ $TCID_{50}$ was optimal.

In monkeys, Imvanex prevented death but there was incomplete suppression of challenge virus replication in the blood of vaccinated monkeys given monkeypox. In contrast, vaccination with ACAM2000 achieved complete viral suppression. It was also a potential concern that the level of antibody response seen in animals was not seen in humans, with unknown implications for the protective efficacy of the vaccine.

In response to concerns regarding the lack of suppression of viraemia and the immune responses to Imvanex (as compared to approved vaccines) and the possibility that Imvanex might not be as effective as approved vaccines the applicant provided results from two additional monkeypox challenge studies. Both studies were dose-response investigations in monkeys of the protective efficacy of Imvanex in the dose range of $1x10^2$ and $1x10^3$ to $1x10^7$ $TCID_{50}$: one study used intravenous challenge and one used inhalational challenge methods. The applicant showed a statistically significant correlation between dose of Imvanex and probability of survival and between dose of Imvanex and each of PRNT and ELISA titres. However, it was evident that vaccinated monkeys that developed PRNT and ELISA titres at levels comparable to those who survived could still succumb to the monkeypox challenge. Despite the statistically significant correlation, survival of an individual monkey could not be accurately predicted based on its PRNT or ELISA response. In one study, there were two monkeys that did not survive despite seroconversion by day 42 and there were two monkeys who had not seroconverted by day 42 but survived. These results suggest that PRNT and ELISA titres are not sufficient correlates of protection and that other immune mechanisms, particularly cellular immunity, may contribute to protection.

An expert scientific panel was convened to consider whether the lack of complete suppression of viraemia was sufficient cause to be concerned that Imvanex might not be as effective as replication competent vaccine and whether the difference in level of antibody response between protected animals and humans is a cause for concern that the vaccine might not be effective.

The panel advised that the evidence indicated that Imvanex produced as good protection as did existing vaccines, but that the nature of protection offered by Imvanex might be different and that the viraemia seen was insufficient reason to deny approval. The panel advised that the preclinical challenge data for Imvanex support expectation of its protective efficacy in humans exposed to variola. In relation to projecting protection in humans based on the antibody titres seen in animals, humans showed reduced titres compared to vaccinated monkeys and it was uncertain that protection in humans could be achieved at the titres obtained.

During the procedure the applicant indicated that additional studies were either ongoing or had been completed. Information was supplied on two further studies that are ongoing and three completed studies. These will be submitted and fully assessed in a post-approval procedure.

Pharmacokinetics

In accordance with CHMP Note for Guidance on the preclinical pharmacological and toxicological testing of vaccines (CHMP/SWP/465/95), pharmacokinetic studies are not normally needed. However, the Note for Guidance indicates that local deposition and retention at the injection site with characterisation of its distribution e.g. into draining lymph nodes and viral shedding or live vaccines should be considered.

The applicant developed methods to allow detection of a vaccinia-specific gene product and used this to test distribution of virus after its subcutaneous injection in rabbits. In this testing, the applicant asserted that there was no vaccinia transcripts identified in the heart: however, the heart of one rabbit did test positive at 48 hours after injection. The signal was weak and at the limits of detection of the assay and the majority of rabbits tested showed no expression of virus in the heart. The distribution profile indicated that there was exposure at the injection site and in the lymph nodes, but that MVA-BN virus was not detected in brain, ovaries and testes. It was also not detected in spleen or kidney. After subcutaneous injection, there appeared to be no exposure to lung or to liver but both these routes tested positive when the route of injection was intramuscular.

2.3.3. Toxicology

The testing done by the applicant was generally consistent with the CHMP Note for Guidance on preclinical pharmacological and toxicological testing of vaccines (CHMP/SWP/465/95). This details that genotoxicity and carcinogenicity studies are not normally needed and these studies were not provided; dedicated fertility studies have also not been done which is in accordance with this guidance which states that histology in toxicity studies may provide sufficient information concerning the integrity of reproductive organs; no signal for concern was identified in this respect.

Toxicity testing addressed general toxicity and reproductive toxicity. The studies were done in suitable species, at suitable doses and dose frequencies and, in general, supported human dosing. There were no major objections identified based on review of these studies. Some minor and reversible effects local to the site of injection were seen – there was inflammation of the dermis which abated over time – but, no major toxicity was identified. However, there was a suggestion that there may have been an effect on the heart in one rabbit in study 254-03. In

the report, the description at post mortem examination is of 'Inflammation, pericardium'. Although this is a finding in a single animal, the possible link to troponin I in the clinical studies is a potential concern, noting that previous vaccines have been associated with rare instances of myocarditis. Its occurrence in one rabbit would not be expected if it occurred in rabbits at the same rate as is suggested in humans and there was also the unexpected finding, since concluded not to be a true finding, of the presence of virus in the heart of one rabbit in the distribution study. The applicant was asked for further consideration of this point. Based on the applicant's response, the finding was concluded to have been resolved as a non-clinical issue, but this (ie myo/pericarditis) remains a concern clinically.

The proposed clinical dosing regime and that used in the majority of studies in animals used two doses given four weeks apart. This was not used in toxicity studies where dosing was at intervals of one, two or three weeks. This difference is not judged to be significant as studies still support the safety of the proposed dosing regime. The primary pharmacology studies provided evidence that the immune response was generated within several days of vaccination.

Concerning the dose, the applicant's proposal is $1x10^8$ $TCID_{50}$/mL. This was used in the toxicity studies; for instance, rabbits were given the full human dose by subcutaneous injection. The studies provide adequate testing at doses sufficiently in excess of that proposed for use in humans. No objection to the dose is raised on preclinical safety grounds.

Neurovirulence testing was not done. This is acceptable as this virus is non-replication competent in mammalian tissue and distribution into the brain was not seen.

In conclusion, the toxicology section of the dossier can be accepted except that additional data should be provided from a study in pregnant rats in which vaccine is given close to implantation.

Genotoxicity, Carcinogenicity

In accordance with the CPMP Note for Guidance on the development of Vaccinia virus based vaccines against smallpox (CPMP/1100/02), the WHO Guideline on nonclinical evaluation of vaccines, and the CPMP Note for Guidance on Preclinical Pharmacological and Toxicological Testing of Vaccines, no genotoxicity and carcinogenicity testing was performed for the vaccine.

Reproduction Toxicity

In pregnant animals, there was no effect of vaccination on gestation, lactation, maternal behaviour or on development of the offspring. The magnitude of the immune response induced in the reproductive toxicity studies in pregnant rats and in rabbits was not especially high. However, a dose-response relation was shown. It is feasible that pregnancy per se resulted in reduced immunogenicity but there is no direct proof of this. There was no vaccination around the time of implantation in these studies. Dosing was at days -14, 0 and 14 in relation to mating. It remains possible that vaccination at this time (i.e. at implantation) could have an adverse effect that has not been identified in these studies. The applicant has committed to provide data on effects of vaccination at the point of implantation in rats by the end of 2014. The below table provides an overview of the studies submitted:

Tabular summary of reproductive toxicity studies

Study type/ Study ID / (GLP Compliant)	Species; Number Female/ group	Route & dose	Dosing period	Major findings	NOAEL
Male fertility	No data, except from general toxicity studies, indicating no concerns				
Female fertility	Rat / 18-22	subcutaneous	Days -14, 0	No toxicity to fertility	$1x10^8$ TCID50
	Rabbit / 13-15	subcutaneous	Days -14, 0	No toxicity to fertility	$1x10^8$ TCID50
Embryofetal development	Rat / 18-22	subcutaneous	Days -14, 0	No embryofetal toxicity	$1x10^8$ TCID50
	Rabbit / 13-15	subcutaneous	Days -14, 0 and +14	No embryofetal toxicity	$1x10^8$ TCID50
Peri & postnatal	Rat	subcutaneous	Days -14, 0 and +14	No impairment to pup development	$1x10^8$ TCID50

Dedicated fertility studies have not been performed, which is in accordance with the CHMP Note for Guidance on preclinical pharmacological and toxicological testing of vaccines (CHMP/SWP/465/95), which states that histology in toxicity studies may provide sufficient information concerning the integrity of reproductive organs. No signal for concern was identified in this respect.

Toxicokinetic data

Not applicable

Local Tolerance

Separate studies to determine local tolerability were not performed. The local tolerance was evaluated as part of the repeat-dose toxicity studies.

Other toxicity studies

Not applicable

2.3.4. Ecotoxicity / environmental risk assessment

Vaccines are exempt from requirements relating to an assessment of environmental risk in accordance with CHMP guidance (EMEA/CHMP/SWP/4447/00 Guideline on the environmental risk assessment of medicinal products for human use).

Nevertheless, the applicant provided such an assessment. In this, the predicted environmental concentration (PEC) was estimated to be 0.0025 µg/ litre (driven by a market penetration estimate of 0.01). The action limit is 0.01 µg/ litre and so the product was considered to pose no environmental concerns.

2.3.5. Discussion on non-clinical aspects

The testing dose by the applicant was generally consistent with the CHMP Note for Guidance on preclinical pharmacological and toxicological testing of vaccines (CHMP/SWP/465/95). This details that genotoxicity and carcinogenicity studies are not normally needed and these studies were not provided; dedicated fertility studies have also not been done which is in accordance with this guidance which states that histology in toxicity studies may provide sufficient information concerning the integrity of reproductive organs; no signal for concern was identified in this respect.

Toxicity testing addressed general toxicity and reproductive toxicity. The studies were done in suitable species, at suitable doses and dose frequencies and, in general, supported human dosing. There were no major objections identified based on reviews of these studies. Some minor and reversible effects local to the site of injection were seen – there was inflammation of the dermis which abated over time – but, no major toxicity was identified. However, there was a suggestion that there may have been an effect on the heart in one rabbit in study 254-03. In the report, the description at post mortem examination is of 'Inflammation, pericardium'. Although this is a finding in a single animal, the possible link to troponin I in the clinical studies is a potential concern, noting that previous vaccines have been associated with rare instances of myocarditis. Its occurrence in one rabbit would not be expected if it occurred in rabbits at the same rate as is suggested in humans. The applicant was asked for further consideration of this point. Based on the applicant's response, the finding was concluded to have been resolved as a non-clinical issue, but this (i.e. myo/pericarditis) remains a concern clinically.

In view of reproduction toxicity no concern arose from the studies in pregnant animals, however, the study designs resulted in no vaccination around the time of implantation. It remains therefore possible that vaccination at the time of implantation could have an adverse effect that has not been identified in these studies. The applicant has committed to provide data on effects of vaccination at the point of implantation in rats by the end of 2014. These data will address missing information on the effect of Imvanex administration during the early phase of pregnancy and are expected in late 2014 (as detailed in the RMP).

The proposed clinical dosing regime and that used in the majority of studies in animals used two doses given four weeks apart. This was not used in toxicity studies where dosing was at intervals of one, two or three weeks. This difference is not judged to be significant as studies still support the safety of the proposed dosing regime. The primary pharmacology studies provided evidence that the immune response was generated within several days of vaccination.

Concerning the dose, the applicant's proposal is $5x10^7$ $TCID_{50}$. This was used in the toxicity studies; for instance, rabbits were given the full human dose by subcutaneous injection. The studies provide adequate testing at doses sufficiently in excess of that proposed for use in humans. No objection to the dose is raised on preclinical safety grounds.

Neurovirulence testing was not done. This is acceptable as this virus is non-replication competent in mammalian tissue and distribution into the brain was not seen.

2.3.6. Conclusion on the non-clinical aspects

The non clinical programme adequately supports the marketing authorisation of Imvanex. However, it remains the possibility that vaccination at the time of implantation could have an adverse effect that has not been identified in the reproductive toxicity studies conducted.

Therefore, the applicant committed to conduct an additional toxicity study in pregnant rats to provide data on the response effect induced by the vaccine on early pregnancy.

2.4. Clinical aspects

2.4.1. Introduction

Overall content of the clinical programme

In the absence of smallpox worldwide since 1977, the evaluation of the potential clinical efficacy of MVA-BN can only be assessed on the basis of non-clinical efficacy data and measurement of immune responses to vaccination in human subjects. The clinical studies included in the application were conducted from 2001 onwards and consisted of dose-finding studies as well as studies with the final selected vaccine virus titre in different populations.

The applicant strategy for filing this application was to demonstrate comparable protection with and immune responses to MVA-BN and replication-competent vaccinia (VV) vaccine in non-clinical studies and then demonstrate comparable immune responses to MVA-BN and to replication-competent NYBH vaccines in humans. Despite this plan the applicant provided only two prospective studies (POX-MVA-002 and -009) in which very small numbers of subjects received Dryvax (15 and 8 per study) with limited blood sampling time points. Neither study compared Dryvax with the final commercial formulation of MVA-BN. There was also one retrospective study that compared immune responses to MVA-BN and to Dryvax or Wetvax using stored sera obtained during a total of 8 studies.

Formulations

Vaccine comprising the final selected virus titre (1 x 10^8 $TCID_{50}$/ml) supplied as lyophilised vaccine or as frozen liquid in 0.5 ml doses is referred to in the following sections simply as MVA-BN. The formulations used by study are listed in the table below. Some data from POX-MVA-BN-028 and 029 were reported at the time of responding to the first list of questions but POX-MVA-030 was still ongoing. These additional three studies and some of the earliest studies in the programme were sponsored by NIH.

Process Step	Early Development I (Lot 021100)	Early Development II (Lot 080902)	Pilot Scale Process (Lot 130303)	Industrial Scale Process (Lot 0170505)	Validated Industrial Scale Process (Lots 0031105, 0120606 & 0061205)	Commercial Scale Process (Lots 0040707, 0030707 & 0020707)	Optimized Commercial Scale Process (Lots 0070808 & 0050808)	Re-validated Optimized Commercial Scale Process (Lots 0101208 to 0131208)
Use in Clinical and Non-clinical studies	Clinical Study POX-MVA-001	Clinical Study POX-MVA-004 Non-clinical Study BN-PRE-2003.020	Clinical Studies POX-MVA-002 POX-MVA-007 POX-MVA-010 HIV-NEF-004 Non-clinical Studies BN-PRE-2004.004 BN-PRE-05-20 BN-PRE-05-24 BN-PRE-07-006 M-249-03 M-254-03 G-216-04	Clinical Studies POX-MVA-011 POX-MVA-008 POX-MVA-005 Non-clinical Studies BN-PRE-05-032 BN-PRE-05-21 BN-PRE-06-004 BN-PRE-06-005	Clinical Studies POX-MVA-011 HIV-POL-002 POX-MVA-009 Non-clinical Studies BN-PRE-07-006 BN-PRE-07-015	Clinical Studies POX-MVA-008 POX-MVA-023 Non-clinical Studies BN-PRE-08-004 BN-PRE-08-005 BN-PRE-07-006	Clinical Studies POX-MVA-024 POX-MVA-028 POX-MVA-029 (ongoing) POX-MVA-030 Non-clinical Studies BN-PRE-09-003 BN-PRE-09-004 BN-PRE-09-024	Clinical Studies POX-MVA-028 (ongoing) POX-MVA-030 (ongoing) (Delivered to US Strategic National Stockpile)

GCP

The applicant has provided a statement to the effect that clinical trials conducted outside the community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

Overview of clinical studies

Three studies evaluated test formulations containing more than one viral titre. POX-MVA-002 included Dryvax within all regimens tested except for one group that received two doses of MVA-BN. POX-MVA-001 and 004 were dose-finding studies with MVA-BN.
Five studies can be regarded as pivotal. In brief, these comprised:

POX-MVA-005: The study was conducted during 2006-2007 at a single centre in Germany. It compared the safety and immunogenicity of one or two doses of MVA-BN in subjects with and without a history of smallpox vaccination. This was a partially randomised, partially double-blind, placebo-controlled, non-inferiority study with a last follow-up to 6 months but with year 2 data from the booster phase.

POX-MVA-023: The study was conducted during 2008-2009 to evaluate the safety and immunogenicity of a booster dose of MVA-BN administered two years after initial vaccination with one or two doses of the same vaccine in POX-MVA-005. The study was open-label with 6 months follow-up.

POX-MVA-008: The study was conducted between 2006 and 2009 at 17 US and 7 Mexican study sites. It compared the safety and immunogenicity of MVA-BN in subjects with atopic dermatitis vs. healthy subjects. There was a final follow-up visit at 26 weeks after the last assigned dose.

POX-MVA-011: The study was conducted between 2006 and 2009 at 34 US and 2 Puerto Rica sites. It compared the safety and immunogenicity of MVA-BN in HIV-infected subjects with CD4 counts 200-750 cells/µl vs. healthy subjects with sub-division into groups according to prior smallpox vaccine. The duration of the follow-up period varied by study group.

POX-MVA-024: The study was conducted between 2009 and 2010 at 4 study sites in the US. It evaluated the safety and immunogenicity of one or two doses of MVA-BN administered to healthy

subjects aged 56-80 years who had received smallpox vaccine in the past. Follow-up was to 6 months.

- Five other studies (including initial studies in subjects with atopic dermatitis or HIV infection) are regarded as supportive.
- In addition the applicant provided a report on a serological study that sought to compare antibody levels elicited by Dryvax or Wetvax in NIH/NIAID-sponsored studies with those elicited by MVA-BN in three of the applicant's studies.

2.4.2. Pharmacokinetics

As mentioned in the Note for Guidance on Clinical Evaluation of New Vaccines (CHMP/VWP/164653/2005), "Pharmacokinetic studies are usually not required for vaccines". It was found acceptable that the applicant did not conduct pharmacokinetic (PK) studies during the clinical development of Imvanex.

2.4.3. Pharmacodynamics

The pharmacological profile of Imvanex is represented by its immunogenicity profile evaluated in the clinical trials submitted. The selection of the dose is further described under the section on clinical efficacy below.

2.5. Clinical efficacy

Design features of the pivotal and other studies

The major design features of the 13 studies in the initial application were as shown in Table 1.

Table 1 Overview of study design

Study Identification	Study Design	Comment
Pivotal Studies		
POX-MVA-005	Partially randomised, partially double-blind, placebo-controlled, Phase II, non-inferiority study	Study Vaccine: IMVANEX® Placebo control (Tris Buffer)
POX-MVA-023	Open-label, phase II study	Study Vaccine: IMVANEX®
POX-MVA-008	Open-label, controlled, phase II study	Study Vaccine: IMVANEX® Control: Healthy Subjects
POX-MVA-011	Open-label, controlled, phase II study	Study Vaccine: IMVANEX® Control: Healthy Subjects
HIV-POL-002	Randomised, single-blind, controlled, phase I/II study	Study Vaccine: MVA-mBN32 (MVA vector expressing HIV-1 epitopes) Control: IMVANEX®
POX-MVA-024	Randomised, double-blind, placebo-controlled, phase II study	Study Vaccine: IMVANEX® Placebo control (Tris Buffer)
Supportive Studies		
POX-MVA-001	**Part I of Study:** Randomised, double-blind, parallel group, dose-finding, phase I study. **Part II of Study:** Open-label, phase I study.	Study Vaccine: IMVANEX® No control
POX-MVA-002	Randomised, double-blind, controlled, dose-finding phase I study	Study Vaccine: IMVANEX® Placebo control (Tris Buffer) and Active control (Dryvax®)
POX-MVA-004	Randomised, double-blind, dose-finding phase I study	Study Vaccine: IMVANEX® No control
POX-MVA-007	Open-label, controlled, phase I study	Study Vaccine: IMVANEX® Control: Healthy Subjects
POX-MVA-010	Open-label, phase I/II study	Study Vaccine: IMVANEX® Control: Healthy Subjects
POX-MVA-009	Randomised, partially blinded, placebo-controlled, phase I/II study	Study Vaccine: IMVANEX® Placebo control (Saline) Active control (Dryvax®)
HIV-NEF-004	Randomised, single-blind, controlled phase II study	Study Vaccine: MVA-nef (MVA vector expressing HIV-1 LAI gene) Control: IMVANEX

Treatments

The formulations used by study are listed in the table below. Some data from studies POX-MVA-BN-028 and 029 were reported during the evaluation but POX-MVA-030 was still ongoing. These additional three studies and some of the earliest studies in the programme were sponsored by NIH.

2.5.1. Dose response studies

POX-MVA-001 was conducted at a single study site using an early liquid frozen formulation.

The primary objective was to evaluate the safety of two doses of MVA-BN by SC and IM administration routes vs. a single SC selected dose group. The assessment of immune responses was a secondary objective. Subjects were vaccinia-naïve (Groups 1-4) and vaccinia-experienced (Group 5; i.e. history of smallpox vaccination [VV]) healthy male adults aged 20-55 years. A single vaccine lot was used but there were three sets of vials containing the different $TCID_{50}$ doses.

In Part I subjects with no history of smallpox vaccination were randomised to receive two injections of MVA-BN at 0 and 4 weeks at one of three doses (there was double-blinding by dose; each being administered as 0.5 ml) and using one of two routes of administration:

- Group 1: dose 10^6 $TCID_{50}$, *subcutaneous*
- Group 2: dose 10^7 $TCID_{50}$, *subcutaneous*
- Group 3: dose 10^8 TCID50, *subcutaneous*
- Group 4: dose 10^8 $TCID_{50}$, *intramuscular*

In Part II one additional group (Group 5) of subjects previously vaccinated against smallpox received one single subcutaneous injection of MVA-BN at the highest dose tested (10^8 $TCID_{50}$).
Follow-up was planned at Year 1 and Year 2 (subsequent to day 128 in the initial phase of the study).

Of the 90 subjects planned (18 per group) there were 88 enrolled and 86 vaccinated. Subjects in part II were ~10 years older vs. Part I due to the requirement for prior smallpox vaccination. Time elapsed since the last VV administration is not reported but compulsory vaccination was abolished in 1976 in Germany.

The ELISA results (note that rates are for titres > 1:100) for groups 1-4 showed that the highest TCID50 dose tested elicited the highest results. At this dose the results were comparable for the two routes of administration and significantly higher GMTs were observed vs. the two lower doses. Within each of Groups 2-4 the second dose of MVA elicited a marked increment in antibody level.

The D28 GMT for Group 5 was ~10-fold higher than the D28 GMTs for Groups 3 and 4 and was numerically higher vs. the D42 GMTs after two doses in the previously naïve subjects. The data showed that MVA-BN boosted immune responses in subjects primed with VV (probably > 20 years earlier). D128 GMTs were also numerically higher in Group 5, in which all subjects retained titres > 1:100.

Table 2 Geometric mean titres and seroconversion rates in the ELISA (completers sample)

Group	Visit		n (%)	Geometric Mean Titre (IgG)	95% confidence interval
1	Day 0	18	2 (11%)	1.80	(0.77-4.24)
	Day 28	18	5 (28%)	4.59	(1.30-16.23)
	Day 42	18	7 (39%)	8.52	(2.13-34.04)
	Day 128	18	5 (28%)	4.30	(1.28-14.49)
2	Day 0	16	2 (13%)	1.96	(0.73-5.23)
	Day 28	16	7 (44%)	14.73	(2.65-81.89)
	Day 42	16	13 (81%)	106.19	(28.55-394.94)
	Day 128	16	8 (50%)	16.55	(3.39-80.82)
3	Day 0	16	1 (6%)	1.33	(0.72-2.46)
	Day 28	16	13 (81%)	114.26	(31.33-416.67)
	Day 42	15	15 (100%)	743.37	(509.94-1083.66)
	Day 128	16	14 (88%)	124.42	(42.22-366.66)
4	Day 0	16	1 (6%)	1.41	(0.68-2.95)
	Day 28	16	14 (88%)	128.37	(39.56-416.50)
	Day 42	15	15 (100%)	899.75	(508.23-1592.90)
	Day 128	16	14 (88%)	162.01	(50.56-519.18)
5	Day 0	18	4 (22%)	3.75	(1.06-13.31)
	Day 28	18	18 (100%)	1165.10	(661.74-2051.37)
	Day 42	18	18 (100%)	979.20	(564.09-1699.80)
	Day 128	18	18 (100%)	488.75	(296.87-804.65)

N = number of subjects with samples for antibody response
n/% = number of subjects with antibody response with titres above 1:100
Group 1: 106 TCID50, subcutaneous in subjects not vaccinated against smallpox
Group 2: 107 TCID50, subcutaneous in subjects not vaccinated against smallpox
Group 3: 108 TCID50, subcutaneous in subjects not vaccinated against smallpox
Group 4: 108 TCID50, intramuscular in subjects not vaccinated against smallpox
Group 5: 108 TCID50, subcutaneous in subjects previously vaccinated against smallpox

The PRNT data gave lower seroconversion rates compared to the ELISA data. However, the pattern of results within and between the four dose/route groups was the same as for the ELISA data. D42 responses to two doses in Groups 3-4 were slightly numerically lower vs. D28 responses to a single dose in Group 5. The D126 titres (% with 1:10 and GMT) were higher in Group 5 vs. all other groups.

Table 3 Geometric mean titres and seroconversion rates in the neutralisation assay (Completers sample)

Group	Visit	N	n (%)	Geometric Mean Titre (IgG)	95% confidence interval
1	Day 0	18	3 (16.7%)	1.62	(0.92-2.87)
	Day 28	18	4 (22.2%)	2.20	(1.01-4.79)
	Day 42	18	6 (33.3%)	3.50	(1.39-8.84)
	Day 126	18	2 (11.1%)	1.54	(0.81-2.91)
2	Day 0	16	3 (18.7%)	2.00	(0.90-4.43)
	Day 28	16	5 (31.2%)	3.41	(1.23-9.50)
	Day 42	16	8 (50.0%)	6.44	(2.24-18.46)
	Day 126	16	4 (25.0%)	3.01	(1.17-7.72)
3	Day 0	16	3 (18.7%)	1.75	(0.90-3.41)
	Day 28	16	9 (56.2%)	9.76	(3.39-28.12)
	Day 42	15	12 (80.0%)	29.30	(10.49-81.80)
	Day 126	16	6 (37.5%)	4.87	(1.54-15.41)
4	Day 0	16	1 (6.2%)	1.52	(0.80-2.92)
	Day 28	16	8 (50.0%)	6.43	(2.17-19.03)
	Day 42	15	13 (86.7%)	30.30	(12.28-74.75)
	Day 126	16	6 (37.5%)	3.57	(1.42-8.97)
5	Day 0	18	3 (16.7%)	1.80	(0.92-3.51)
	Day 28	18	16 (88.9%)	41.29	(19.63-86.86)
	Day 42	18	16 (88.9%)	34.03	(16.38-70.68)
	Day 126	18	13 (72.2%)	17.06	(6.60-44.07)

N = number of subjects with samples for neutralisation assay
n/% = number of subjects with neutralisation assay with titres above 1:10
Group 1: 106 TCID50, subcutaneous in subjects not vaccinated against smallpox
Group 2: 107 TCID50, subcutaneous in subjects not vaccinated against smallpox
Group 3: 108 TCID50, subcutaneous in subjects not vaccinated against smallpox
Group 4: 108 TCID50, intramuscular in subjects not vaccinated against smallpox
Group 5: 108 TCID50, subcutaneous in subjects previously vaccinated against smallpox

The ELISPOT data were generated using lysed MVA-infected CEF cells as the stimulating antigen and fixed numbers of CD4+ and CD8+ cells. The pattern of results (see below) was not entirely consistent with that for humoral immunity, in particular with regard to Group 1 in which one subject had unexpected results over time. The study report comments on high inter-subject variability and variable sample availability at different time points. Nevertheless, means demonstrated the effect of two vs. a single dose and Group 5 showed a robust response to a single dose.

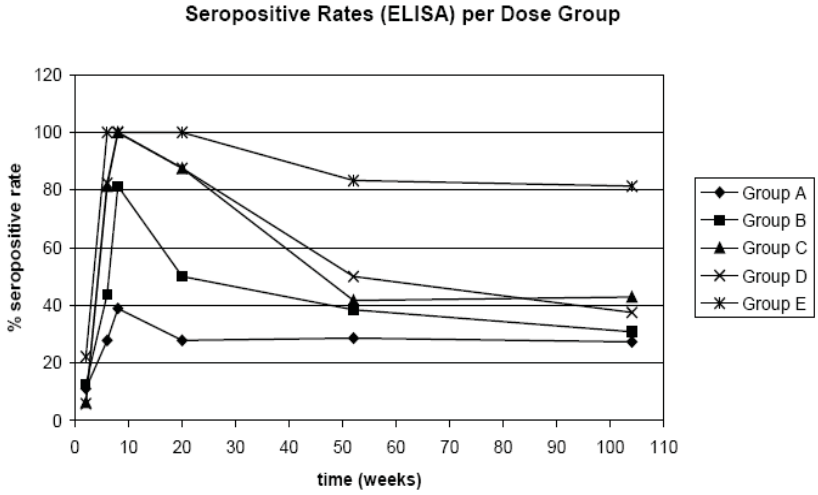
Rates for T-cell-responses above 50 SFU/106 cells were higher after the second dose in Groups 1-4 and in the highest dose group the rates were comparable between routes of administration.

Table 4 Summary statistics for T-Cell response (ELISPOT) for subjects of the completers sample (N=84)

Group	Visit	N	Arithmetic Mean / SD	(Range)
1	Day 0	9	54.9 / 67.15	(0.00 - 210.00)
	Day 28	15	52.0 / 46.51	(0.00 - 153.33)
	Day 42	17	108.8 / 150.9	(0.00 - 493.00)
	Day 126	14	106.4 / 173.71	(0.00 - 673.00)
2	Day 0	8	21.7 / 17.64	(0.00 - 53.33)
	Day 28	14	26.9 / 29.25	(0.00 - 100.00)
	Day 42	16	52.5 / 72.76	(0.00 - 286.67)
	Day 126	12	34.4 / 58.71	(0.00 - 200.00)
3	Day 0	8	47.9 / 38.34	(6.67 - 116.67)
	Day 28	13	74.8 / 114.36	(0.00 - 413.00)
	Day 42	14	101.9 / 85.17	(10.00 - 257.00)
	Day 126	13	55.2 / 39.60	(0.00 - 137.00)
4	Day 0	9	41.6 / 34.65	(0.00 - 120.00)
	Day 28	14	48.0 / 79.52	(0.00 - 250.00)
	Day 42	14	152.2 / 183.79	(0.00 - 667.00)
	Day 126	11	79.1 / 71.43	(0.00 - 257.00)
5	Day 0	11	47.3 / 29.73	(3.33 - 100.00)
	Day 28	16	109.2 / 108.06	(3.33 - 336.67)
	Day 42	17	87.4 / 124.09	(0.00 - 533.33)
	Day 126	14	70.6 / 65.23	(0.00 - 176.67)

Group 1: 106 TCID50, subcutaneous in subjects not vaccinated against smallpox
Group 2: 107 TCID50, subcutaneous in subjects not vaccinated against smallpox
Group 3: 108 TCID50, subcutaneous in subjects not vaccinated against smallpox
Group 4: 108 TCID50, intramuscular in subjects not vaccinated against smallpox
Group 5: 108 TCID50, subcutaneous in subjects previously vaccinated against smallpox
N = number of subjects with samples for T-cell immune response

The follow-up portion of this study reported data from 10-18 subjects per group at Year 1 and 7-16 per group at Year 2. The ELISA data showed decreasing seropositivity rates over time except for those who received the lowest dose (Group 1), who seemed to have comparable seropositivity rates at Week 20 (33.3%) and at the follow-up visits (28.6% and 27.3%). The seropositive rate for Group 5 subjects decreased slightly from Week 20 (100%) to 81.3% at week 104.



Taking into account the safety profiles of the various doses the applicant concluded that both routes of administration and the middle and highest doses tested merited further evaluation.

POX-MVA-004

The primary objective of this monocentric study was to identify a dose of MVA-BN considered to have an optimal immunogenicity and reactogenicity profile by evaluating three doses in a double-blind, randomised and parallel group study. Subjects were healthy males and females aged 18-30 years with no prior vaccination against smallpox. The planned sample size was 165 (55 subjects per Group) to achieve 150 subjects in the fully evaluable datasets for the immunogenicity evaluations.

Follow-up was initially planned to week 12. The follow-up was extended to year 2 and a supplementary study report provides limited data on seropositivity rates over time.

Subjects received two injections of MVA-BN with a 4-week interval at one of three doses as follows:
Group 1: dose 2 x 10^7 $TCID_{50}$, subcutaneous
Group 2: dose 5 x 10^7 $TCID_{50}$, subcutaneous
Group 3: dose 1 x 10^8 $TCID_{50}$, subcutaneous
A single lot of early process MVA-BN was supplied in freeze-dried form to be reconstituted in sterile water and then diluted in specific vaccine diluent (Tris/saline, pH 7.4) so that the required TCID50 dose could be administered in 0.5 ml volumes.

In this study the cut-off for ELISA seropositivity was at 1:50. The results for subjects seronegative at day 0 (only one subject in the ITT population was seropositive at D0) showed increments in seropositivity rates and GMTs with the second dose in all three groups. The GMTs on each of D28, D42 and D84 were highest in the 1x10^8 $TCID_{50}$ group.

Table 5 PP Population: ELISA specific total IgG antibody titer to MVA: Seroconversion rates, by Visit - Frequency table

		Seroconversion				TOTAL (N=155)	
		seronegative		seropositive			
		n	%	n	%	n	%
Group 1 (N=54)	Day 0	54	100.0	0	0	54	100.0
	Day 28	22	40.7	32	59.3	54	100.0
	Day 42	0-	0	54	100.0	54	100.0
	Day 84	3	5.6	51	94.4	54	100.0
Group 2 (N=49)	Day 0	49	100.0	0	0	49	100.0
	Day 28	9	18.4	40	81.6	49	100.0
	Day 42	0-	0	49	100.0	49	100.0
	Day 84	0-	0	49	100.0	49	100.0
Group 3 (N=52)	Day 0	52	100.0	0-	0	52	100.0
	Day 28	3	5.8	49	94.2	52	100.0
	Day 42	0-	0	52	100.0	52	100.0
	Day 84	0-	0	52	100.0	52	100.0

Group 1: 2x10^7 TCID50 / Group 2: 5x10^7 TCID50 / Group 3: 1x10^8 TCID50; seropositive: a titer above or at the assay cut-off value of 1:50; seronegative: a titer below the assay cut-off value of 1:50.
N = total Number of subjects

Table 6 PP Population: ELISA specific total IgG antibody titer to MVA: Descriptive Statistics including GMT and 95% Confidence Interval, by Visit

		n	Arithm. Mean	CV	Lower 95% CL (Arithm. Mean)	Upper 95% CL (Arithm. Mean)	Geome-tric mean titer (GMT)	Lower 95% CL (Geom. Mean)	Upper 95% CL (Geom. Mean
Group 1 (N=54)	Day 0	54	1.00	0.00			1.00		-
	Day 28	54	66.39	127.46	43.29	89.49	14.37	7.71	26.80
	Day 42	54	628.20	143.88	381.49	874.92	377.22	288.33	493.53
	Day 84	54	256.31	181.65	129.23	383.40	134.33	91.05	198.19
Group 2 (N=49)	Day 0	49	1.00	0.00	-	-	1.00		-
	Day 28	49	146.76	142.61	86.64	206.87	53.21	29.85	94.87
	Day 42	49	860.51	117.24	570.73	1150.29	583.62	461.58	737.94
	Day 84	49	345.90	116.55	230.10	461.70	227.76	176.40	294.07
Group 3 (N=52)	Day 0	52	1.00	0.00	-	-	1.00		-
	Day 28	52	162.33	90.27	121.53	203.12	98.52	67.57	143.65
	Day 42	52	1286.46	120.85	853.65	1719.28	813.77	628.74	1053.26
	Day 84	52	534.35	132.96	336.55	732.14	323.63	246.84	424.30

Group 1: 2 x10^7 TCID50 / Group 2: 5x10^7 TCID50 / Group 3: 1x10^8 TCID50;
CL: Confidence Limit: CV: Coefficient of Variation
N = Total Number of Subjects

All subjects with PRNT data were seronegative pre-vaccination. In the PP population the maximum seropositivity rate and the highest GMT after two doses occurred in the highest $TCID_{50}$ group. The rates and GMTs showed rapid waning from D42 to D84 (from weeks 6-12 on study).

Table 7 PP Population: PRNT specific antibody titer to MVA: Seroconversion rates, by Visit - Frequency table

		Seroconversion				TOTAL (N=155)	
		seronegative		seropositive			
		n	%	n	%	n	%
Group 1 (N=54)	Day 0	54	100.0	0	0	54	100.0
	Day 28	50	92.6	4	7.4	54	100.0
	Day 42	31	57.4	23	42.6	54	100.0
	Day 84	44	81.5	10	18.5	54	100.0
Group 2 (N=49)	Day 0	49	100.0	0	0	49	100.0
	Day 28	43	87.8	6	12.2	49	100.0
	Day 42	20	40.8	29	59.2	49	100.0
	Day 84	38	77.6	11	22.4	49	100.0
Group 3 (N=52)	Day 0	52	100.0	0	0	52	100.0
	Day 28	47	90.4	5	9.6	52	100.0
	Day 42	15	28.8	37	71.2	52	100.0
	Day 84	37	71.2	15	28.8	52	100.0

Group 1: 2x107 TCID50 / Group 2: 5x107 TCID50 / Group 3: 1x108 TCID50 seropositive: a titer above the assay cut–off value of 1:20; seronegative: a titer below the assay cut–off value of 1:20.
N = Number of subjects

Table 8 PP Population: PRNT specific antibody titer to MVA: Descriptive Statistics including GMT and 95% Confidence Interval, by Visit

		n	Arith. Mean	CV	Lower 95% CL (Arithm. Mean)	Upper 95% CL (Arithm. Mean)	Geometric mean titer (GMT)	Lower 95% CL (Geom. Mean)	Upper 95% CL (Geom. Mean)
Group 1 (N=54)	Day 0	54	1.00	0.00	-	-	1.00	-	-
	Day 28	54	3.83	276.53	0.94	6.73	1.31	1.01	1.70
	Day 42	54	27.81	151.42	16.32	39.31	5.51	3.17	9.59
	Day 84	54	7.89	199.47	3.59	12.18	1.94	1.32	2.85
Group 2 (N=49)	Day 0	49	1.00	0.00	-	-	1.00	-	-
	Day 28	49	5.92	260.75	1.49	10.35	1.55	1.10	2.19
	Day 42	49	38.51	132.92	23.81	53.21	10.31	5.78	18.40
	Day 84	49	11.35	196.98	4.93	17.77	2.32	1.47	3.66
Group 3 (N=52)	Day 0	52	1.00	0.00	-	-	1.00	-	-
	Day 28	52	3.90	233.47	1.37	6.44	1.39	1.05	1.85
	Day 42	52	68.29	155.46	38.73	97.84	19.43	11.05	34.16
	Day 84	52	15.33	213.10	6.23	24.42	2.94	1.81	4.76

Group 1: 2x107 TCID50 / Group 2: 5x107 TCID50 / Group 3: 1x108 TCID50;
CL: Confidence Limit, CV: Coefficients of Variation
N = Total Number of Subjects

The CTL response was assessed in subsets by measuring IFN-producing cells by intracellular cytokine staining (ICS). It is not clear from the study reports how the subjects/samples were selected for determination of CTL responses. Numbers with data are small. For CD4+ cells the peak positive response rates were <20% and there was no appreciable difference between dose groups. For CD8+ cells there was a trend for rates of positive responses to increase from the lowest dose but with no appreciable difference between the two higher dose groups at D42 while the D84 rates showed a dose-related trend.

Table 9 PP Population: MVA-specific gamma interferon production measured by ICS (CD4+ T cells): Response rates by Visit-Frequency table

		Response rate						TOTAL (N=155)	
		response positive		response negative		NA			
		n	%	n	%	n	%	n	%
Group 1 (N=54)	Visit 2 (Day 0)	4	7.4	49	90.7	1	1.9	54	100.0
	Visit 3 (Day 28)	5	9.3	49	90.7	0	0	54	100.0
	Visit 4 (Day 42)	10	18.5	44	81.5	0	0	54	100.0
	Visit 5 (Day 84)	5	9.3	49	90.7	0	0	54	100.0
Group 2 (N=49)	Visit 2 (Day 0)	4	8.2	45	91.8	0	0	49	100.0
	Visit 3 (Day 28)	8	16.3	41	83.7	0	0	49	100.0
	Visit 4 (Day 42)	8	16.3	41	83.7	0	0	49	100.0
	Visit 5 (Day 84)	8	16.3	40	81.6	1	2.0	49	100.0
Group 3 (N=52)	Visit 2 (Day 0)	1	1.9	51	98.1	0	0	52	100.0
	Visit 3 (Day 28)	4	7.7	48	92.3	0	0	52	100.0
	Visit 4 (Day 42)	8	15.4	44	84.6	0	0	52	100.0
	Visit 5 (Day 84)	5	9.6	47	90.4	0	0	52	100.0

Group 1: 2x107 TCID50 / Group 2: 5x107 TCID50 / Group 3: 1x108 TCID50
Response rate positive: a titer above or at the assay cut-off value of 0.14%
Response rate negative: a titer below the assay cut-off value of 0.14%
N = Number of Subjects

Table 10 PP Population: MVA-specific gamma interferon production measured by ICS (CD8+ T cells): response rates, by Visit-Frequency table

		Response rate						TOTAL (N=155)	
		response positive		response negative		NA			
		n	%	n	%	n	%	n	%
Group 1 (N=54)	Visit 2 (Day 0)	2	3.7	51	94.4	1	1.9	54	100.0
	Visit 3 (Day 28)	10	18.5	44	81.5	0	0	54	100.0
	Visit 4 (Day 42)	25	46.3	29	53.7	0	0	54	100.0
	Visit 5 (Day 84)	16	29.6	38	70.4	0	0	54	100.0
Group 2 (N=49)	Visit 2 (Day 0)	5	10.2	44	89.8	0	0	49	100.0
	Visit 3 (Day 28)	19	38.8	30	61.2	0	0	49	100.0
	Visit 4 (Day 42)	25	51.0	24	49.0	0	0	49	100.0
	Visit 5 (Day 84)	22	44.9	26	53.1	1	2.0	49	100.0
Group 3 (N=52)	Visit 2 (Day 0)	3	5.8	49	94.2	0	0	52	100.0
	Visit 3 (Day 28)	14	26.9	38	73.1	0	0	52	100.0
	Visit 4 (Day 42)	27	51.9	25	48.1	0	0	52	100.0
	Visit 5 (Day 84)	28	53.8	24	46.2	0	0	52	100.0

Group 1: 2x107 TCID50 / Group 2: 5x107 TCID50 / Group 3: 1x108 TCID50
Response rate positive: a titer above or at the assay cut-off value of 0.25%
Response rate negative: a titer below the assay cut-off value of 0.25%
N = Number of Subjects

The applicant concluded that the immune response to MVA-BN is highly dose dependent. Results after the first vaccination and at day 84 indicated that the immune response elicited with the low doses was inferior to the highest dose administered.

In the follow-up phase of this study all participants in the initial phase were invited to provide a blood sample for assay at 2 years (± 10 weeks) after the last assigned vaccine dose. Overall 74 subjects provided a sample for ELISA and PRNT of which 50 submitted a second sample for determination of CMI. Numbers per dose group who were followed up were inevitably small, which limits the interpretation of the data.

The ELISA seropositivity rates (titre at least 1:50) and GMTs at year 2 did not show a consistent trend according to the virus titre in the test vaccine received although the lowest values did occur in the lowest titre group.

Similarly, there was no consistent trend for PRNT titres according to the initial virus titre received.

If anything, only the CMI data at Year 2 suggested an advantage for the lowest virus titre administered initially.

2.5.2. Main studies

Study POX-MVA 005 – Groups 1-3 enrolled vaccinia-naïve subjects who received one or two doses of MVA-BN and/or placebo in a double-blind fashion. Group 4 subjects were vaccinia-experienced and received a single dose of MVA-BN in an open-label fashion.

Group		1. Vaccination	2. Vaccination	Abbrev. used in tables/listings
1	Subjects without history of smallpox vaccination	MVA-BN®	MVA-BN®	MM
2		MVA-BN®	Placebo	MP
3		Placebo	Placebo	PP
4	Subjects with history of smallpox vaccination	MVA-BN®	-	M-

Study POX-MVA 023 – The first 75 eligible subjects from each of the original Groups 1 and 2 were to receive a single dose of MVA-BN approximately two years (-2 to +3 months) after their last dose. Other subjects from Groups 1, 2 and 4 were not vaccinated but all available subjects attended a single study visit when a sample was taken to assess immune persistence.

Studies POX-MVA 008 and 011 – All subjects (healthy, AD and HIV-infected) received two doses of MVA-BN. In study 008 all subjects were vaccinia-naïve. In study 011 both the vaccinia-naïve and experienced subjects received two doses of MVA-BN regardless of their HIV status.

Study 024 - Group 1 (n=60) received two doses of MVA-BN. Group 2 (n=60) received placebo first followed by a single dose of MVA-BN. All subjects were vaccinia-experienced.

Assessment of the immune response

Humoral immune response

The humoral response was assessed by ELISA and by determination of functional (neutralising) antibody titres using a plaque reduction neutralisation test (PRNT). Briefly:

The human ELISA was initiated as a manual test and was used at a research level and for the testing of samples from POX-MVA-001. To allow higher throughput testing, the assay was transitioned to automated status, following which there were a number of reagent lot switches and some optimisation steps were implemented. Six validations have been performed on the human ELISA since 28 August 2002, since when the applicant stated that the assay was used under a validated status and performance characteristics have remained consistent since 2002. Antibody titres are calculated by linear regression and defined as the serum dilution resulting in OD 0.30 (end point titres). End point titres of the samples are determined by generating a logarithmic plot. Samples with a titre ≥8000 are repeated with a higher starting dilution. In the final assay, a titre of 50 (corresponding to a dilution of 1:50) is the lowest detectable antibody level (ACV) and when this titre is reached, the subject is considered seropositive. Titres below the cut-off value of the assay are assigned a value of 1 for the purpose of calculations and subjects are considered seronegative.

The human PRNT was initiated and validated using IHD-J virus. The PRNT was then subcontracted to Focus Diagnostics (Cypress, California USA) to allow for centralised testing of BN and NIH samples. At this time the assay virus inoculum was transitioned to the VV Western Reserve (VV-WR) strain. Because Focus Diagnostics had limited laboratory capacity the PRNT testing was later returned to BN. During this transition BN made efforts to establish an assay capable of reproducing the PRNT results generated at Focus Diagnostics by validation and cross validation against the Focus Diagnostics results for the POX-MVA-007 study. The applicant reported that this exercise confirmed the comparability of titres obtained from the two assays. Following the return of PRNT testing to BN a number of reagent lot switches and optimisation steps were implemented. Four validations have been performed on the human PRNT since 28 July 2003. The applicant reports that performance characteristics have remained consistent since 2003.

The BN PRNT assay was used to test for vaccinia responses in the BN and NIH studies except for POX-MVA-004 FU phase, -007 and -010 and HIV-NEF-004, in which the testing was performed at Focus Diagnostics using a non-validated assay. The antibody titre is calculated based on the plaque counts generated from the qualified Neural Network plaque counting package. The qualified Excel macro is then used to generate titres, where the number of plaques is fitted as a linear function of the log10 of the dilution. This linear curve is then used to determine PRNT titres as the point where the virus was neutralised by 50% compared to the 100% virus control (the 100% virus control is based on the average number of plaques in 40 wells of a 48-well plate). A PRNT value ≥ 15 is considered seropositive.

Antigens and viruses used in the assays

During the procedure the applicant addressed the ability of the ELISA to bind to, and the PRNT to neutralise, the extracellular (EV) form of vaccinia virus as well as the mature virion (MV) form.

The applicant also justified routine use of the VV-WR strain in the validated PRNT.

The applicant stated that protective antibody responses have been shown to be directed against the viral A33 and B5 glycoproteins of the EV and against at least six viral membrane proteins of the MV form. MVA and VV express the same MV and EV antigens in infected cells. Microarray analysis of rabbit, non-human primates (NHP) and human sera showed that MVA-BN and Dryvax induced comparable immune responses to viral antigens; in particular, to membrane and core antigens *(Davies et al., 2008*) that are known to be involved in protection *(Moss, 2007*). The applicant provided data from EV protein specific ELISA using recombinant variola virus (VARV) proteins as antigens and comet reduction induced by sera from MVA-BN vaccinated subjects. These data showed that MVA-BN generates substantial antibody responses to EV proteins.

Sera from MVA-immunized mice and NHP were reported to show EV-neutralizing activity in the comet reduction assay as well as in an EV-specific PRNT (Earl, 2007; Wyatt, 2004). The applicant provided data from a comet reduction assay for demonstration of EV-specific neutralizing activity in sera of MVA-BN vaccinated subjects.

The VV-WR strain is a derivative of the NYCBH strain. Phylogenetic analysis revealed that MVA and Dryvax, including its derivatives, were more closely related to each other than to VV-WR (Meisinger-Henschel, 2007) but the protein sequences of MVA are 97 to 99% identical to those of other VV strains (Davies, 2008). The seroreactive antigens of VV-WR have been identified. With the exception of one antigen, the 30 genes that code for an orthologous protein showed high (≥ 93) percentages in protein identity when VV-WR was compared to Dryvax, VV-WR to MVA and MVA to Dryvax.

Comparisons between ELISA and PRNT titres

Within the majority of study reports there were analyses reported on the correlation between the ELISA and PRNT titres.

During the procedure the applicant reported on two additional NHP studies that were claimed to support the applicant's position that antibody titres reported from each of PRNT and ELISA are correlates of protection against lethal poxvirus challenge (one study used IV and one used inhalation routes of infection). These studies used MVA-BN at a range of $TCID_{50}$ in order to be able to have sufficient cases to be able to examine the correlation between $TCID_{50}$ in the vaccine and protection as well as between antibody titres and protection. A replication-competent vaccine control group was not employed.

Cell-mediated immune response (CMI)

Evidence points to a conclusion that humoral immune responses constitute only one component of immunity conferring protection against smallpox and that CMI responses are also important. In the two clinical dose-finding studies the evaluation of CMI was limited. In the pivotal clinical studies an interferon-gamma ELISPOT was used to provide a quantitative determination of IFN-γ producing cells in cryopreserved PBMCs after stimulation with live MVA-BN.

A vaccinia-specific signal was defined by a frequency of at least 50 Spot Forming Units (SFUs) per 1 x 10^6 PBMC after correction for background (subtraction of SFU/1 x 10^6 non-stimulated

cells). In addition, any value which was not at least twice the background level was set to zero. The ELISPOT number of SFUs was reported as mean, median, maximum and minimum. A response at any post-Baseline visit was defined as either a signal in subjects that had no signal at Baseline or a relative increase by a factor of at least 1.7 over the Baseline value in subjects that had a signal at Baseline. A subject was considered a responder if responses were available for at least two post-Baseline visits. The number of SFUs/1 x 10^6 PBMC after correction for background was reported.

Study Participants

The table below summarises the features of the study populations.

Study Identification	Study Populations: Health Status Vaccinia Status	Gender	Age
Pivotal Studies			
POX-MVA-005	Healthy vaccinia-naïve Healthy vaccinia-experienced	Male / Female	18 to 55
POX-MVA-023	Healthy vaccinia-experienced Healthy IMVANEX®-experienced	Male / Female	20 to 57
POX-MVA-008	Healthy vaccinia-naïve without AD Vaccinia-naïve with AD	Male / Female	18 to 40
POX-MVA-011	Healthy vaccinia-naïve and vaccinia-experienced HIV-1 infected vaccinia-naïve with CD4 ≥ 200 to 750 cells/µL HIV-1 infected vaccinia-experienced with CD4 ≥ 200 to 750 cells/µL	Male / Female	18 to 55
HIV-POL-002	HIV-1 infected vaccinia-naïve with CD4 ≥ 250 cells/µL HIV-1 infected vaccinia-experienced with CD4 ≥ 250 cells/µL	Male / Female	18 to 50
POX-MVA-024	Healthy vaccinia-experienced	Male / Female	56 to 80
Supportive Studies			
POX-MVA-001	Healthy vaccinia-naïve Healthy vaccinia-experienced	Male	20 to 55
POX-MVA-002	Healthy vaccinia-naïve	Male / Female	18 to 32
POX-MVA-004	Healthy vaccinia-naïve	Male / Female	18 to 30
POX-MVA-007	Healthy vaccinia-naïve without AD Vaccinia-naïve with a history of AD Vaccinia-naïve with active AD Vaccinia-naïve with AR	Male / Female	18 to 40
POX-MVA-010	HIV-1 infected vaccinia-naïve with CD4 > 350 cells/µL HIV-1 infected vaccinia-experienced with CD4 > 350 cells/µL Healthy vaccinia-naïve Healthy vaccinia-experienced	Male / Female	18 to 49 (Male) 18 to 55 (Female)*
POX-MVA-009	Healthy, vaccinia-naïve	Male / Female	18 to 35
HIV-NEF-004	HIV-1 infected vaccinia-naïve with CD4 > 250 cells/µL HIV-1 infected vaccinia-experienced with CD4 > 250 cells/µL	Male / Female	18 to 60

* In POX-MVA-010, different age ranges for male and female subjects were introduced to cover for the higher cardiac risk in male subjects.

Some common features to the patient selection criteria were as follows:

Main inclusion criteria

- Male and female (non-pregnant and with adequate contraception from 30 days pre- to 28 days post-vaccination) subjects aged 18 - 57 years of age (005/023, 011), 18 - 40 years (008) or 56-80 years (024).
- Laboratory tests for healthy subject groups required: troponin I WNL or defined as < 2-fold ULN; WBC ≥ 2500/mm3 and ≤ 11,000/ mm3; ANC ≥ 1000/mm3 or WNL; negative urine glucose; Hb ≥ LLN; platelets 100 – 450/nL or WNL; serum creatinine WNL or CrCl > 60 ml/min; urine protein ≤ 30 mg/dL; total bilirubin ≤ 1.5 x ULN; normal AST/ALT/ALP; ECG without abnormal findings.

Study POX-MVA 008 enrolled only vaccinia-naïve subjects and 024 enrolled only subjects with history of receipt of a replication-competent smallpox vaccine [VV]. In other studies the requirements regarding prior or no prior receipt of VV were defined by study group. Prior VV was to be documented and/or there was to be a typical vaccinia scar. A minimum time elapsed since last VV was not specified in study 011. In 005 it was specified that prior VV was to have been given at least 5 years ago and in 024 at least 10 years ago.

Other study-specific inclusion criteria by study and/or group were applicable as follows:

Study POX-MVA 008 – Vaccinia-naïve subjects with diagnosed atopic dermatitis (AD) were to have a history of or currently active AD (defined as SCORAD] ≤ 30 (Kunz et al., 1997).

The study report states that currently active AD included relapsing forms of AD, also when subjects were enrolled during inflammation-free intervals.

Calcineurin inhibitor use was restricted to 10 g/week of tacrolimus (0.1% ointment) or pimecrolimus (1% cream) on up to 25% of the face.

Low dose topical cortisone was not to exceed 30 g/week of an intermediate potency (class IV) topical steroid (or equivalent) applied to ≤ 10% of total body surface.

Study POX-MVA 011 – Subjects were enrolled into groups according to the following additional eligibility criteria:

Group 1: healthy subjects with and without previous VV; after amendment #3 only vaccinia-naïve subjects were eligible.

HIV-infected subjects:

Group 2a: CD4 350 – 500 cells/µl with and without a history of VV

Group 2b: CD4 200 – 750 cells/µl without a history of VV

Group 3: CD4 200 – 750 cells/µl with a history of VV

In Groups 2 (a, b) and 3:

- HIV-1 infection was to be documented by ELISA and confirmed by Western blot. Also acceptable were positive PCR for HIV-1 DNA, HIV-1 culture, HIV-1 antigen, plasma HIV-1 RNA or a second antibody test other than ELISA.
- Subjects were to have been on stable antiretroviral therapy (ARV) for > 6 months with a sustained response at < 400 copies/ml or not on ARV for at least 8 weeks prior to entry provided that they had ≤ 50,000 copies/ml and CD4 ≥ 350 cells/µl or ≤ 10,000 copies/ml and CD4 < 350 cells/µl.
- HIV-infected subjects also had to have: Hb ≥ 9.0 g/dl; platelets ≥ 100,000/mm3; normal serum glucose; CrCl > 60 ml/min; ≤ +1 proteinuria; total bilirubin ≤ 2 x ULN (unless due to ARV); AST, ALT and ALP all ≤ 2.5 x ULN.

Study POX-MVA 023 – Subject from Groups 1, 2 or 4 who had completed study 005 without major protocol violations were eligible for the booster phase study.

Study POX-MVA 024 – Subjects were aged 56-80 years. The first 30 were to be aged 56-70 years. Up to 25% of the total study population was projected to be aged 71-70 years.

Main exclusion criteria

- Pregnant or breast-feeding women, uncontrolled serious infection, history of any serious medical condition, history of or active autoimmune disease (except vitiligo and those on thyroid replacement), known or suspected impairment of immunologic function and history of malignancy (other than local skin).
- Specifically regarding cardiac risk, subjects were excluded if they had a history of coronary heart disease, myocardial infarction, angina, congestive heart failure, cardiomyopathy, stroke or transient ischemic attack, uncontrolled high blood pressure or any other heart condition under the care of a doctor. Also, if they had an immediate family member who died due to ischaemic heart disease before age 50 years of if they had a 10% or greater risk of developing a myocardial infarction or coronary death within 10 years.
- Other exclusions were history of IVDA, known allergy to egg or aminoglycoside (gentamicin) and any history of anaphylaxis or severe allergic reaction.
- In some studies there was a specific exclusion regarding an ongoing acute infection with or without documented fever and ongoing tuberculosis.
- Any other live vaccine was precluded within 30 days prior or after study vaccination and any killed vaccine within 14 days prior or after study vaccination. Use of immunoglobulin or blood products within 3 months prior to vaccination or expected at any time during study participation.

Objectives

Study POX-MVA 005 - To compare the ELISA response between the group with history of smallpox vaccination (Group 4: 1 dose MVA-BN) vs. the group without history of smallpox vaccination (Group 1: 2 doses MVA-BN) in order to demonstrate that a single dose is sufficient as a booster in a previously vaccinated population.

Study POX-MVA 023 - To evaluate the immune response to a booster dose of MVA-BN two years after completing vaccination with either one or two doses of MVA-BN in study 005. An important secondary objective was to compare the immune responses between subjects according to their initial assignment in study 005 (i.e. 1 or 2 doses of MVA-BN in the previously vaccinia-naïve Groups 1 and 2).

Study POX-MVA 008 - To assess the ELISA response to MVA-BN in subjects with atopic dermatitis (AD) compared to healthy subjects.

Study POX-MVA 011 - To investigate the safety of MVA-BN in HIV infected subjects with CD4 counts ≥ 200–750 cells/µl compared to healthy subjects.

Study POX-MVA 024 - To expand the database on safety in a vaccinia-experienced population 56 to 80 years of age after administration of either one or two doses of MVA-BN.

Outcomes/endpoints

Studies POX-MVA 005, 008 and 011

The primary immunogenicity variable was the vaccinia-specific ELISA seroconversion rate.

Seroconversion based on ELISA was defined as either:

- Appearance of a titre ≥ 50 for initially seronegative subjects or
- ≥ 2-fold increase in titre vs. baseline for initially seropositive subjects

The PRNT response was the secondary immunogenicity variable. Seroconversion was defined as:

- Appearance of a titre ≥ 15 (008) or > 6 (005 and 011) in initially seronegative subjects or
- ≥ 2-fold increase in titre vs. baseline for initially seropositive subjects

Depending on the study and in some cases the study group, immune responses were assessed at D0 (first dose of assigned treatment) and then at weeks 1 (some groups/studies), 2 and 4 (i.e. at 2 and 4 weeks after the first dose), 6 and 8 (i.e. at 2 and 4 weeks after the second dose, if a second dose was administered). The last visit was at ~6 months after the second assigned dose in studies 005, 008 and 011. In study 011 the footnotes to the tables state that immune responses were assessed only for subjects enrolled into the main phase of the study and for the HIV-infected vaccinia-experienced subjects. It seems this refers to 55 healthy subjects and 110 HIV-infected subjects.

Study POX-MVA 023 - The peak booster rate was the primary immunogenicity endpoint. This was defined as the individual peak response at either Visit 2, 3 or 4 (i.e. weeks 1, 2 or 4 post-dose) given as percentage of subjects with either an appearance of antibody titers ≥ 50 in a vaccinia-specific ELISA (for initially seronegative subjects) or an increase of the antibody titer compared to the baseline titer (for subjects with a pre-existing antibodies).

Study POX-MVA 024 - The primary endpoint was the rate of SAEs. The secondary immunogenicity endpoints are described as ELISA and PRNT seroconversion rates (defined using the same criteria applied in 008 as mentioned above) and GMTs. A “response” to the vaccine using a particular assay was defined as either the appearance of titres ≥ assay cut-off value for

initially seronegative subjects or an increase in titre compared to baseline for subjects with a pre-existing measurable titre.

Sample size

Study POX-MVA 005 - The study aimed to demonstrate that the Group 4 seroconversion rate was not worse than the Group 1 seroconversion rate using a pre-specified non-inferiority margin of 5%. It was anticipated that the seroconversion rate after two doses would be 98-100%. Assuming a significance level of 5%, a power of 80% and expected seroconversion rates of 98% in both groups, the required sample size was 175 subjects per group (700 subjects in total).

Study POX-MVA 023 – The first 75 eligible subjects from each of the original Groups 1 and 2 in study 005 were enrolled to receive a single dose of MVA-BN.

Study POX-MVA 008 – The sample size calculation for the main study was based on a primary hypothesis that the humoral immune response of the AD group was not statistically inferior vs. healthy subjects at Week 6 (Visit 4) using a pre-defined non-inferiority margin of 5%. Assuming a significance level of 5%, a power of > 80%, and an expected seroconversion rate of 99% in both groups (from the pilot study POX-MVA-007) the required sample size was 124 subjects per group and 130 were planned to account for drop outs. In the final protocol 560 individuals were to be enrolled. The number included 260 in the main phase [130 healthy and 130 AD subjects] plus 100 healthy and 200 AD subjects in the extension phase.

It was projected that 300 subjects with diagnosed AD (enrolment was tracked to ensure that at least 50% had active AD rather than only history) would allow the detection of one unexpected AE in 100 subjects with 95% confidence in the absence of a background incidence. The number was increased to 330 to account for dropouts. Due to the exchange of MVA-BN lots the number of healthy subjects was increased to 230 so that at least 130 were vaccinated with the new lot.

Study POX-MVA 011 – Target enrolment in the main study was 165 subjects - 55 healthy subjects in Group 1 and 110 HIV-infected subjects in Groups 2a/b. Target enrolment for the extension was 385 subjects - 35 added in Group 1, 250 added in Groups 2a/b plus enrolment of 100 into Group 3.

Group 1 90 provided a probability of 95% to detect SAEs with an incidence of at least 3.3%.

Group 2a/b 360 provided a probability of 95% to detect SAEs with an incidence of at least 0.9%.

Group 3 100 provided a probability of 95% to detect SAEs with an incidence of at least 2.7%.

Study POX-MVA 024 - The sample size calculation was based on the primary endpoint to evaluate the incidence of SAEs. A sample size of 60 subjects was to provide a probability of 95% to detect serious ADRs with an incidence of at least 5%.

Randomisation

Study POX-MVA 005 – The randomisation list was provided by the CRO and was included in the eCRF. By entering the subject number in the eCRF, the investigator received the randomisation number from the eCRF system.

Study POX-MVA 023 – There was no randomisation step.

Study 008 - There was stratification of Group 2 subjects (AD) according to active AD vs. history of AD with an aim to include at least 50% with active disease.

Study POX-MVA 011 – There was no randomisation step. Groups were enrolled according to baseline characteristics until the pre-defined numbers were achieved.

Study POX-MVA 024 – The method of randomisation was as for study 005. There was stratification of enrolment according to age groups 56 – 70 years and 71 – 80 years.

Blinding (masking)

Studies 005 and 024 involved double-blind designs for most or all groups.

Studies 008, 011 and 023 were conducted in an open label fashion.

Statistical methods

Studies pre-specified the Full Analysis (FA) Set (FAS = all treated subjects) and the Per Protocol (PP) population (received all the assigned doses and had no major protocol violations). SAPs were provided.

Study POX-MVA 005 - The primary hypothesis was tested based on an exact, unconditional test for binomial differences. In addition an exact one-sided 97.5% unconditional confidence interval for the difference of proportions was calculated. If the lower limit of this confidence interval was greater than 5% then the null hypothesis was rejected. In order to limit the overall type-I error to a nominal level of 5%, a hierarchical test procedure was chosen. The primary null hypothesis was tested on the measurements of samples collected two weeks after the last vaccination and, if this showed a significant result, the comparison of data at four weeks after vaccination was performed. The same approach was taken for analyses of the PRNT titres.

Study POX-MVA 008 – For the test of non-inferiority if the lower limit of the exact one-sided 97.5% unconditional CI for the difference in ELISA seroconversion rates between Group 2 and Group 1 at week 6 (visit 4) was greater than -5% the primary hypothesis of non-inferiority of the AD group with respect to the healthy group was confirmed. This inference was repeated for all post vaccination time points for the PPS and the subset of initially seronegative subjects (all subjects in the FAS with ELISA titres < 50 at baseline).

Studies POX-MVA 011 and 024 – In these studies immunogenicity was assessed only in the secondary endpoints.

Results

Vaccinia-naïve subjects (i.e. those with no history of VV receipt) were younger than the VV-experienced cohorts and showed very low rates of pre-vaccination seropositivity by ELISA or PRNT. For example, in study 005 6/545 vaccinia-naïve subjects had ELISA titres > 100 at baseline and 7 had PRNT50 titres > 6. Baseline seropositivity rates varied in VV-experienced populations. For example, in study 024 in subjects with a history of VV receipt 115/119 were seropositive by ELISA (>50) at baseline while 84/119 were seropositive by PRNT. However, not all study reports and supplemental tabulations allow for an assessment of baseline seropositivity rates.

The following tabulations summarise the ELISA and PRNT data across all 13 studies, with separate tabulations of the initial study phase responses and the follow-up data.

Peak responses are shown i.e. highest titre based on all available time points for the initial and later phases.

The tables have been divided according to the five pivotal studies described above and then the other studies included in the initial application. Summary observations accompany each table.

Pivotal studies - ELISA responses in the initial study phases (M=MVA-BN dose; P=placebo dose)

Note that results are shown for the FAS except for PP in POX-MVA-008.

Table 11 Comparison of Peak Seroconversion Rate (ELISA), Peak GMT Responses (ELISA) and Time to Peak Values (Week 1 to Week 10) in All Studies

Study (Data Set)	Dose	IMVANEX® Dose	Peak SC % (95% CI)	Day to Peak SC %	Peak GMT (95% CI)	Day to Peak GMT	Assay Antigen
Pivotal Studies							
POX-MVA-005 FA Set, N = 745							
Group 1, Vaccinia-naive	MM	1 x 10^8 $TCID_{50}$	98.9 (96.0, 99.9)	42	**495.8 (431.9, 569.3)**	42	MVA-BN®
Group 2, Vaccinia-naive	MP	1 x 10^8 $TCID_{50}$	87.4 (81.5, 91.9)	28	60.3 (47.6, 76.5)	28	
Group 3, Vaccinia-naive	PP	NAP	3.4 (1.3, 7.4)	42	1.3 (1.1, 1.4)	28	
Group 4, Vaccinia-experienced	M-	1 x 10^8 $TCID_{50}$	95.5 (91.6, 97.9)	14	**568.8 (473.3, 683.7)**	14	
POX-MVA-008 PP Set, N = 451							
Group 1, Vaccinia-naive	MM	1 x 10^8 $TCID_{50}$	98.5 (95.5, 99.7)	42	**499.4 (417.1, 598.0)**	42	MVA-BN®
Group 2, Vaccinia-naive, Diagnosed AD	MM	1 x 10^8 $TCID_{50}$	97.3 (94.5, 98.9)	42	532.9 (452.9, 627.1)	42	
POX-MVA-011 FA Set, N = 579							
Healthy,Vaccinia-naive	MM	1 x 10^8 $TCID_{50}$	98.7 (93.1, 100)	42	**560.6 (441.3, 712.3)**	42	MVA-BN®
HIV, Overall, Vaccinia-naive	MM	1 x 10^8 $TCID_{50}$	96.2 (93.4, 98.0)	42	282.9 (241.2, 331.7)	42	
HIV, CD4 ≥ 501-750, Vaccinia-naive	MM	1 x 10^8 $TCID_{50}$	98.9 (94.2, 100)	42	351.1 (277.0, 445.0)	42	
HIV, CD4 ≥ 350-500 , Vaccinia-naive	MM	1 x 10^8 $TCID_{50}$	95.9 (91.2, 98.5)	42	268.2 (209.2, 343.9)	42	
HIV, CD4 ≥ 200-349, Vaccinia-naive	MM	1 x 10^8 $TCID_{50}$	93.5 (85.5, 97.9)	42	240.5 (167.8, 344.6)	42	
Healthy,Vaccinia-experienced	MM	1 x 10^8 $TCID_{50}$	100.0 (63.1, 100)	28	**610.2 (211.3, 1762.0)**	42	
HIV, Overall, Vaccinia-experienced	MM	1 x 10^8 $TCID_{50}$	92.7 (86.6, 96.6)	42	525.8 (435.2, 635.2)	42	
HIV, CD4 ≥ 501-750, Vaccinia-experienced	MM	1 x 10^8 $TCID_{50}$	90.7 (77.9, 97.4)	42	574.4 (440.4, 749.1)	42	
HIV, CD4 ≥ 350-500, Vaccinia-experienced	MM	1 x 10^8 $TCID_{50}$	94.8 (85.6, 98.9)	42	534.9 (384.3, 744.4)	42	
HIV, CD4 ≥ 200-349, Vaccinia-experienced	MM	1 x 10^8 $TCID_{50}$	90.9 (70.8, 98.9)	42	421.1 (290.2, 611.2)	42	
POX-MVA-023 Booster FA Set, N = 152							
Group 1, IMVANEX®-experienced	M-	1 x 10^8 $TCID_{50}$	100 (95.2, 100)	7	**1688.2 (1381.5, 2062.9)**	14	MVA-BN®
Group 2, IMVANEX®-experienced	M-	1 x 10^8 $TCID_{50}$	100 (95.3, 100)	14	**1608.9 (1285.9, 2013.0)**	14	
POX-MVA-024 FA Set, N = 119							
Group 1, Vaccinia-experienced	MM	1 x 10^8 $TCID_{50}$	83.6 (71.9, 91.8)	14	804.1 (636.3, 1016.0)	42	MVA-BN®
Group 2, Vaccinia-experienced	PM	1 x 10^8 $TCID_{50}$	82.8 (70.6, 91.4)	42*	605.8 (479.6, 765.2)	42*	MVA-BN®

- After two doses in vaccinia-naïve healthy subjects and those with AD the peak seroconversion rates exceeded 90% and the GMTs were close to or higher than 500.
- After two doses in vaccinia-naïve HIV-infected subjects the peak seroconversion rates exceeded 90% but the GMTs were in the range 240-350.

- After a single dose in vaccinia-naïve healthy subjects the seroconversion rate was 87% and the GMT was 60.
- After two doses in VV-experienced healthy subjects and HIV-infected subjects the seroconversion rate was >90% (except for 84% in 024) and the GMTs were in the range 420-805.
- After a single dose in VV-experienced subjects in 005 and 024 the seroconversion rates were 96% and 83% and the GMTs were 569 and 606.
- After a single dose in MVA-BN experienced subjects (2 doses given 2 years prior to boosting) all subjects seroconverted and the GMT was > 1600.

Pivotal studies - PRNT responses in the initial study phases

Table 12 Comparison of Peak Seroconversion Rate (PRNT), Peak GMT Responses (PRNT) and Time to Peak Values (Week 1 to Week 10) in All Studies

Study (Data Set)	Dose	IMVANEX® Dose	Peak SC % (95% CI)	Day to Peak SC %	GMT Peak (95% CI)	Day to Peak GMT	Assay Antigen
Pivotal Studies							
POX-MVA-005 FA Set, N = 745							
Group 1, Vaccinia-naïve	MM	1 x 10^8 $TCID_{50}$	89.2 (83.7, 93.4)	42	**45.6 (35.1, 59.2)**	42	VVWR
Group 2, Vaccinia-naïve	MP	1 x 10^8 $TCID_{50}$	62.1 (54.4, 69.3)	28	7.2 (5.5, 9.4)	28	
Group 3, Vaccinia-naïve	PP	NAP	1.1 (0.1, 4.0)	14	1.1 (1.0, 1.1)	14	
Group 4, Vaccinia-experienced	M-	1 x 10^8 $TCID_{50}$	78.5 (72.2, 84.0)	14	**175.2 (140.0, 219.1)**	14	
POX-MVA-008 PP Set, N = 451							
Group 1, Vaccinia-naïve	MM	1 x 10^8 $TCID_{50}$	86.6 (81.0, 91.1)	42	**34.6 (26.4, 45.3)**	42	VVWR
Group 2, Vaccinia-naïve, Diagnosed AD	MM	1 x 10^8 $TCID_{50}$	90.3 (86.0, 93.6)	42	47.7 (38.1, 59.8)	42	
POX-MVA-011 FA Set, N = 579							
Healthy,Vaccinia-naïve	MM	1 x 10^8 $TCID_{50}$	77.2 (66.4, 85.9)	42	**21.7 (13.7, 34.6)**	42	VVWR
HIV, Overall, Vaccinia-naïve	MM	1 x 10^8 $TCID_{50}$	60.3 (54.7, 65.8)	42	13.1 (10.1, 17.0)	42	
HIV, CD4 ≥ 501-750, Vaccinia-naïve	MM	1 x 10^8 $TCID_{50}$	57.0 (46.3, 67.2)	42	13.4 (8.2, 22.0)	42	
HIV, CD4 ≥ 350-500 , Vaccinia-naïve	MM	1 x 10^8 $TCID_{50}$	57.9 (49.5, 66.1)	42	11.9 (8.1, 17.6)	42	
HIV, CD4 ≥ 200-349, Vaccinia-naïve	MM	1 x 10^8 $TCID_{50}$	68.8 (57.3, 78.9)	42	15.4 (9.4, 25.1)	42	
Healthy,Vaccinia-experienced	MM	1 x 10^8 $TCID_{50}$	85.7 (42.1, 99.6)	42	**358.4 (117.5, 1093.3)**	42	
HIV, Overall, Vaccinia-experienced	MM	1 x 10^8 $TCID_{50}$	75.6 (67.0, 82.9)	42	69.0 (48.3, 98.6)	42	
HIV, CD4 ≥ 501-750, Vaccinia-experienced	MM	1 x 10^8 $TCID_{50}$	75.6 (60.5, 87.1)	56	63.4 (36.8, 109.3)	42	
HIV, CD4 ≥ 350-500, Vaccinia-experienced	MM	1 x 10^8 $TCID_{50}$	77.6 (64.7, 87.5)	42	100.8 (58.9, 172.6)	42	
HIV, CD4 ≥ 200-349, Vaccinia-experienced	MM	1 x 10^8 $TCID_{50}$	72.7 49.8, 89.3)	42	30.0 (11.6, 77.6)	42	
POX-MVA-023 Booster FA Set, N = 152							
Group 1, IMVANEX®-experienced	M-	1 x 10^8 $TCID_{50}$	98.7 (92.8, 100)	14	**125.3 (89.5, 175.3)**	14	VVWR
Group 2, IMVANEX®-experienced	M-	1 x 10^8 $TCID_{50}$	96.1 (89.0, 99.2)	14	**80.7 (54.4, 119.7)**	14	
POX-MVA-024 FA Set, N = 119							
Group 1, Vaccinia-experienced	MM	1 x 10^8 $TCID_{50}$	90.0 (79.5, 96.2)	42	210.3 (146.1, 302.7)	42	VVWR
Group 2, Vaccinia-experienced	PM	1 x 10^8 $TCID_{50}$	77.6 (64.7, 87.5)	42*	126.7 (82.4, 194.8)	42	

- After two doses in vaccinia-naïve healthy subjects and those with AD the peak seroconversion rates were 77-90% and the GMTs were in the range 22-46.
- After two doses in vaccinia-naïve HIV-infected subjects the peak seroconversion rates were from 57-69% with GMTs 12-15.
- After a single dose in vaccinia-naïve healthy subjects the seroconversion rate was 62% and the GMT was 7.
- After two doses in VV-experienced healthy subjects and HIV-infected subjects the seroconversion rates were 73-90% (73-78% for HIV-infected) and the GMTs were in the

range 30-358 (30-100 for HIV-infected). In contrast to the ELISA, the PRNT data showed differential responses between the healthy and HIV-infected groups.

- After a single dose in VV-experienced subjects in 005 and 024 the seroconversion rate was 78% and the GMTs were 127 and 175.
- After a single dose in MVA-BN experienced subjects (2 doses given 2 years prior to boosting) >95% seroconverted while the GMT with GMTs of 81 and 125 in the groups primed with one and two doses of MVA-BN, respectively.

Other studies – ELISA data from the initial phases (D=Dryvax)

Note that the analysis population varies by study and that subject numbers per group were often small.

- The three studies that evaluated two doses of different $TCID_{50}$ in vaccinia-naïve healthy subjects suggested that at least 2 x 10^7 TCID50 should be used and showed higher (but variable) GMTs at 1 x 10^8 $TCID_{50}$.
- A single dose at the selected $TCID_{50}$ in VV-experienced subjects showed a comparable response to two doses in the vaccinia-naïve.
- Presence of AD in vaccinia-naïve subjects did not seem to affect the responses to MVA-BN.
- In the HIV-infected subjects two doses in the vaccinia-naïve and one dose in the VV-experienced gave seroconversion >90% but GMTs were lower compared to corresponding healthy cohorts.
- In study 009 there was a protocol amendment after 20 subjects to eliminate the Dryvax groups. Only Groups A, B and F provide useful data (although available information on Dryvax is reported in the clinical assessment). The study did not support a 7-day interval between MVA-BN doses.
- In HIV-NEF-004 in HIV-infected subjects with a good response to ART and of mixed VV history the third dose of MVA-BN administered at 8 week intervals was shown to elicit a further increment in GMT from 193 at week 8 to 564 at week 16 and to 864 at week 24.

Other studies – PRNT data from the initial phases

- The three studies that evaluated two doses of different $TCID_{50}$ in vaccinia-naïve healthy subjects suggested that at least 1 x 10^8 $TCID_{50}$ should be used although even at this dose the seroconversion rates after two doses ranged from 71% to 100% with GMTs from 19 – 126.
- A single dose at the selected $TCID_{50}$ in VV-experienced subjects showed a comparable response to two doses in the vaccinia-naïve completers in POX-MVA-001. A single dose in VV-experienced subsets in the PP population in study 010 gave seroconversion at least 90% and higher GMTs compared to two doses in the vaccinia-naïve subjects in the same study.
- Presence of AD in vaccinia-naïve subjects did not seem to affect the responses to MVA-BN although GMTs were numerically lower.

- In the HIV-infected subjects two doses in the vaccinia-naïve and one dose in the VV-experienced gave seroconversion >90% but GMTs were lower compared to corresponding healthy cohorts.
- In study 009, the study did not support a 7-day interval between MVA-BN doses.
- In HIV-NEF-004 in HIV-infected subjects with a good response to ART the third dose of MVA-BN administered at 8 week intervals was shown to elicit a further increment in GMT from 125 at week 8 to 403 at week 16 and to 545 at week 24.

Antibody persistence data

All studies in the tables above except for POX-MVA-004 and POX-MVA-001 collected GMT response data at approximately 6 months.

Two studies - POX-MVA-002 and POX-MVA-001 - collected data at Year 1, while studies - POX-MVA-001 and POX-MVA-004 - collected data at Year 2.

POX-MVA-023 collected data pre-boost, which was at Year 2 after initial dosing in POX-MVA-005. These data are therefore shown as 24 months persistence below but the rates are for seropositivity at year 2 and not for seroconversion (despite the column heading). Please also note that the post-booster response data in study 023 are shown in the initial phase tables above.

ELISA data up to year 2

In the pivotal studies:

The Month 6 data showed marked drops in seroconversion rates and in GMTs for all groups that were vaccinia-naïve prior to receiving two doses of MVA-BN. The pre-boost data from 023 suggested that the drop occurred sometime within the first 6 months and then reached a plateau, which is compatible with the pattern observed for many other vaccines.

The two VV-experienced groups prior to receiving a single dose of MVA-BN showed seroconversion rates at Month 6 of 68% and 59% with GMTs at 180 and 258. Study 024 did not suggest an advantage for two doses in terms of antibody persistence. The Month 24 data from study 005 suggested much better antibody persistence in those who were VV-experienced at baseline even though they received only one dose. In addition, study 023 showed that antibody persistence at Month 6 after a third (booster) dose of MVA-BN resulted in much higher seroconversion rates and GMTs compared to Month 6 after two doses of MVA-BN.

In the other studies:

- The data from studies 001 and 009 supported a conclusion that the rapid drop in ELISA titres occurred within the first 6 months after MVA-BN.
- Studies 001 and 004 did not show a consistent trend for better antibody persistence according to the $TCID_{50}$ delivered.
- The Month 6 findings were very variable. However, the PP population in study 007 suggested good persistence at Month 6 regardless of AD and study 010 suggested no significant difference between healthy and HIV-infected subsets.

- Study 009 pointed to a numerical advantage for two doses 28 days apart compared to two doses 7 days apart or a single dose.

- The subgroups that were VV-experienced before receiving a single dose of MVA-BN also showed variable results at Month 6 and Month 12 although the picture suggested that even a single dose resulted in generally better antibody persistence compared to two doses in the previously vaccinia-naïve.

According to the ELISA data the seroconversion rate in those who were vaccinia naïve before the initial two doses of MVA-BN will be < 50% at 2 years and the seropositivity rate will be < 70%.

PRNT data up to year 2

In the pivotal studies:

The Month 6 data showed drops in seroconversion rates and in GMTs for all groups that were vaccinia-naïve prior to receiving two doses of MVA-BN. The pre-boost data from 023 suggested that the drop in PRNT was not confined to the initial 6-month period.

The two VV-experienced groups prior to receiving a single dose of MVA-BN showed seroconversion rates at weeks 30 and 35 of 64% and 41% with GMTs at 106 and 28 at these respective time points. Study 024 did not suggest a significant advantage for two doses in terms of antibody persistence. The Month 24 data from study 005 suggested better antibody persistence in terms of GMTs but not seroconversion rates after a single dose in VV-experienced compared to two doses in vaccinia-naïve.

Study 023 showed that antibody persistence at Month 6 after a third (booster) dose of MVA-BN resulted in higher seroconversion rates and GMTs compared to Month 6 after two doses of MVA-BN.

In the other studies:

- The data from study 001 at the selected dose suggested little change in PRNT beyond month 12.

- Study 001 (Groups 1-3) and study 004 suggested a trend for better antibody persistence according to the $TCID_{50}$ delivered.

- The Month 6 findings were variable. However, the PP population in study 007 suggested comparable persistence at Month 6 regardless of AD.

- Study 010 suggested lesser antibody persistence in the HIV-infected vs. healthy vaccinia-naïve groups and a lower GMT for the HIV-infected vs. healthy VV-experienced groups.

- Study 009 did not point to a convincing benefit for two doses 28 days apart compared to two doses 7 days apart but two doses appeared better than a single dose.

- The subgroups that were VV-experienced before receiving a single dose of MVA-BN also showed variable results at Month 6 and Month 12 but study 001 suggested that even a single dose resulted in generally better antibody persistence compared to two doses in the previously vaccinia-naïve.

According to the PRNT data the seroconversion rate in those who were vaccinia naïve before the initial two doses of MVA-BN and in those who were VV-experienced before a single dose of MVA-BN will be <50% at 2 years.

Additional data on dose and formulation

During the procedure the applicant provided preliminary data from two NIH-sponsored studies as follows:

POX-MVA-029 directly compared the freeze-dried formulation of MVA-BN (lot 130303; as used in POX-MVA-002) to the liquid frozen formulation (final commercial scale process). The comparison was to be based on the GMTs derived from individual peak PRNTs following 2 doses of 1 x 10^8 $TCID_{50}$ administered at Day 0 and 28 as liquid frozen and as freeze-dried MVA-BN. Based on the data generated using the applicant's ELISA and PRNT assays and on the pre-defined acceptance margin of ≤2.0-fold difference in GMTs it was concluded that non-inferiority was demonstrated. However, both the applicant's and the SLU assays showed numerically higher GMTs with lyophilised vaccine and the 95% CI did not overlap at several time points.

POX-MVA-028 compared a single high dose of MVA-BN with the standard 2-dose regimen using 2 lots - 2x10^8 $TCID_{50}$/mL (for standard dose) and 5x10^8 $TCID_{50}$/mL (for high dose).

The SLU ELISA gave maximal seroconversion rates around 50% after a single dose but 96% after two standard doses and proportions with positive titres (>50) closely matched these rates. The GMTs showed a marked increase after the second standard dose. In contrast, the BN ELISA showed that all subjects seroconverted after a single high dose but the GMTs showed a clear difference between groups after the second standard dose.

Table 13 BN ELISA – ITT population – Summary of geometric mean titers

	Group			
	A: One High Dose IMVAMUNE® Day 0, Placebo Day 28		B: Two Standard Doses IMVAMUNE® Days 0, 28	
Study Day	n	GMT (95% CI)	n	GMT (95% CI)
Day 0*	46	25.8 (24.7, 26.9)	45	27.0 (25.3, 28.8)
Day 4	46	25.8 (24.7, 26.9)	45	27.0 (25.3, 28.8)
Day 8	46	31.6 (26.9, 37.2)	45	30.3 (25.1, 36.5)
Day 14	46	224.3 (173.8, 289.4)	45	110.1 (79.7, 152.2)
Day 21	46	263.7 (207.8, 334.5)	45	145.3 (106.9, 197.4)
Day 28**	46	236.6 (187.4, 298.6)	45	129.7 (98.2, 171.5)
Day 42	45	179.3 (142.0, 226.5)	45	996.7 (740.5, 1341.7)
Day 56	46	143.8 (114.6, 180.3)	45	627.5 (463.5, 849.4)
Day 208	43	73.9 (58.1, 93.9)	44	90.6 (71.4, 115.1)

* First vaccination, ** Second vaccination

The SLU PRNT GMTs showed the differential effect of the second standard dose but there was no substantial difference by Day 208. Proportions with positive titres (at least 1:20) showed an early advantage for the high dose but there was a benefit from the second dose. However, by Day 208 there was no substantial difference. As in POX-MVA-029 the GMTs obtained with the BN PRNT were notably lower vs. the SLU PRNT. Nevertheless, the patterns were comparable with the SLU data and both assays showed no substantial difference between dose groups at Day 208.

Table 14 SLU PRNT - ITT population – Summary of geometric mean titers

	Group			
	A: One High Dose IMVAMUNE® Day 0, Placebo Day 28		B: Two Standard Doses IMVAMUNE® Days 0, 28	
Study Day	n	GMT (95% CI)	n	GMT (95% CI)
Day 0*	46	8.0 (7.1, 9.0)	45	7.6 (7.4, 7.9)
Day 4	46	7.9 (7.1, 8.9)	45	7.6 (7.4, 7.9)
Day 8	46	8.0 (7.2, 8.9)	45	8.3 (6.8, 10.0)
Day 14	46	12.5 (10.5, 14.8)	45	10.5 (8.4, 13.2)
Day 21	46	13.1 (11.2, 15.3)	45	10.8 (8.6, 13.6)
Day 28**	46	14.0 (11.6, 17.0)	45	10.3 (8.5, 12.4)
Day 42	45	12.3 (10.4, 14.5)	45	44.6 (30.7, 64.7)
Day 56	46	10.8 (9.7, 11.9)	45	32.4 (22.2, 47.3)
Day 208	43	10.6 (8.8, 12.8)	44	12.2 (9.2, 16.2)

* First vaccination, ** Second vaccination

Proportions with titres at least 1:15 showed augmentation after the second standard dose but there was no appreciable difference by Day 208.

It was concluded that vaccination with a single high dose produces higher titres more rapidly than a single standard dose but the titres do not reach the same magnitude as after the second standard dose. Based on the median time to seroconversion, use of a single high dose vaccine may be an alternative to a 2-dose vaccine series during a post-event emergency and may warrant further study.

CMI data

The results of the non-clinical efficacy studies pointed to the importance of both humoral and cell-mediated immunity. The following is a brief summary of the data from some of the clinical studies:

In the dose-finding study **POX-MVA-001** ELISPOT data were generated using lysed MVA-infected CEF cells as the stimulating antigen and fixed numbers of CD4+ and CD8+ cells. The pattern of results was not entirely consistent with that for humoral immunity. The study report comments on high inter-subject variability and variable sample availability at different time points. Nevertheless, means demonstrated the effect of two vs. a single dose and the VV-experienced group showed a robust response to a single dose.

Rates for T-cell-responses above 50 SFU/106 cells were higher after the second dose in the previously vaccinia-naïve subjects.

In the dose-finding study **POX-MVA-004** the CTL response was assessed in subsets by measuring IFN-producing cells by intracellular cytokine staining (ICS). It is not clear from the study reports how the subjects/samples were selected for determination of CTL responses. Numbers with data are small. For CD4+ cells the peak positive response rates were < 20% and there was no appreciable difference between dose groups. For CD8+ cells there was a trend for rates of positive responses to increase from the lowest dose but with no appreciable difference between the two higher dose groups at D42 while the D84 rates showed a dose-related trend.

In the pivotal studies (note that CMI data were not reported for 005/023 and 024):

In **POX-MVA-008** a lower percentage in the healthy group (28.3%) showed a vaccinia-specific ELISPOT response at Week 1 vs. the AD group (47.9%) but the 95% CI overlapped. Response rates increased to 56.5% and 63.4% at Week 6.

Table 15 Vaccinia-specific ELISPOT: IFN-γ producing cells – response rates (EAS, N=117)

Healthy (=N=46)					Diagnosed AD (N=71)			
Week (Visit)	n	R	%	95% CI (%)	n	R	%	95% CI (%)
Week 1(Visit 2)	46	13	28.3	(16.0, 43.5)	71	34	47.9	(35.9), 60.1)
Week 6(Visit 4)	46	26	56.5	(41.1, 71.1)	71	45	63.4	(51.1, 74.5)

In addition, cells from 67% of healthy and 78% of AD subjects responded at least once to stimulation in the vaccinia-specific ELISPOT.

In **POX-MVA-011,** 20 - 27% of vaccinia-naïve healthy (n=24) and HIV-infected (n=34) subjects in the PP population with data showed a vaccinia-specific ELISPOT response at weeks 1 and 4. Response rates increased to 30% and 40% in respective groups at week 6. In the FAS, ELISPOT

data were available for 40 vaccinia-naïve healthy subjects and 75 vaccinia-naïve HIV-infected subjects. Throughout the study the response rates were not significantly different between vaccinia-naïve healthy and overall HIV-infected subjects. The highest response rates were generally seen at week 6.

In the VV-experienced PP population there were data from up to 71 HIV-infected subjects. The vaccinia-specific ELISPOT response rate in HIV-infected subjects increased from 27% of 71 subjects at week 1 to 35% of 68 subjects at week 4. Only 12 subjects were followed to week 32, when 3 were still classified as responders. In the FAS, ELISPOT data were available for 5 healthy and up to 96 HIV-infected subjects. The peak responder rate (33%) was again observed at week 4.

Comparisons between MVA-BN and replication-competent VV vaccines

Retrospective analysis of sera obtained from MVA-BN and NIH studies

The application included ELISA and PRNT data generated on testing sera obtained from subjects who had received MVA-BN, Dryvax (Wyeth; NYBH strain, freeze-dried; manufactured 1978) or Wetvax (Sanofi-Pasteur; NYBH strain, liquid; manufactured 1958). Antibody levels in sera from 251 vaccinia-naïve healthy subjects that received a single dose of Dryvax or Wetvax during five NIH/NIAID-sponsored clinical studies (2000-2002) were compared to those in sera obtained from 419 vaccinia-naïve subjects during three MVA-BN studies POX-MVA-005, 008 and 011) that used commercial scale liquid frozen vaccine and were conducted between 2006-2010. Subjects were healthy, young and vaccinia-naïve of both genders. Vaccinees in each set of studies received a range of lots (some data were missing).

All samples were tested from July 20 2006 to May 20 2010 at the applicant's laboratories. The highest antibody titres measured in samples obtained on day 28 or 56 from subjects who received Dryvax or Wetvax and on day 42 or 56 for subjects who received MVA-BN were used for the comparisons between vaccines. Data were provided to support stability of antibody titres during storage at -70°C

The ELISA data for Dryvax and Wetvax demonstrated a 15-26% decline in GMT from Day 28 to 56 but 190 subjects had a peak ELISA titre on day 28, 47 on day 56 and 14 had the same titre on both days.

Table 16 ELISA Titer by Study Dryvax/Wetvax Immunised Subjects

Trial ID	N	Total Antibody Titer (GMT)			Individual peak			
		Baseline	Day 28	Day 56	Individual peak	95% CI lower limit	95% CI upper limit	CV (%)
DMID# 00-005	17	3	265	NA	265	112	631	30
DMID#01-632	26	3	261	194	270	206	353	12
DMID#01-651	10	1	203	NA	203	137	302	10
DMID#02-009	86	2	384	327	405	349	469	12
DMID#02-054	112	2	375	297	377	342	415	9

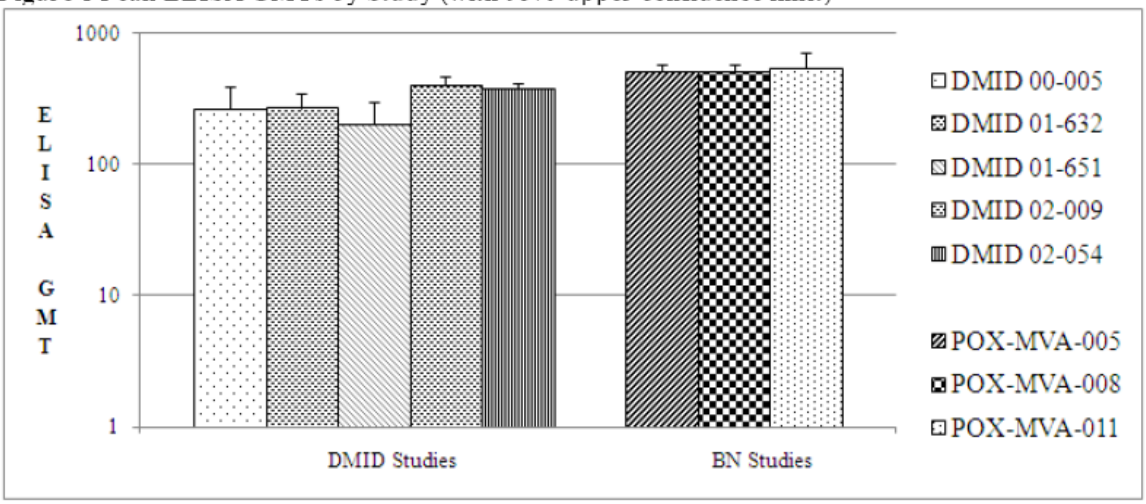
The peak ELISA titre was observed 2 weeks post-dose 2 of MVA-BN (i.e. Day 42). There were 364 (86.9%) with a peak ELISA response on day 42, 28 (6.7%) on day 56 and 21 (5.0%) had the same titre on both days. The CV% for the peak ELISA titres ranged from 9-30% across the NIH and MVA-BN studies.

Table 17 Elisa Titer by Study Imvamune Immunised Subjects

Trial ID	N	Total Antibody Titer (GMT)			Individual peak			
		Baseline	Day 42	Day 56	Individual peak	95% CI lower limit	95% CI upper limit	CV (%)
POX-MVA 005 DMID#05-0128	168	1	492	327	515	456	581	13
POX-MVA 008 DMID#05-0133	194	1	499	298	511	428	611	20
POX-MVA 011 DMID#05-0132	57	2	526	313	541	405	721	17

There were slightly higher GMTs after MVA-BN vs. those resulting from Dryvax and Wetvax.

Figure 1 Peak ELISA GMTs by Study (with 95% upper confidence limit)



Based on individual peak data the ELISA response to MVA-BN was concluded to be non-inferior vs. responses to the NYBH vaccines.

Table 18 Summary of the ELISA Titer by Treatment

Vaccine	N	Individual peak					
		GMT	CV (%)	95% CI lower limit	95% CI upper limit	Ratio of GMTs (95% CI)	Pr > 1
Imvamune	419	517	17	466	573	1,453	< 0.0001
Dryvax/Wetvax	251	356	13	324	390	(1,250, 1,690)	

PRNT data showed significant variability in the timing of the individual peak neutralising antibody response. For Dryvax and Wetvax 109 (43.3%) had a peak PRNT titre on day 28, 113 (45.0%) on day 56 and 29 (11.6%) had the same titre on both days.

In MVA-BN studies the peak response was on day 42 for 224 (53.5%) subjects, day 56 for 96 (22.9%) and the same titre occurred on both days for 93 (22.2%). The CV% ranged from 42 to 131%.

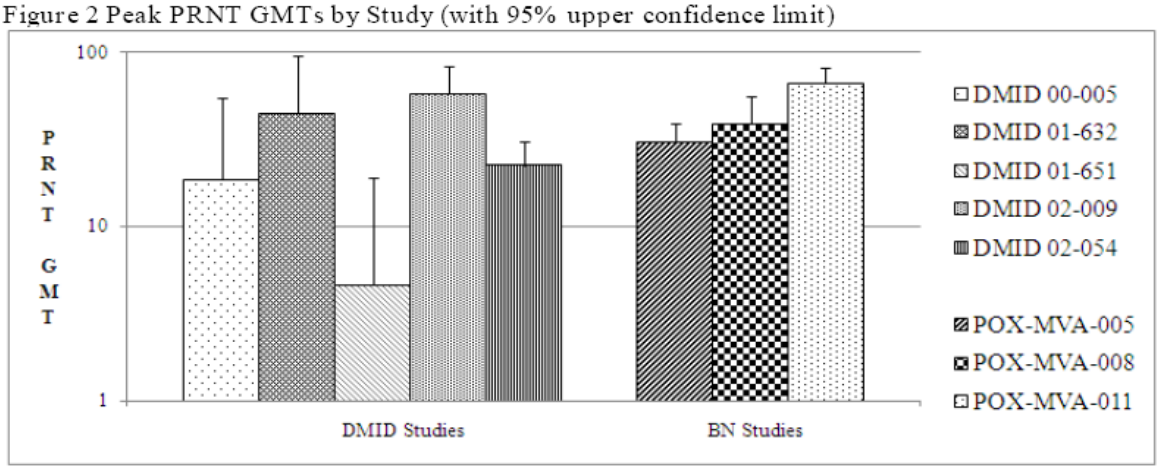
Table 19 PRNT Titer by Study Dryvax/Wetvax Immunised Subjects

Trial ID	N	Total Antibody Titer (GMT)			Individual peak			
		Baseline	Day 28	Day 56	Individual peak	95% CI lower limit	95% CI upper limit	CV (%)
DMID# 00-005	17	1	19	NA	19	6	55	72
DMID#01-632	26	1	17	15	45	21	96	50
DMID#01-651	10	1	5	NA	5	1	19	131
DMID#02-009	86	1	29	35	58	40	83	42
DMID#02-054	112	1	8	12	23	16	31	54

Table 20 PRNT Titer by Study Imvamune Immunised Subjects

Trial ID	N	Total Antibody Titer (GMT)			Individual peak			
		Baseline	Day 42	Day 56	Individual peak	95% CI lower limit	95% CI upper limit	CV (%)
POX-MVA 005 DMID#05-0128	168	1	45	33	82	67	100	30
POX-MVA 008 DMID#05-0133	194	1	35	16	39	30	50	50
POX-MVA 011 DMID#05-0132	57	1	21	15	36	24	56	45

Figure 2 Peak PRNT GMTs by Study (with 95% upper confidence limit)

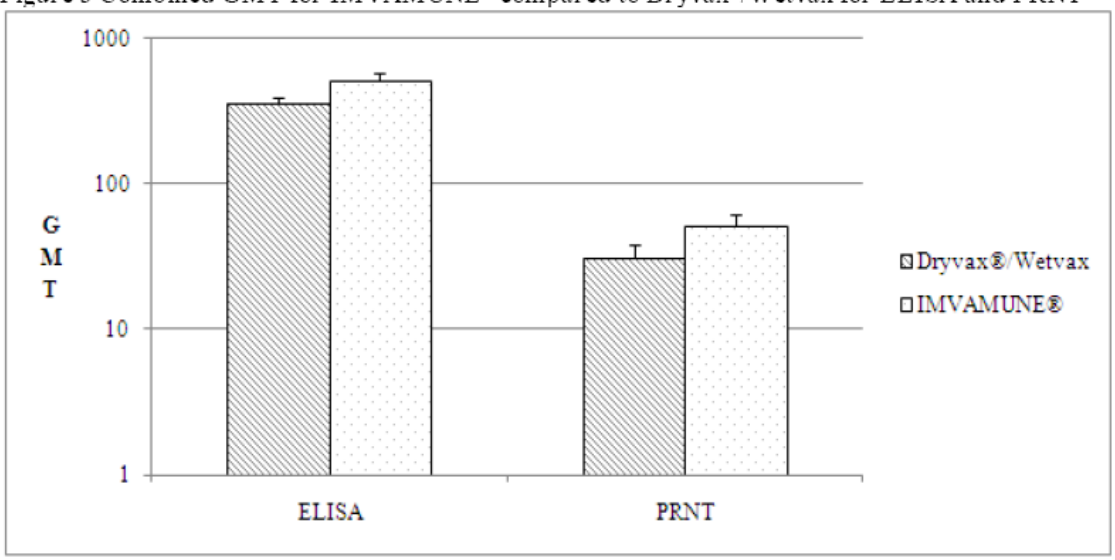


The peak GMT was 31 for 251 subjects vaccinated with Dryvax or Wetvax vs. 52 for the 419 that received MVA-BN. The applicant concluded that the neutralising antibody response to MVA-BN was non-inferior vs. that to the NYBH strain vaccines.

Table 21 PRNT Titer by Treatment

Vaccine	N	Individual peak					
		Peak GMT	Peak CV (%)	95% CI lower limit	95% CI upper limit	Ratio of GMTs (95% CI)	Pr > 1
Imvamune	419	52	42	44	61	1,681	< 0.0001
Dryvax/Wetvax	251	31	54	25	39	(1,282, 2,204)	

Figure 3 Combined GMT for IMVAMUNE® compared to Dryvax®/Wetvax for ELISA and PRNT



Subsequently, the applicant added to the analyses of these data by repeating the assessments of non-inferiority using each of a fixed effect model, mixed effect model and marginal model to account for study and subject variability and missing samples. These analyses were adjusted for

potential baseline differences to account for possible bias from different study populations and individual peak titres were imputed to account for missing samples. All analyses led to the same interpretation of results that humoral immune responses induced by MVA-BN are non-inferior to those induced by Dryvax/Wetvax. The analyses were applied to ELISA and neutralising antibody datasets.

In response to questions regarding the timing of sampling and possibility that the peak responses to Dryvax/Wetvax had not actually been captured the applicant stated that the humoral immune response to replication-competent vaccine develops about 10 days after scarification, with almost all vaccinees having seroconverted by day 14 and neutralizing antibody titers being significantly developed by day 16 (McCarthy 1958; Henderson 1999). NIH sponsored clinical trials performed since 2000 with remaining stocks of Dryvax applied sample time points at week 4 (day 28), while some of the studies additionally included immunogenicity assessments at week 8 (day 56). The applicant asserted that the peak response to Dryvax is observed at day 28 after vaccination and that this is supported by the lower GMTs at day 56 compared to the day 28 time point reported by Belshe 2004 but in fact Belshe *et al*. obtained samples only at day 28 and Day 56.

POX-MVA-002

This study was sponsored by NIH under the number DMID 02-017.

- The CDC published (Damon *et al*., 2009) on the variola neutralisation titres following administration of MVA-BN and Dryvax in this study (see below).
- The original CSR, including the long term follow-up report, were prepared by the NIH and included ELISA, PRNT and ELISPOT data.
- Three separate ELISA assays and two PRNT assays based on different poxvirus antigens were used but none was validated except for the BN ELISA using MVA-BN as the antigen.
- In response to a request from FDA the applicant went on to develop a validated PRNT using VV-WR as the test strain. Subsequently the applicant analysed the sera from POX-MVA-002 using this validated PRNT in BN's laboratories.

The study was conducted during 2004-2006 in the US in 90 vaccinia-naïve healthy subjects aged 18-32 years. Subjects (90) were randomised in a double-blind study design (with the exception of Group F) to one of six treatment groups, each of 15 subjects.

There were three MVA-BN dose groups (M/M/D) as follows:

Group A: MVA-BN $2x10^7$ $TCID_{50}$ SC on Days 0 and 28

Dryvax by scarification (15 strokes bifurcated needle) on Day 112

Group B: MVA-BN $5x10^7$ $TCID_{50}$ SC on Days 0 and 28

Dryvax by scarification on Day 112

Group C: MVA-BN $1x10^8$ $TCID_{50}$ SC on Days 0 and 28

Dryvax by scarification on Day 112

Two groups included placebo controls and two used alternative routes of administration:

Group D (P/P/D): **Placebo** SC on Days 0 and 28

Dryvax by scarification on Day 112

Group E (M/M/P): MVA-BN 1x108 TCID50 SC on Days 0 and 28

Placebo by scarification on Day 112

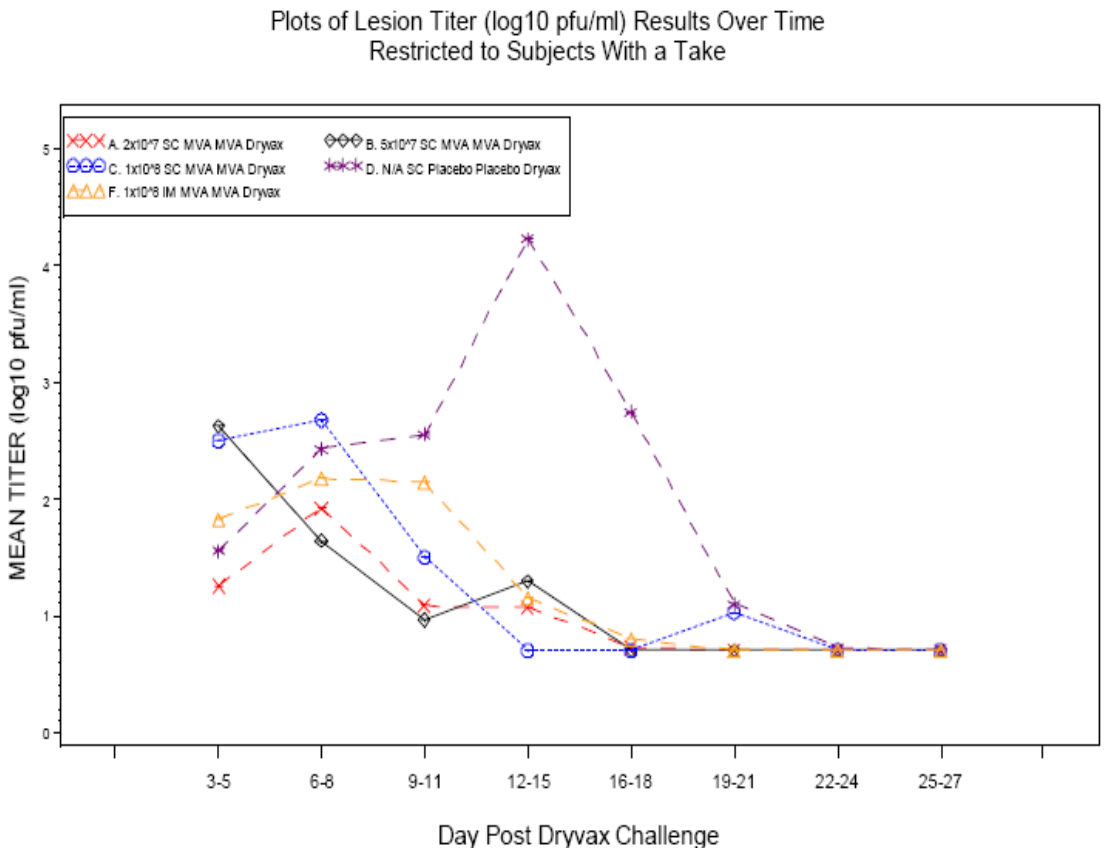
Group F (M/M/D): MVA-BN 1x108 TCID50 **IM** on Days 0 and 28

Dryvax by scarification on Day 112

All 13 subjects with no prior MVA-BN (Group D) had a "take" compared to 10/11 when Dryvax was given after two sc doses of MVA-BN at the selected TCID50 (Group C).

Table 22 Summary of Clinical Take rates by Group

Treatment group	# Subjects evaluated	# with a take	Take rate In %	95% Lower CI	95% Upper CI
Group A 2x10^7 Sc MMD	14	13	92.9	66.1	99.8
Group B 5x10^7 Sc MMD	13	7	53.8	25.1	80.8
Group C 1x10^8 Sc MMD	11	10	90.9	58.7	99.8
Group D N/A SC PPD	13	13	100	75.3	100
Group E 1x10^8 Sc MMD	9	0	0.0	0.0	33.6
Group F 1x10^8 Sc MMD	12	8	66.7	34.9	90.1

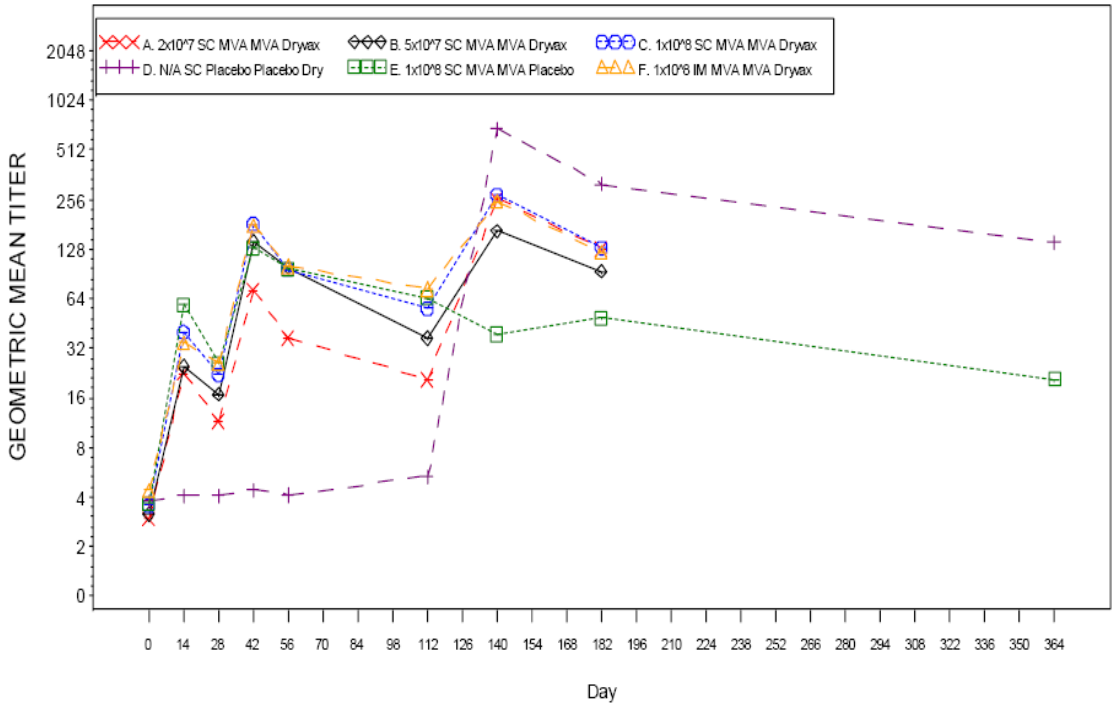


The vaccination lesion size and the time to healing (6-10 days for M/M/D vs. 14 days for P/P/D) were significantly reduced when Dryvax was given after MVA-BN. There were significantly larger diameters for erythema, induration and lesion size in the P/P/D group vs. the combined M/M/D groups. Based on culture of viral swabs of the vaccination site on Days 3-5 and 6-8 and any further visits before lesion healing, prior MVA-BN significantly reduced the post-Dryvax lesion viral titres at the site of scarification. There was no significant trend for lesion size or titre across the three MVA-BN dose groups.

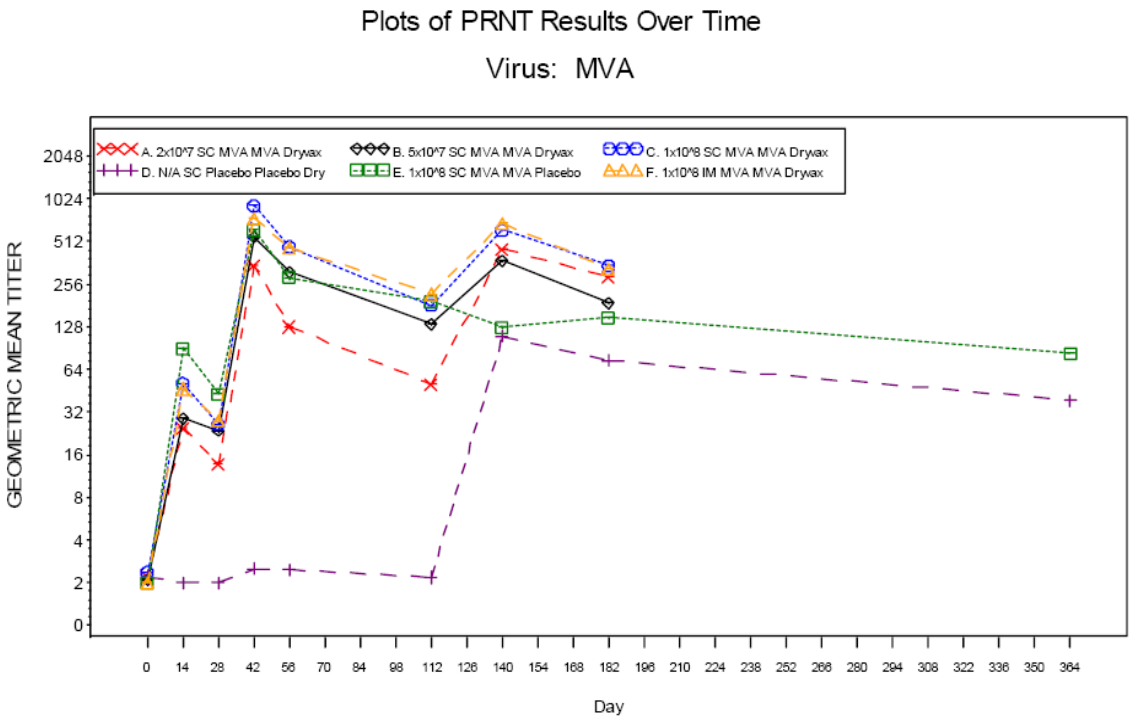
PRNT using MVA VR-1508 or Dryvax in the assay

- The GMTs increased in the M/M/D groups after each dose and after Dryvax when using either antigen in the assay. The report states that there were significant linear dose response correlations.

Plots of PRNT Results over Time: Virus: Dryvax®



Plots of PRNT Results over Time: Virus: MVA



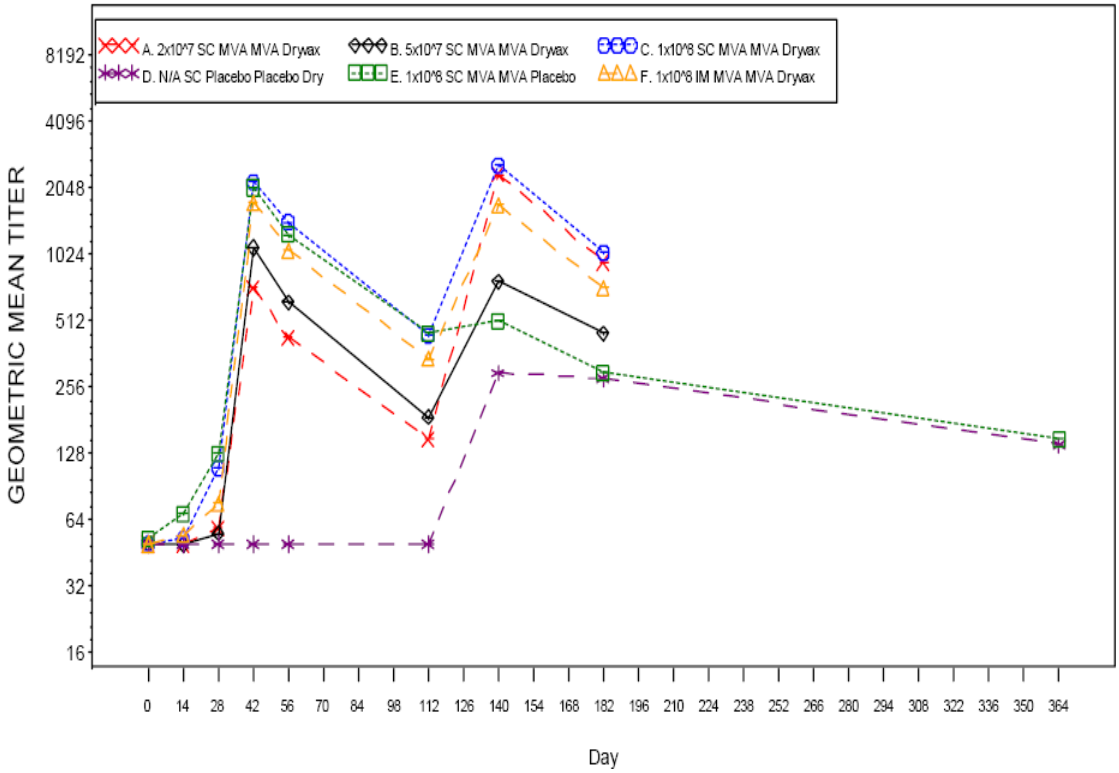
- At 4 weeks and 10 weeks after administration of Dryvax the GMTs were significantly lower in the combined M/M/D groups compared to the P/P/D group when Dryvax was used as the antigen assay.

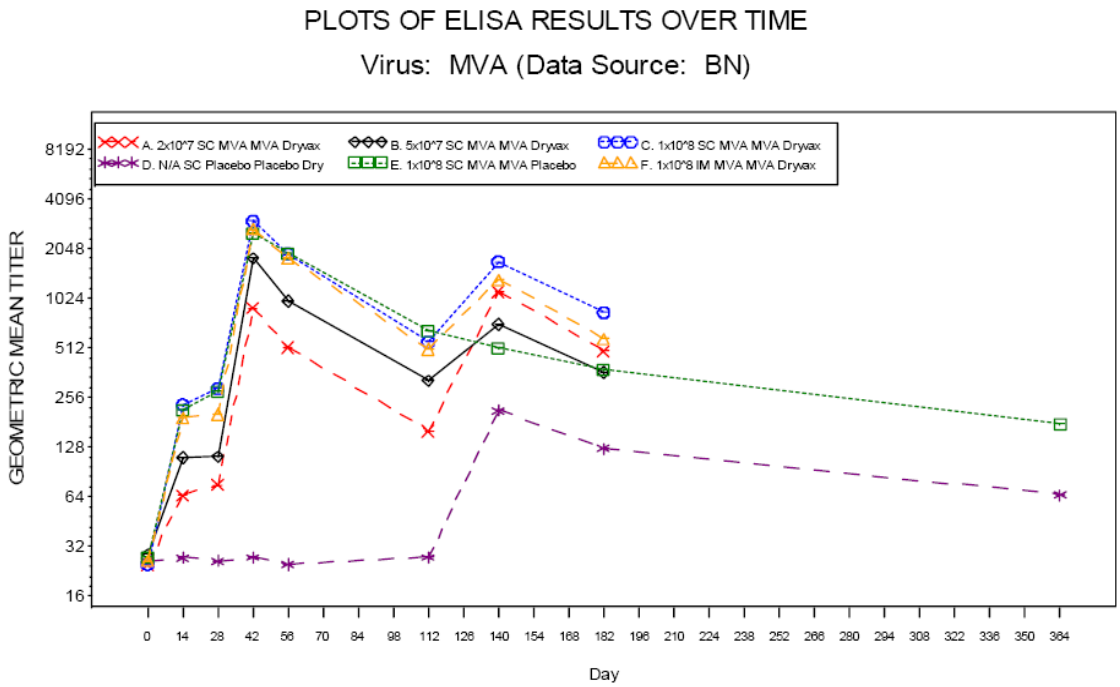
- In contrast the GMTs were significantly higher at these time points in the combined M/M/D groups compared to the P/P/D group when MVA VR-1508 was used as the assay antigen.
- Using Dryvax as the assay antigen and a titre ≥ 20 to define seroconversion 86.7 to 100% in the MVA-BN groups seroconverted at 2 weeks after the second dose (D42) and all M/M/D and P/P/D subjects seroconverted at 4 weeks after Dryvax.
- When MVA VR-1508 was used as the antigen all M/M/D subjects seroconverted within 2 weeks of the second dose (D42) while 85% in the P/P/D group seroconverted 4 weeks after Dryvax.
- One-year follow-up of groups D (P/P/D) and E (M/M/P) showed that between Day 140 and 364 the GMT dropped from 692 to 141 after P/P/D (Group D) and from 39 to 18 after M/M/P (Group E) when Dryvax was used as the assay antigen. When MVA VR-1508 was used as the antigen the drops were from 110 to 39 and from 127 to 83 in respective groups.

ELISA using MVA-BN, MVA VR-1508 or Dryvax in the assay

- At Day 28 there was no significant difference in GMTs among all the groups regardless of the assay antigen but there was a difference between the M/M/D and P/P/D groups at D42. GMTs in the P/P/D group were significantly lower vs. the combined M/M/D group at 4 weeks after Dryvax.

Plots of ELISA Results over Time: Virus: Dryvax®





- Significant linear correlations of increasing GMT were observed for increasing MVA-BN doses after each dose. The route of administration of MVA-BN (IM or SC) did not affect the antibody titres.

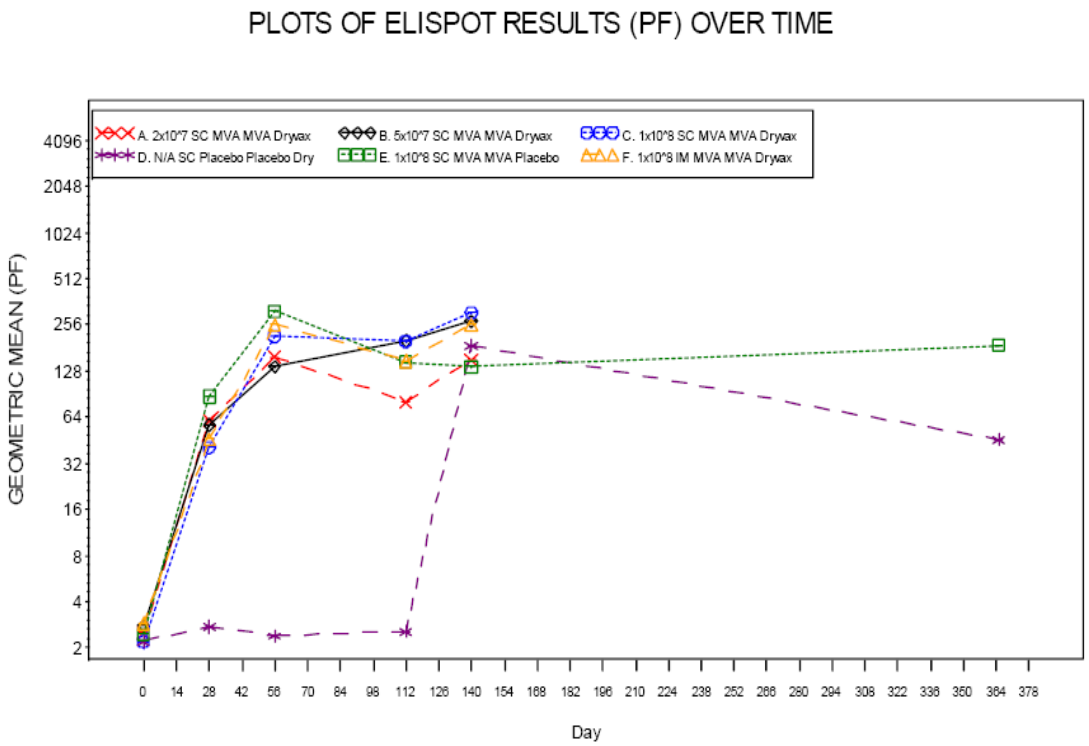
- Using a cut-off of ≥ 50 to define seroconversion all M/M/D subjects had seroconverted by 2 weeks post-dose 2 when Dryvax was used as the assay antigen with rates of 92-100% when MVA-BN was used as the assay antigen. In the P/P/D group 62% seroconverted at 4 weeks post Dryvax.

- One-year follow-up showed no difference in GMTs (141 for Group D and 148 for Group E) or seroconversion rates (73% vs. 86%, respectively) when Dryvax was used as the assay antigen. GMTs were 66 for group D and 177 for Group E and seroconversion rates were 82% versus 100%, respectively, when MVA was used as the assay antigen.

ELISPOT using Dryvax as the stimulating antigen

There were no significant differences in the GMTs between the P/P/D and M/M/D groups after Dryvax was given. Significant trends of higher amounts of IFN-γ secreting T cells (methodology not specified except for the stimulating antigen) at increasing doses of MVA-BN were observed on Day 28 and at 56 and 112 days after the second dose.

Plots of ELISPOT Results (PF) over Time



Using a cut-off of >15 spots to define a response 93 - 100% in the M/M/D groups showed T-cell responses after the second dose. At 4 weeks after Dryvax seroconversion rates were at least 92% for the M/M/D groups and 100% for the P/P/D group. After 1 year, all of group E were still responders compared to 80% in group D.

Re-assay using the applicant's validated PRNT assay and using WR-VV as the assay antigen

After the first dose of MVA-BN the Day 28 seroconversion rates were from 40-70%. The highest GMT was recorded in Group F (11). On Day 42 all except one subject in each of groups B and C (who took until Day 56 to seroconvert) demonstrated seroconversion. The GMTs increased after the second dose but there was no trend with respect to virus titre in the vaccine.

At Day 112 seroconversion rates were 92% and 100% for the highest vaccine virus titre compared to 62% and 85% at the lower titres. The lowest GMT occurred in group A. All subjects in M/M/D and P/P/D groups seroconverted by D140 (4 weeks after Dryvax).

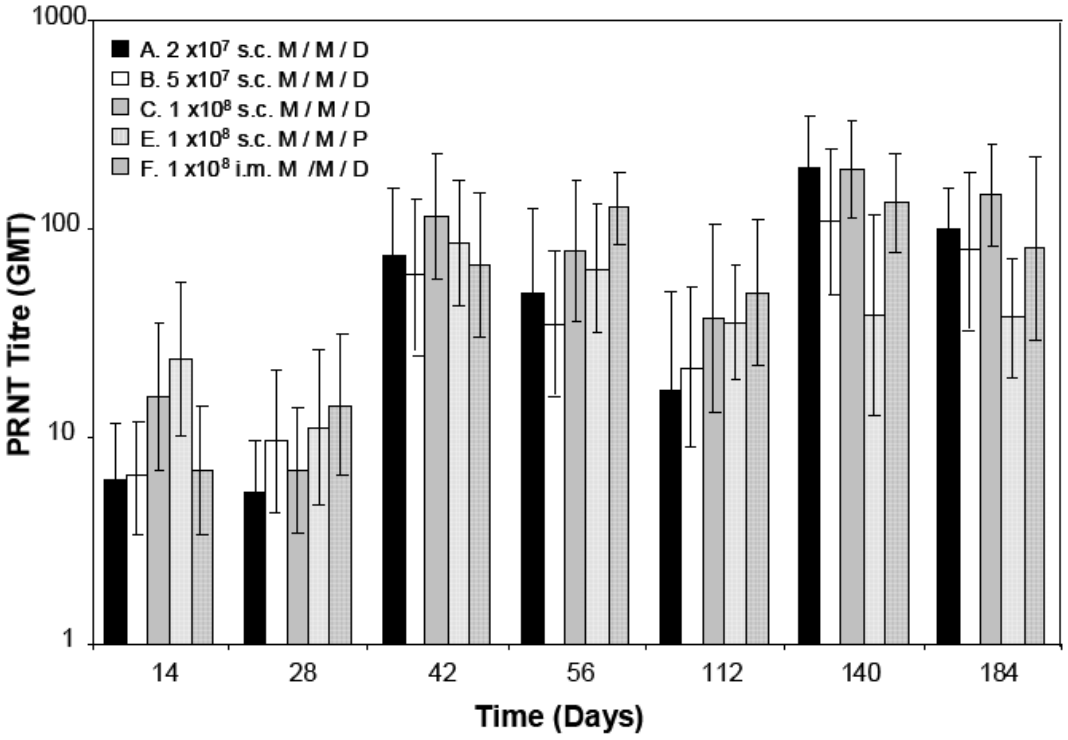


Figure 2. Neutralizing Antibody Titers by a validated PRNT following various vaccination regimes in healthy subjects

The trends in PRNT titres were comparable with those observed using the other (non-validated) assays.

At 4 weeks after the first dose of MVA-BN the seroconversion rates (40% - 73%) were lower than the 100% observed after Dryvax. As shown in the figure below, for the highest dose of MVA-BN the GMTs were 108 (group C) and 86 (group E) on Day 42. These values were not significantly different from the GMT induced by Dryvax (GMT 149; Day 140).

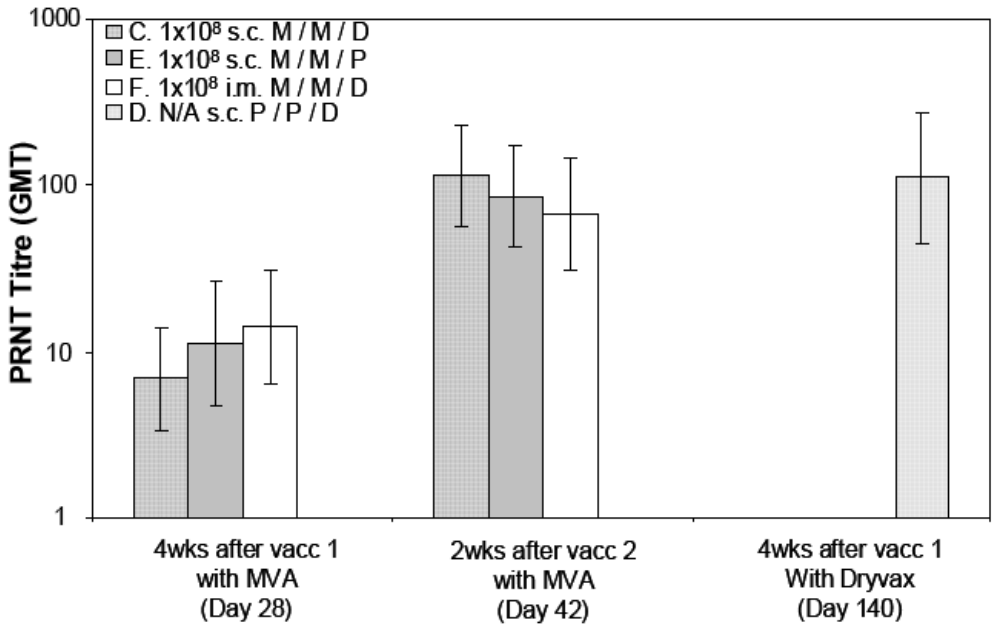


Figure 5. Comparison of the peak neutralizing antibody responses induced by IMVAMUNE® compared to Dryvax® in healthy subjects.

The comparison of titres reported from the validated ELISA (MVA-BN as antigen) and validated PRNT (VV-WR antigen) showed a highly significant correlation.

Table 23 Linear regression analysis of the PRNT and ELISA data

Time (Day)	p-value
42(2 weeks after 2nd Imvamune vaccination	0.004
56(4 weeks after 2nd Imvamune vaccination	0.003
112(12 weeks after 2nd Imvamune vaccination	0.0008
140(4 weeks after Dryvax vaccination	0.0002
184(10 weeks after Dryvax vaccination	1.7E-09

PRNT against variola virus

The CDC paper from Damon et al. 2009 (Evaluation of smallpox vaccines using variola neutralization) reports PRNT titres against variola strain Solaimen.
The paper describes results on testing 106 sera from 53 subjects in DMID02-017 (i.e. POX-MVA-002) including 14 who received Dryvax (sera obtained 28-30 days post-dose) plus 26 and 15 who received two doses 28 days apart of SC or IM MVA-BN (sera obtained 14 days after the second dose) at the selected $TCID_{50}$. The assessor presumes that the 26 sera tested from subjects who received SC MVA-BN comprised Groups C+E, which conflicts with the applicant's summary description that refers only to Group C in this regard. VIG was used as a positive control.

It is stated that the sera obtained represented peak response sera. However, as mentioned above, humoral antibody was measured on D0 and at D14 and D28 after each dose of MVA-BN, immediately prior to administration of Dryvax and then at D28 and D70 after Dryvax. These data do not substantiate a claim that the comparison between vaccines represents a comparison of peak titres.

It appears that four technicians performed the assays but it is not stated how the four sets of results were used to derive the reported data. It is stated that non-parametric statistics were used for comparisons between groups because the data were not normally distributed. Percentage plaque reductions were compared between groups for each dilution using a Wilcoxon rank-sum test. Linear regression was applied to a log transformation of serum dilutions per individual to facilitate linear interpolation of their actual PRNT60 and PRNT90 titres. PRNT60 beyond 1:1280 was extrapolated based on 6 dilution points.

Subjects in this study (n=90) were to have been vaccinia-naïve and aged 18-32 years. However, the pre-vaccination PRNT60 GMTs using this assay ranged from 15-28 while PRNT90 GMTs were from 3-6 (see Table below).

Table 24 Number of persons achieving 60 and 90% neutralisation at each dilution by vaccine treatment group and GMTs using log linear transformation for 60 and 90% neutralisation at pre and post-vaccination by vaccine group

Vaccine treatment	**N**	**Dilution**						
		1:40	1:80	1:160	1:320	1:640	1:1280	> 1:1280
Number (%) persons achieving 60 % neutralisation								
MVA SC	26	26(100)	25(96.2)	23(88.5)	20(76.9)	11(42.3)	10(38.5)	9(34.6)
Dryvax	12	12(100)	12(100)	10(83.3)	7(58.3)	5(41.6)	3(25)	2(16.7)
MVA IM	15	15(100)	15 (100.0)	14(93.3)	10(66.7)	6(40)	4(26.7)	4(26.7)
Number (%) persons achieving 90 % neutralisation								
MVA SC	26	18(69.2)	11(42.3)	8(30.8)	4(15.4)	1(3.8)	0(0)	0(0)
Dryvax	12	4(33.3)	2(16.7)	1(8.3)	0(0)	0(0)	0(0)	0(0)
MVA IM	15	11(73.3)	8(55.3)	1(6.7)	1(6.7)	0(0)	0(0)	0(0)
Vaccine treatment	**N**	**Pre-vaccination**			**Post-vaccination**			
		GMT	**95% LCL**	**95% UCL**	**GMT**	**95% LCL**	**95% UCL**	
GMTs at 60% neutralisation								
MVA SC	25	27.83	16.96	46.68	1735.62	551.59	5461.28	
Dryvax	10	27.00	14.70	49.61	688.23	247.82	1915.95	
MVA IM	14	15.34	9.94	23.66	1617.50	289.82	9027.28	
GMTs at 90% neutralisation								
MVA SC	16	4.15	3.15	5.46	79.23	55.77	112.56	
Dryvax	12	5.96	3.79	9.39	43.81	29.34	65.41	
MVA IM	15	2.96	2.13	4.13	73.60	47.82	113.30	

The footnote of the table below shows that four subjects had PRNT60 titres of at least 1:40 prior to vaccination and were not included in the analysis. After exclusion, the authors state that there was no difference between vaccines in proportions with 4-fold and 8-fold increases in PRNT60 titres but there was a difference for the PRNT90 titres as shown below.

Vaccine treatment	60% neutralization			90% neutralization		
	n*	4-fold increase	8-fold increase	n	4-fold increase	8-fold increase
MVA SC	25	24 (96.0)	22 (88.0)	26	26 (100.0)†	23 (88.5)
Dryvax	10	10 (100.0)	10 (100.0)	12	9 (75.0)†	7 (58.3)
MVA IM	14	14 (100.0)	13 (92.9)	15	15 (100.0)	14 (93.3)

*Two persons in the Dryvax arm and one each in the MVA SC and MVA IM arms had 60% VAR PRNT titres ≥ 1:40 prior to vaccination and were not included in the analysis.
†A statistically significantly higher proportion of MVA SC vaccinees achieved a fourfold rise compared with Dryvax vaccinees (P=0.03).

At dilutions > 1:40 all sera neutralised > 60% of the variola virus used per neutralisation. The Table below shows that there was a difference between groups that reached significance only at the 1:40 dilutions (p = 0.02).

Table 25 Aggregate mean percentage plaque reductions at each dilution level by vaccine treatment group

Vaccine treatment	N	Dilution					
		1:40	1:80	1:160	1:320	1:640	1:1280
MVA SC	26	90.86	85.63	78.47	69.53	59.98	52.42
Dryvax	12	86.77	82.23	74.68	64.58	54.99	44.05
MVA IM	15	91.10	86.61	78.79	71.34	60.09	47.26

The authors concluded that the data suggest the vaccines (2 doses MVA-BN; one dose Dryvax) elicited at least comparable variola neutralising antibody.

The applicant was requested to provide the vaccinia PRNT data and ELISA data for individual subjects for whom variola neutralisation titres were available. It was not possible to use the same method for the determination of neutralization titres for all samples for the two assays. The CDC used a fixed series of dilutions for pre- (1:10 - 1:40) and post-immunisation sera (1:40 - 1:1,280). Due to the limitations imposed re use of variola, repeat testing was not performed. Instead extrapolations were used for the determination of 60% and 90% PRNT titres for sera that did not provide plaque counts that allowed direct quantitation of titres.
BN routinely uses a linear regression method for the determination of 50% PRNT titres and does not extrapolate titres for subjects not achieving 50% plaque counts; only direct quantitation is allowed.

For the purpose of the requested analysis, BN calculated 50% PRNT titres from the CDC variola PRNT raw data using the "closest dilution method". All available quantitative results (log10 titres) obtained from the variola PRNT were subjected to a correlation analysis against the corresponding results from the BN PRNT and ELISA and r2 value, slope and intercept were calculated. However, due to the limitations imposed by the variola PRNT (lack of repeat testing at alternative specimen dilutions), quantitative PRNT titres could not be obtained from all samples. Some samples resulted in discrete PRNT titres (e.g. < 10, > 40 and > 1,280) and these data could not be used in the correlation analysis, which was confined to the numbers shown in Table 26.

Table 26 Pre- and post-vaccination sera used for the correlation analysis

Group	Treatment	Post-vaccination	Number of pre-vaccination sera	Number of post-vaccination sera
C	SC MVA MVA Dryvax	D42, 2 weeks post 2nd MVA	8	10
D	SC Placebo Placebo Dryvax	D140, 28 d-30 days post Dryvax	8	9
E	SC MVA MVA Placebo	D42, 2 weeks post 2nd MVA	5	5
F	IM MVA MVA Dryvax	D42, 2 weeks post 2nd MVA	9	9
All groups combined	NA	D42, 2 weeks post 2nd MVA or 28-20 days post Dryvax	30	33

The Figure below shows the pooled results from all treatment groups and all time points for which quantitative variola PRNT titres were available. A Spearman Rank Order Correlation analysis was performed on the CDC variola PRNT titres vs. the BN PRNT titres (n=63) resulting in a correlation coefficient of 0.82 and a p-value <0.0001.

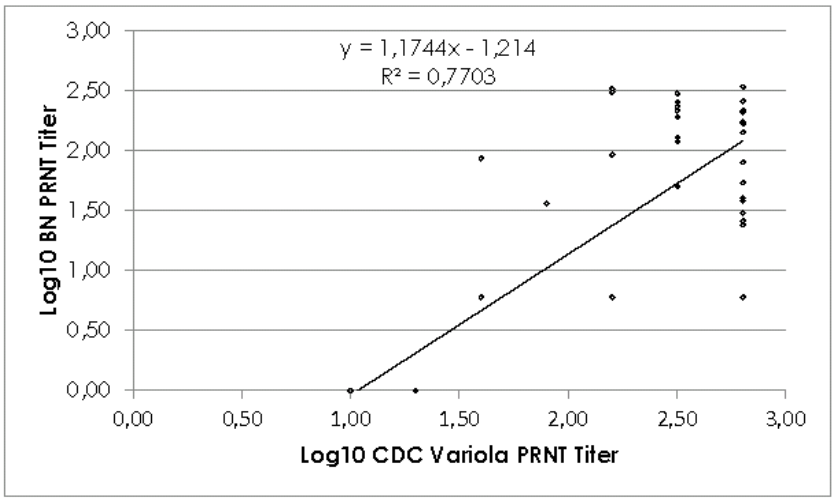


Figure 1 Correlation between CDC variola PRNT titers and BN PRNT titers - All treatment groups combined (n=63)

A correlation analysis was also performed separating the individual treatment groups. Although the number of results was limited, the r2 values obtained from the MVA-BN and Dryvax groups were > 0.73. The slopes and intercepts were very similar for the two groups, indicating that the correlation between the two PRNTs was not dependent upon the vaccine.

Table 27 Correlation analysis – BN PRNT vs variola PRNT

Group	Number of sera (n)	Treatment	R^2	Slope	Intercept
C+E	28	SC Imvanex	0.8113	1.2227	-1.267
D	17	Dryvax	0.8113	1.4027	-1.4950
F	18	IM Imvanex	0.7332	0.9831	-1.0209

A Spearman Rank Order Correlation analysis between the CDC variola PRNT titres vs. the BN ELISA titres (n=63) resulted in a correlation coefficient of 0.85 and a p-value <0.0001.

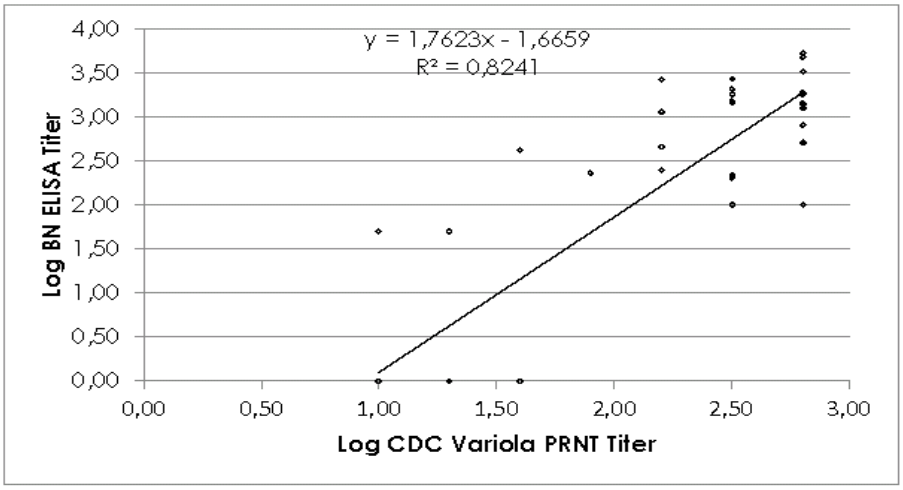


Figure 2 Correlation between CDC variola PRNT titers and BN ELISA titers - All treatment groups combined (n=63)

Table 28 showed r2 values > 0.79 for results obtained from MVA-BN and Dryvax subjects and the slopes and intercepts were very similar, indicating that the correlation between assays was not dependent upon the vaccine.

Table 28 Correlation analysis – BN ELISA vs. variola PRNT

Group	Number of sera (n)	Treatment	R^2	Slope	Intercept
C+E	28	SC Imvanex	0.8269	1.8564	-1.6764
D	17	Dryvax	0.7945	1.4963	-1.5181
F	18	IM Imvanex	0.8847	1.7480	-1.6038

This additional analysis concerned 63 sera, which is less than the 106 sera reported on by Damon *et al*. 2009. The pre-vaccination PRNT60 GMTs using the CDC's assay ranged from 15-28 while PRNT90 GMTs were from 3-6 although subjects in POX-MVA-002 were to have been vaccinia-naïve and aged 18-32 years. While there were several issues regarding how Damon and co-workers analysed the results the authors pointed out that there was no difference between vaccines in proportions with 4-fold and 8-fold increases in PRNT60 titres but there was a difference for the PRNT90 titres.

It can be observed that for the most part individual subjects that showed increments in titres post-vaccination did so whether the CDC or BN PRNT or BN ELISA assay was applied. However,

some subjects in both the MVA-BN and the Dryvax groups showed quite large differences between assay results.

POX-MVA-009

The study was conducted during 2007-2009 at 7 US study sites. The initial study protocol was amended after 20 subjects had been enrolled (8 received Dryvax not following or with MVA-BN; antibody response data are included in the figures below) due to FDA concern regarding the use of Dryvax. The final protocol required that 195 vaccinia-naïve healthy subjects were to be randomly assigned to one of three MVA-BN dose groups as shown below.

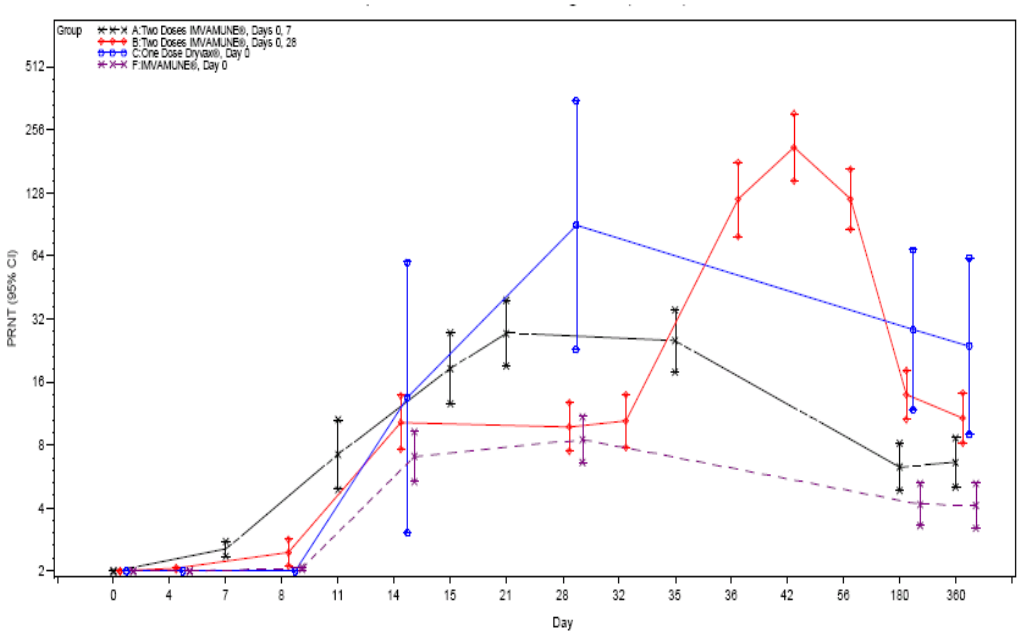
Group	Vaccine/Day	N = Vaccine/ Placebo	Dose/ Volume[1]	Day 0	Day 7	Day 28
Group A	Two Doses IMVAMUNE® Days 0, 7	60	1×10^8/ 0.5 mL	Vaccine	Vaccine	
		5	Saline Placebo 0.5 mL	Placebo	Placebo	
Group B	Two Doses IMVAMUNE® Days 0, 28	60	1×10^8/ 0.5 mL	Vaccine		Vaccine
		5	Saline Placebo 0.5 mL	Placebo		Placebo
		*2**	*Previously enrolled and remained blinded as to IMVAMUNE® or Placebo*			
Group F	IMVAMUNE® Day 0	60	1×10^8/ 0.5 mL	Vaccine		
		5	Saline Placebo 0.5 mL	Placebo		
Totals 3 Groups		N = 197 180/15 + *2* Previously Enrolled in Group B (Vaccine or Placebo)* = 197				

[1] IMVAMUNE® was administered SC. Placebo was administered SC.

Sera were assayed using both ELISA and PRNT but with different methodologies at Saint Louis University (SLU) and in the applicant's laboratories. Comparisons between groups were made on days 4, 8, 14, 28, 180 and 365 after the second assigned dose. In the 8 subjects who received Dryvax on Day 0 sampling was at days 4, 8, 14, 28 and then on days 180 and 365.
PRNT performed by SLU: The comparison of GMTs showed that non-inferiority of Group A vs. B was not demonstrated at any of the six time points after the second dose, including day 14. The figure also shows GMTs in the original protocol Group C, in which 8 subjects were enrolled and received Dryvax on D0.

14.2.5.1 Geometric Mean Titers by Treatment Group – SLU PRNT

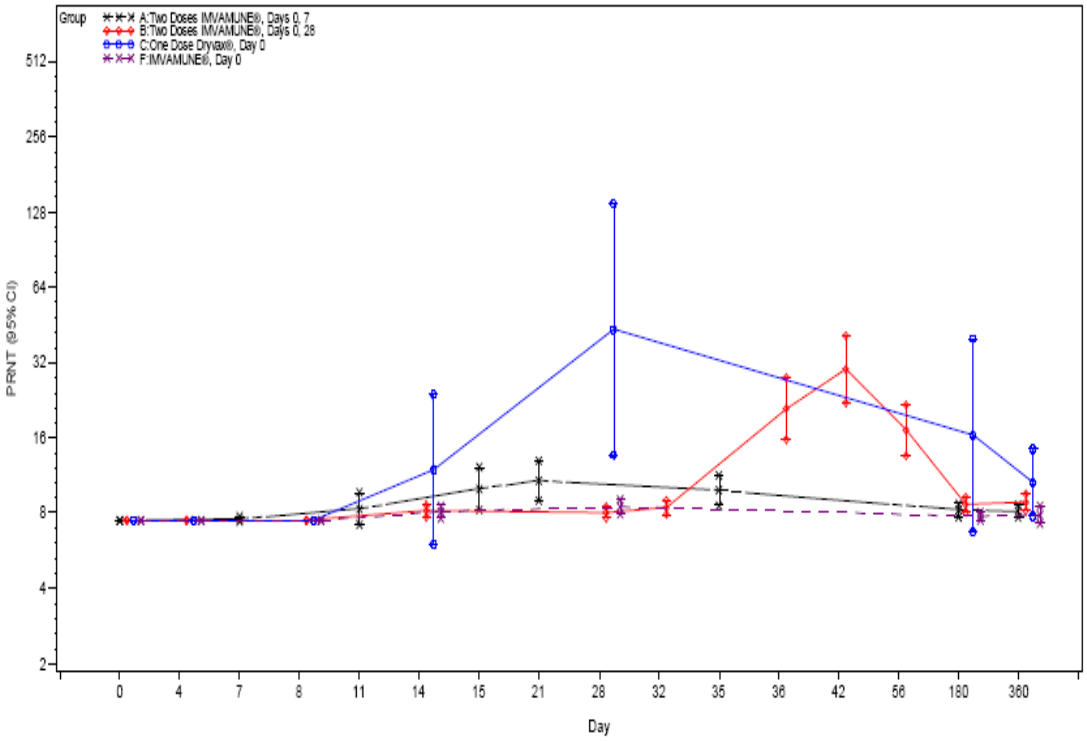
Groups A, B, C and F



PRNT performed by Bavarian Nordic: The comparison of GMTs showed that Group A was non-inferior to Group B only on Days 4, 180 and 365 after the second assigned dose. The proportions with positive titres (at least 1:15) in Group B were statistically significantly greater than in Group A on Days 8, 14 and 28.

14.2.5.2 Geometric Mean Titers by Treatment Group – BN PRNT

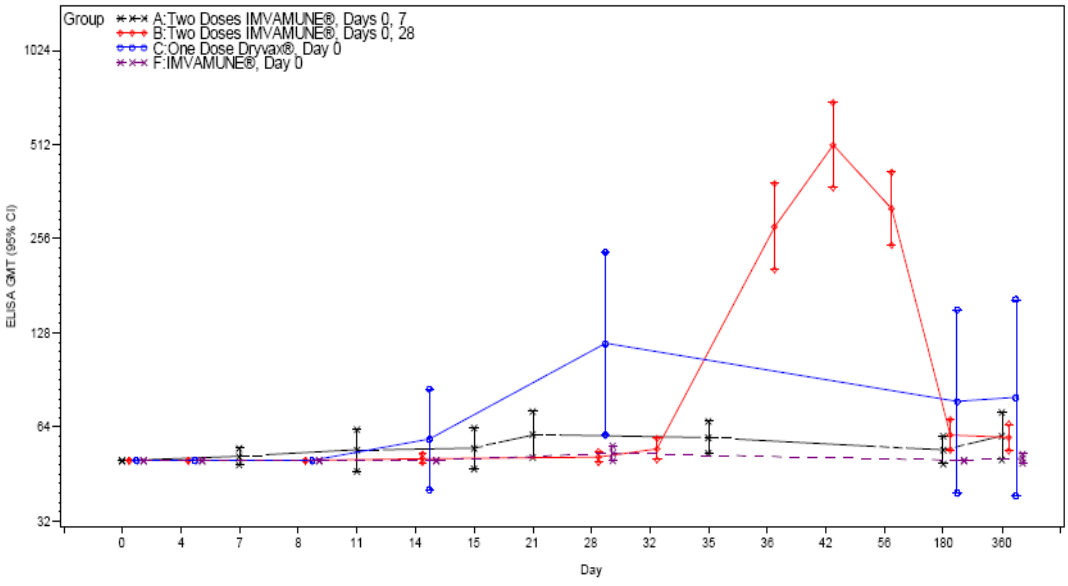
Groups A, B, C, and F



ELISA performed by SLU: Based on the comparison of GMTs, Group A was non-inferior to Group B on Days 4, 180 and 365. The proportions with positive titres were statistically significantly greater in Group B vs. Group A at Days 8, 14, 28 and 180 after the second dose.

14.2.5.3 Geometric Mean Titers by Treatment Group - SLU ELISA

Groups A, B, C, and F

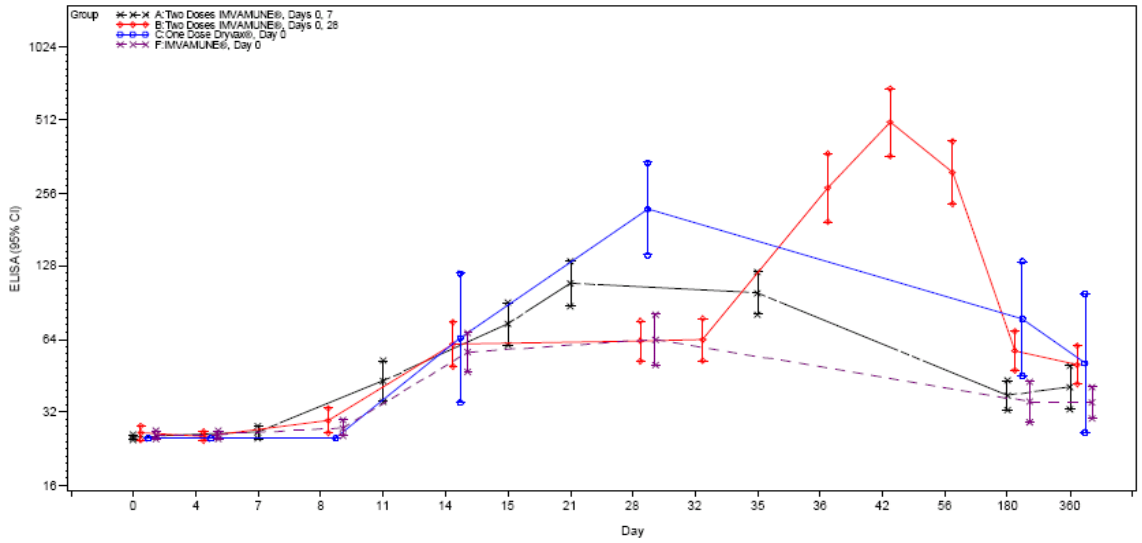


ELISA performed by Bavarian Nordic

Based on comparisons of GMTs, Group A was non-inferior to Group B on Days 4, 180 and 365. The proportions with a positive titre in Group B were significantly greater than in Group A on Days 4 and 180 after the second dose.

14.2.5.4 Geometric Mean Titers by Treatment Group - BN ELISA

Groups A, B, C, and F



IFN-γ ELISPOT performed by CTL

The applicant stated that a second dose of MVA-BN at Day 28 compared to Day 7 provided a greater antibody response and the maximal number of responders with at least a 4-fold rise. CMI data were not provided for the Dryvax group.

Summary of main studies

The following tables summarise the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Summary of Immunogenicity for trial POX-MVA-005

A partially randomized, partially double-blind, placebo-controlled Phase II non-inferiority study to evaluate immunogenicity and safety of one and two doses of MVA-BN (IMVANEX) smallpox vaccine in 18-55 year old healthy subjects		
Study identifier	POX-MVA-005	
Design	Partially randomised, partially double-blind, placebo-controlled	
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:	8 weeks 4 weeks 6 months
Hypothesis	Non-inferiority	
Treatments groups	Group 1 (blinded), vaccinia-naïve	2 doses IMVANEX®; 4 weeks apart N = 183 (randomised) N = 183 (vaccinated)

	Group 2 (blinded), vaccinia-naïve		1st dose IMVANEX® , 2nd dose placebo; 4 weeks apart; N = 184 (randomised) N = 181 (vaccinated)
	Group 3 (blinded), vaccinia-naïve		2 doses placebo; 4 weeks apart; N = 182 (randomised) N = 181 (vaccinated)
	Group 4 (open label vaccinia-experienced		1 dose IMVANEX®; N = 204 (randomised) N = 200 (vaccinated)
Endpoints and definitions	Primary endpoint	Immunogenicity	Vaccinia-specific seroconversion rate by ELISA at the peak visit
	Secondary endpoint	Immunogenicity	Vaccinia-specific seroconversion rate by ELISA 4 weeks after the last vaccination Vaccinia-specific seroconversion rate by PRNT at the peak visit and 4 weeks after the last vaccination
Database lock	15-Nov-2007		

Results and Analysis

Analysis description	**Primary Analysis**			
Analysis population and time point description	Intent to treat (Full Analysis Set)			
Descriptive statistics and estimate variability	Treatment group	Group 1	Group 3	Group 4
	Number of subject	183	181	200
	Peak visit ELISA SC (%)	98.9%	3.4%	95.5%
	95% exact confidence interval	(96.0,99.9)	(1.3, 7.3)	(91.6, 97.9)
	Peak visit PRNT SC (%)	89.2%	1.1%	78.5%

	95% exact confidence interval	(83.7, 93.4)	(0.1, 4.0)	(72.2, 84.0)

Effect estimate per comparison	Primary endpoint Peak Visit ELISA SC	Comparison groups	Group 4 – Group 1
		Difference SC rate (%)	-3.4 %
		Lower limit 95% CI	-7.36%
		P-value (Non-inferiority)	0.2162
	Secondary endpoint Peak Visit PRNT SC	Comparison groups	Group 4 – Group 1
		Difference SC rate (%)	-10.7 %
		Lower limit 95% CI	-18.18%
		P-value (Non-inferiority)	0.9353
Notes	SC = Seroconversion (defined as an at least two-fold rise in titres, or becoming seropositive in originally seronegative subjects) Non-inferiority analysis using a 5% non-inferiority margin (delta)		
Analysis description	**Other Secondary Immunogenicity analyses**		

Seroconversion rates and Geometric Mean Titers were calculated at each visit for both ELISA and PRNT

Seroconversion rates by ELISA and PRNT at each visit

Week (Day)	**Seroconversion Rate % (95% CI)**			
	Group 1 (N=183)	**Group 2 (N=181)**	**Group 3 (N=181)**	**Group 4 (N=200)**
ELISA				
Week 0 (0)	NAP	NAP	NAP	NAP
Week 2 (14)	70.9 (63.7, 77.4)	72.6 (65.5, 79.0)	2.8 (0.9, 6.4)	95.5 (91.6, 97.9)
Week 4 (28)	88.9 (83.4, 93.1)	87.4 (81.5, 91.9)	2.9 (0.9, 6.5)	93.0 (88.5, 96.1)
Week 6 (42)	98.9 (96.0, 99.9)	82.2 (75.7, 87.6)	3.4 (1.3, 7.3)	NAP
Week 8 (56)	98.9 (96.0, 99.9)	72.0 (64.7, 78.5)	2.8 (0.9, 6.5)	NAP
Up to Week 35 (245): FUV – Groups 1, 2 and 3 Up to Week 30 (210): FUV – Group 4	73.0 (65.9, 79.4)	37.9 (30.7, 45.6)	2.3 (0.6, 5.7)	67.8 (60.9, 74.3)
PRNT				
Week 0 (0)	NAP	NAP	NAP	NAP
Week 2 (14)	45.1 (37.7, 52.6)	52.0 (44.4, 59.5)	1.1 (0.1, 4.0)	78.5 (72.2, 84.0)
Week 4 (28)	56.7 (49.1, 64.0)	62.1 (54.4, 69.3)	0.6 (0.0, 3.1)	69.8 (63.0, 76.1)
Week 6 (42)	89.2 (83.7, 93.4)	56.3 (48.6, 63.8)	0.0 (0.0, 2.1)	NAP
Week 8 (56)	86.0 (80.0, 90.7)	47.4 (39.8, 55.1)	0.0 (0.0, 2.1)	NAP
Up to Week 35 (245): FUV – Groups 1, 2 and 3 Up to Week 30 (210): FUV – Group 4	65.2 (57.7, 72.1)	23.6 (17.5, 30.6)	0.6 (0.0, 3.1)	63.8 (56.7, 70.5)

N= number of subjects in specified group, NAP = Not applicable ,

GMT Responses by ELISA and PRNT at all visits				
Week (Day)	**GMT Response (95% CI)**			
	Group 1 (N=183)	**Group 2 (N=181)**	**Group 3 (N=181)**	**Group 4 (N=200)**
ELISA				
Week 0 (0)	1.4 (1.2, 1.7)	1.3 (1.1, 1.5)	1.1 (1.0, 1.3)	38.8 (29.4, 51.3)
Week 2 (14)	27.8 (20.1, 38.3)	29.3 (21.5, 39.9)	1.2 (1.1, 1.4)	568.8 (473.3, 683.7)
Week 4 (28)	71.8 (56.9, 90.7)	60.3 (47.6, 76.5)	1.3 (1.1, 1.4)	452.3 (388.8, 526.2)
Week 6 (42)	495.8 (431.9, 569.3)	42.3 (32.7, 54.8)	1.2 (1.1, 1.4)	NAP
Week 8 (56)	328.7 (288.5, 374.4)	23.2 (17.4, 31.0)	1.2 (1.1, 1.4)	NAP
Up to Week 35 (245): FUV – Groups 1, 2 and 3 Up to Week 30 (210): FUV – Group 4	27.9 (20.7, 37.6)	5.3 (3.9, 7.2)	1.2 (1.0, 1.3)	179.9 (148.9, 217.4)
PRNT				
Week 0 (0)	1.1 (1.0, 1.2)	1.1 (1.0, 1.1)	1.0 (1.0, 1.1)	21.6 (16.3, 28.5)
Week 2 (14)	4.8 (3.6, 6.3)	5.1 (3.9, 6.6)	1.1 (1.0, 1.1)	175.2 (140.0, 219.1)
Week 4 (28)	7.5 (5.7, 10.0)	7.2 (5.5, 9.4)	1.0 (1.0, 1.1)	144.3 (117.9,176.5)
Week 6 (42)	45.6 (35.1, 59.2)	5.9 (4.5, 7.6)	1.0 (1.0, 1.1)	NAP
Week 8 (56)	34.0 (26.4, 43.9)	4.2 (3.3, 5.4)	1.0 (1.0, 1.1)	NAP
Up to Week 35 (245): FUV – Groups 1, 2 and 3 Up to Week 30 (210): FUV – Group 4	7.2 (5.6, 9.4)	1.9 (1.6, 2.2)	1.0 (1.0, 1.1)	106.5 (89.1, 127.2)

N= number of subjects in specified group, NAP = Not applicable

Summary of Immunogenicity for trial POX-MVA-008

A multicenter, open-label, controlled phase II study to evaluate safety and immunogenicity of MVA-BN (IMVANEX) smallpox vaccine in 18-40 year old subjects with diagnosed atopic dermatitis		
Study identifier	POX-MVA-008	
Design	Open-label, controlled	
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:	8 weeks 4 weeks 6 months
Hypothesis	Non-inferiority	
Treatments groups	Group 1, vaccinia-naïve, healthy	2 doses IMVANEX®; 4 weeks apart N = 282 (vaccinated)

	Group 2; vaccinia-naïve, Diagnosed Atopic Dermatitis		2 doses IMVANEX®; 4 weeks apart N = 350 (vaccinated)
Endpoints and definitions	Primary endpoint	Immunogenicity	Vaccinia-specific seroconversion rate by ELISA 2 weeks after the last vaccination (visit 4)
	Secondary endpoint	Immunogenicity	Vaccinia-specific seroconversion rate by ELISA at all other time points Vaccinia-specific GMT by ELISA at all blood sampling time points Vaccinia-specific seroconversion rate and GMT by PRNT at all blood sampling time points IFN gamma producing T-cells in response to stimulation with MVA-BN® detected by Enzyme-Linked Immunospot (subgroup only)
Database lock	09-Apr-2010		

Results and Analysis

Analysis description	**Primary Analysis**		
Analysis population and time point description	Per Protocol		
Descriptive statistics and estimate variability	Treatment group	Group 1	Group 2
	Number of subject	194	257
	ELISA SC rate (%) Visit 4	98.5%	97.3%
	95% exact CI	(95.5, 99.7)	(94.5, 98.9)
	PRNT SC rate (%) Visit 4	86.6%	90.3%
	95% exact CI	(81.0, 91.1)	(86.0, 93.6)
Effect estimate per comparison	Primary endpoint	Comparison groups	Group 2 – Group 1
		Difference SC rate (%)	-1.2%
		Lower 95% CI limit	-4.31
		P-value	Non-inferiority passed
	Secondary endpoint	Comparison groups	Group 2 – Group 1
		Difference SC rate (%)	3.7%
		Lower 95% CI limit	-2.31%
		P-value	Non-inferiority passed
Notes	SC = Seroconversion (at least a two fold rise in titers, or becoming seropositive in originally seronegative subjects) Non-inferiority analysis using a 5% non-inferiority margin (delta)		
Analysis description	**Secondary immunogenicity analysis**		
	Geometric Mean titers at Visit 4 were: ELISA: Group 1 GMT = 499.4 (417.1, 598.0) Group 2 GMT = 532.9 (452.9, 627.1) PRNT: Group 1 GMT = 34.6 (26.4, 45.3) Group 2 GMT = 47.7 (38.1, 59.8)		

Summary of Immunogenicity for trial POX-MVA-011

A multicenter, open-label, controlled phase II study to evaluate safety and immunogenicity of MVA-BN (IMVANEX) smallpox vaccine in 18-55 year old naive and previously vaccinated HIV infected subjects with CD4 counts > 200 – 750/µl.			
Study identifier	POX-MVA-011		
Design	Multicenter, open-label, controlled, phase II study		
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:	8 weeks 4 weeks 6 months	
Hypothesis	Exploratory: Safety and Immunogenicity		
Treatments groups	Group 1 Healthy Vaccinia-naïve or vaccinia-experienced	2 doses IMVANEX®; 4 weeks apart N= 88 (vaccinia-naïve) N= 9 (vaccinia-experienced)	
	Group 2 a HIV-1, CD4 cells ≥ 350-500 cells/µL Vaccinia-naïve or vaccinia-experienced	2 doses IMVANEX®; 4 weeks apart N= 351 (vaccinia-naïve) N= 131 (vaccinia-experienced) HIV (CD4 750-501/µl) N= 99 (vaccinia-naïve) N= 46 (vaccinia-experienced) HIV (CD4 500-350/µl) N= 163 (vaccinia-naïve) N= 61 (vaccinia-experienced) HIV (CD4 349-200/µl) N= 89 (vaccinia-naïve) N= 24 (vaccinia-experienced)	
	Group 2 b HIV-1, CD4 cells ≥ 200-750 cells/µL Vaccinia-naïve		
	Group 3 HIV-1, CD4 cells ≥ 200-750 cells/µL, Vaccinia-experienced		
Endpoints and definitions	Primary endpoint	Safety	Occurrence, relationship and intensity of any serious and or unexpected adverse reaction at any time during the study.
	Secondary endpoint	Immuno-genicity	Vaccinia-specific seroconversion rate and GMT by PRNT at all blood sampling time points. Vaccinia-specific seroconversion rate and GMT by ELISA at all blood sampling time points. IFN gamma producing T-cells in response to stimulation with MVA-BN® detected by Enzyme-Linked Immunospot (subgroup only).
Database lock	30-Jun-2009		

Results and Analysis

Analysis description	**Secondary Immunogenicity Analysis**				
Analysis population and time point description	Intent to treat (FAS) Visit 4 (2 weeks after second vaccination)				
Descriptive statistics and estimate variability	Treatment group	Healthy Vaccinia-naive	HIV Vaccinia-naive	Healthy Vaccinia-experienced	HIV Vaccinia-experienced
	Number of subject	88	351	9	131
	ELISA SC rate (%)	98.7%	96.2%	100%	92.7%
	95% exact CI	(93.1, 100)	(93.4, 98.0)	(59.0, 100)	(86.6, 96.6)
	PRNT SC rate (%)	77.2%	60.3%	85.7%	75.6%

	95% exact CI	(66.4, 85.9)	(54.7, 65.8)	(42.1, 99.6)	(67.0, 82.9)
	ELISA GMT	560.6	282.9	610.2	525.8
	95% CI	(441.3, 712.3)	(241.2, 331.7)	(211.3, 1762.0)	(435.2, 635.2)
	PRNT GMT	21.7	13.1	358.4	69.0
	95% CI	(13.7, 34.6)	(10.1, 17.0)	(117.5, 1093.3)	(48.3, 98.6)
Effect estimate per comparison	No hypothesis tests of comparison were made between the above subject strata.				
Notes	SC = Seroconversion (at least a two fold rise in titers, or becoming seropositive in originally seronegative subjects) GMT = Geometric Mean Titer. The Confidence Interval (CI) was calculated under the assumption that the log titers have a normal distribution.				
Analysis description	**Tertiary Analysis**				
	No significant differences (trends) were detected across the HIV CD4 substrata for either the naïve or experienced subjects for PRNT or ELISA				

Summary of Immunogenicity for trial POX-MVA-023

An Open-Label Phase II Study to Evaluate Immunogenicity and Safety of a Single IMVANEX Booster Vaccination Two Years after the Last IMVANEX Vaccination in Former POX-MVA-005 Vaccinees			
Study identifier	POX-MVA-023		
Design	Open label		
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:		4 weeks 6 weeks 6 months
Hypothesis	Non-inferiority		
Treatments groups	Group 1 IMVANEX®-experienced / vaccinia-naive		One booster dose N = 75 (vaccinated) Blood draw only N = 17
	Group 2 IMVANEX®-experienced / vaccinia-naive		One booster dose N = 77 (vaccinated) Blood draw only N = 14
	Group 4 IMVANEX®-experienced / vaccinia-experienced		Blood-draw only N = 121
Endpoints and definitions	Primary endpoint	Immunogenicity	Vaccinia-specific seroconversion rate (booster rate) by ELISA after booster vaccination with IMVANEX® from the individual peak response
	Secondary endpoint	Immunogenicity	Vaccinia-specific seroconversion rate and GMTs by ELISA after booster vaccination with IMVANEX® measured for all individual blood sampling time-points. Vaccinia-specific seroconversion rate and GMTs by PRNT for all individual blood sampling time-points.
Database lock	02-Oct-2009		
Results and Analysis			
Analysis description	**Primary Analysis**		
Analysis population and time point description	Intent to treat (Full Analysis Set)		

Descriptive statistics and estimate variability	Treatment group	Group 1	Group 2
	Number of subject	75	77
	ELISA individual peak SC rate (%)	100%	100%
	95% exact CI	(95.2, 100)	(95.3, 100)
	PRNT individual peak SC rate (%)	98.7%	96.1%
	95% exact CI	(92.8, 100)	(89.0, 99.2)
Effect estimate per comparison	Primary endpoint Difference in individual peak ELISA SC rate	Comparison groups	Group 1 – Group 2
		Difference SC rate (%)	0.0%
		95% exact CI	NAP
		P-value	NAP
	Secondary endpoint Difference in individual peak PRNT SC rate	Comparison groups	Group 1 – Group 2
		Difference SC rate (%)	2.6%
		95% exact CI	(-2.5, 7.6)
		P-value	0.620
Notes	SC = Seroconversion (at least a two fold rise in titers, or becoming seropositive in originally seronegative subjects)		
Analysis description	**Other, specify:** Long-term persistence of anti-vaccinia antibody titers after two years in former POX-MVA-005 study Groups 1, 2 and 4.		

Seropositivity rates (ELISA and PRNT) and GMT Responses (ELISA and PRNT) 2 years after priming vaccination with 1 or 2 doses of IMVANEX® in vaccinia-naïve subjects (Group 1 and Group 2) and 2 years after one booster dose in vaccinia-experienced subjects (Group 4)

		ELISA		PRNT	
	N	**GMT (95% CI)**	**S+% (95% CI)**	**GMT (95% CI)**	**S+% (95% CI)**
Group 1	**92**	23.3 (15.2, 35.9)	71.7 (61.4, 80.6)	1.3 (1.0,1.5)	5.4 (1.8, 12.2)
Group 2	**91**	6.2 (4.0, 9.7)	42.9 (32.5, 53.7)	1.1 (1.0,1.1)	1.1 (0.0, 6.0)
Group 4	**121**	134.6 (111.9, 162.0)	97.5 (92.9, 99.5)	10.3 (7.2, 14.6)	68.6 (59.5, 76.7)

GMT= Geometric Mean Titer
S+ = Seropositivity
CI = confidence interval
N = total number of subjects

Summary of Immunogenicity for trial POX-MVA-024

A randomized, double-blind, placebo-controlled Phase II study to evaluate safety and immunogenicity of one and two doses of IMVANEX smallpox vaccine in 56-80 year old vaccinia-experienced subjects		
Study identifier	POX-MVA-024	
Design	randomized, double-blind, placebo-controlled, phase II study	
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:	8 weeks 4 weeks 6 months
Hypothesis	Exploratory: Safety and Immunogenicity	
Treatments groups	Group 1 (MM)	2 doses IMVANEX®; 4 weeks apart N= 62 (randomised) N= 61 (vaccinated)

	Group 2 (PM)		1 dose Placebo and 1 dose IMVANEX® 4 weeks apart, N= 58 (randomised) N= 58 (vaccinated)
Endpoints and definitions	Primary endpoint	Safety	Occurrence of any serious adverse events associated with the study vaccine occurring until the last active study visit.
	Secondary endpoint	Immuno-genicity	Vaccinia-specific seroconversion rate by ELISA at the peak visit. Vaccinia-specific seroconversion rate and GMTs by ELISA for all individual blood sampling time-points. Vaccinia-specific seroconversion rate by PRNT at the peak visit. Vaccinia-specific seroconversion rate and GMTs by PRNT for all individual blood sampling time-points.
Database lock	20-Oct-2010		

Results and Analysis

Analysis description	**Secondary Immunogenicity Analysis**		
Analysis population and time point description	Intent to treat (Full Analysis Set)		
Descriptive statistics and estimate variability	Treatment group	Group 1 (MM)	Group 2 (PM)
	Number of subject	61	58
	ELISA SC rate (%) (peak visit)	83.6%	82.8%
	95% exact CI	(71.9, 91.8)	(70.6, 91.4)
	PRNT SC rate (%) (2 weeks after final vaccination)	90.0%	77.6%
	95% exact CI	(79.5, 96.2)	(64.7, 87.5)

Effect estimate per comparison	Primary endpoint	Comparison groups	Group 1 – Group 2
		Difference Response rate	0.6%
		95% Exact CI	(-15.8, 17.0)
		P-value (superiority)	0.9828
	Secondary endpoint	Comparison groups	Group 1 – Group 2
		Difference Response rate	12.4%
		95% Exact CI	(-3.1, 28.7)
		P-value (superiority)	0.0700
Notes	SC = Seroconversion (at least a two fold rise in titers, or becoming seropositive in originally seronegative subjects)		
Analysis description	**Secondary Immunogenicity analysis**		
	Geometric Mean titers of the individual peak titres were: ELISA: Group 1 GMT = 804.1 (636.3, 1016.0) Group 2 GMT = 605.8 (479.6, 765.2) PRNT: Group 1 GMT = 210.3 (146.1, 302.7) Group 2 GMT = 126.7 (82.4, 194.8)		

2.5.3. Discussion on clinical efficacy

1. General comment on the dossier

Taking into account the particularities of this condition and that the studies were performed during the last decade it is accepted by the CHMP that only non-clinical efficacy and immunogenicity data and human immune response data can be provided to support the likely efficacy of MVA-BN against smallpox. As a consequence of the inability to provide comprehensive clinical data on the efficacy under normal conditions of use of the vaccine the CHMP requested specific post-authorisation obligations to gather effectiveness data in observational prospective non-interventional cohort studies (see section 2.8). Status update reports are expected annually. In addition, post-authorisation studies will be conducted in children to provide effectiveness data in this population.

2. Non-clinical efficacy

The additional NHP studies supported correlations between each of dose and antibody titres and survival but the results clearly showed some discrepancies between each of ELISA and PRNT titres and survival, indicating that humoral antibody does not alone predict protection.

3. Formulations of MVA-BN

The dose-finding studies and the two studies with a comparison to Dryvax were not performed using final commercial process vaccine. In particular, POX-MVA-002 (dose-finding vs. Dryvax [n=15]) used the freeze-dried version while POX-MVA-009 (regimen-finding vs. Dryvax [n=8]) did not use the final industrial process vaccine. The CHMP recommended to provide data on immune responses induced by different vaccine formulations (liquid frozen and freeze dried, see also POX-MVA-027).

4. Assays

The results of the PRNT assay were more variable than the ELISA, which is not an uncommon finding when both functional and total binding antibody assays are applied to the same sera. In non-clinical models and in humans the post-vaccination ELISA titres are always higher than the PRNT titres, as would be expected.

Agreement between assays in terms of seropositivity rates is rather better post-vaccination than pre-vaccination. This is not an unusual finding and most likely reflects the prevalence of samples with titres very close to the assay cut-off limits.

With regard to the timing of sampling, details of the kinetic of the antibody response to MVA-BN in man were explored in some studies with additional early samplings. However, in POX-MVA-002 and 009 it cannot be concluded that the sampling times necessarily captured the peak immune response in the Dryvax group.

Although there is no standardised methodology, the non-clinical data and the past experience with replication-competent vaccines suggest that the role of CMI could be very important. However, only one study used alternative antigens to MVA-BN to stimulate the T-cells. Overall, the CMI data were not obtained in a uniform fashion and it is not possible to draw any conclusions.

5. Humoral immune responses

There was evidence of a dose-related increment in antibody titres in the short term but longer-term data did not show a consistent advantage for the higher TCID50 formulations, including the selected titre in MVA-BN. However, POX-MVA-028 compared two standard doses with a single dose of a slightly higher TCID50. The NIH concluded from this study that vaccination with the latter formulation produced higher titres more rapidly than a single standard dose but the titres did not reach the same magnitude as after the second standard dose. Based on the median time to seroconversion, NIH stated that use of a single high dose vaccine may be an alternative to a 2-dose vaccine series during a post-event emergency and may warrant further study.

More than two initial doses have not been evaluated except in two small studies in HIV-infected subjects.

POX-MVA-009 showed that a dose interval of one week was sub-optimal. No other dose intervals have been assessed except in one study in HIV-infected subjects (8 weeks).

In vaccinia-naïve subjects the data across all the studies showed low responses to a first dose of MVA-BN with a considerable increase in titre following a second dose administered four weeks later. The PRNT seroconversion rates (which approximate to the seropositivity rates in most cases) were at a maximum of 90% and mostly less than this at Day 42. Both ELISA and PRNT data indicated a very rapid waning of antibody titres within the first 3-4 months after a second dose. One study (POX-MVA-023) evaluated the response to a third dose of MVA-BN but only at two years after an initial course of two doses administered 4 weeks apart.

A single dose of MVA-BN was administered to VV-experienced subjects in POX-MVA-001, 005, 010, 011 and 024. The data from POX-MVA-001 and 005 showed that a single dose of MVA-BN in VV-experienced subjects elicited greater immune responses compared to a first dose of MVA-BN in the vaccinia-naïve study groups. The data suggest that the antibody response to MVA-BN in these VV-experienced subjects was rapid, with high seroconversion rates at 14 days post-dose, indicating effective stimulation of memory B-cells. The applicant compared immune responses by age group only in POX-MVA-024, which was confined to VV-experienced subjects and compared administrations of one or two doses of MVA-BN. Only 15 subjects were aged > 70 years and the data did not show a clear trend for immune responses by age but the PRNT titres showed better responses to two doses compared to one dose in this older VV-experienced population (e.g. overall seroconversion rates 90% vs. 77%; the difference was maintained at follow-up – 65% vs. 49%), which is not reflected in the SmPC.

It is not possible to conclude that the timing of sampling after Dryvax in POX-MVA-002 and 009 or in the past NIH studies was adequate to capture the peak immune response. In contrast, the data from MVA-BN studies suggested that this likely was captured. Therefore it is considered that the comparisons made are very likely inherently biased in favour of MVA-BN regardless of the antigen or strain used in the assay.

In POX-MVA-002 the PRNT GMT as measured against the NYBH strain was greater after Dryvax than after two doses of MVA-BN. The PRNT titres measured against MVR showed a lower GMT after Dryvax compared to after MVA-BN while the PRNT titres against VV-WR were 149 at week 4 post-Dryvax compared to 86 and 108 at 2 weeks after the second dose of MVA-BN. The PRNT

titres against variola reported by CDC raise several issues in terms of study conduct and design beyond the authors' assumption that the comparison is valid based on the timing of the samples.

POX-MVA-009 provided data on 8 subjects who received only Dryvax. PRNT data indicated that neutralising antibody first appeared at about day 14 with a linear increase across the day 8, 14 and 28 time points. The next sample after day 28 was not until day 180. As measured, the peak titre after Dryvax was lower than after two doses of MVA-BN when measured at St. Louis University (SLU) but the opposite observation applied using the applicant's PRNT assay.

The retrospective study compared antibody titres (using the applicant's assays only) after administration of Dryvax or Wetvax in 5 NIH studies and after MVA-BN in three MVA-BN studies (POX-MVA-005, 008 and 011). It is not appropriate to place too much weight on this retrospective analysis of samples of different ages, with assays conducted over a 4-year period. In addition, the sera were obtained from several different study populations although subjects were to be healthy young and vaccinia-naïve adults. Peak immune responses were determined from samples obtained on days 28 or 56 for subjects who received Dryvax or Wetvax and on days 42 or 56 for subjects who received MVA-BN.

It is of value to note that the ELISA data for Dryvax and Wetvax demonstrated that 190 subjects had a peak ELISA titre on day 28, 47 on day 56 and 14 had the same titre on both days. The spread for MVA-BN was narrow and 87% had a peak at day 42. Not surprisingly the ELISA GMTs (measured against MVA-BN) were slightly higher in the MVA-BN recipients compared to the NIH study subjects. Even more important is the finding that PRNT data showed significant variability in the timing of the individual peak neutralising antibody response. These observations indicate that a comparison of immune responses between MVA-BN and Dryvax based on very limited sampling time points cannot support a conclusion that the humoral immune response to MVA-BN is at least as good as that to a replication-competent vaccine.

6. Use in populations in which the use of VV is problematical

POX-MVA-008 enrolled only vaccinia-naïve subjects and the data suggest that AD subjects can be dosed as healthy subjects.

POX-MVA-011 enrolled vaccinia-naïve and VV-experienced subjects and all received two doses of MVA-BN 4 weeks apart. After two doses the PRNT seroconversion rates were just under 80% in the healthy but from about 55-68% in the HIV-infected although by week 32 there was no appreciable difference between subsets. The PRNT data for the VV-experienced showed seroconversion rates in the 70% range after two doses, with much higher rates at week 32 compared to the corresponding vaccinia-naïve groups. The applicant agreed that two doses should be recommended for VV-experienced HIV-infected individuals to boost the memory response.

Currently, there are insufficient data to determine whether a third dose would be useful in clinically immunocompromised individuals, therefore the CHMP requested to evaluate a higher dose and/or different time interval in this patient population. This study will generate important information that is currently missing and the results are expected by in 2016 (as detailed in the RMP).

7. Cell Mediated Immune response data

The MVA-BN strain does not replicate after injection. This would imply that its ability to elicit a useful cell mediated immune (CMI) response would be less than a replication-competent VV strain. Overall, even between the studies that appear to have used the same methodology, the results have been very variable. In the pivotal studies the method used mostly suggested lack of any detectable response in the majority of subjects. In those studies that have reported data separately for interferon gamma-producing CD4 and CD8 cells very few subjects showed any response among CD4 cells.

The currently available CMI data are variable and inconclusive. The CHMP considered that more reliable data from planned studies should be collected, therefore CMI data will be generated during study POX-MVA-027. The results of the T cell analysis are planned to be reported in Q2/2016 (as detailed in the RMP).

8. Effect of prior MVA-BN on the response to Dryvax

There was attenuation of takes when Dryvax was administered after MVA-BN in POX-MVA-002. However, takes still occurred in most subjects and virus was still detected in swabs up to day 8. Importantly, the time interval between MVA-BN and administration of Dryvax in the four groups that included two doses of MVA-BN followed by Dryvax was 84 days (12 weeks), which is too short to assess the duration of the immune response against NYBH elicited by MVA-BN.

Prior MVA-BN could interfere with subsequent responses to a replication competent vaccine. In this regard, the PRNT data against NYBH that are available from the groups that received Dryvax on day 112 indicate lower GMTs at day 140 and day 184 in the 4 groups that had received MVA-BN before Dryvax vs. the group that received only placebo on days 0 and 28. This finding has important implications for any government or institution that might consider using MVA-BN for priming in a non-emergency setting with intent to give a single dose of a replication-competent vaccine in the case of an emergency situation.

9. Impact of MVA-BN vaccination on other vaccines

Strains of MVA (including specifically MVA-BN) are under evaluation as live viral vector vaccines (LVVVs) intended to elicit immunity to infections unrelated to the poxvirus diseases. The applicant was requested to consider whether prior MVA-BN could seriously interfere with the development of immunity to these other infections or at least reduce the magnitude of the immune responses to the foreign antigens encoded by the vectors. The applicant responded by stating that pre-existing immunity against MVA does not hamper the ability of this virus to significantly boost the B and T cell memory originally stimulated by a smallpox vaccine, even in the presence of high circulating antibodies against MVA.

One may argue that the same effect may not occur when MVA-BN is being used as a LVV to stimulate and/or boost immunity against one or more encoded antigens (or transgenes). In this case the antigen load of the transgene(s) would be lower compared to the backbone LVV and may be inhibited by vector immunity stimulated by earlier vaccination(s). However, there is really no evidence that pre-existing immunity against MVA hampers the ability to stimulate immune response to encoded genes within MVA-BN. BN has recently published data that show certain poxvirus promoters are able to stimulate a higher T cell response to an encoded antigen compared to the normally dominant response to MVA. This was only achieved by repeat

vaccination with the recombinant MVA-BN-based vaccine in the presence of high circulating antibodies against MVA. These findings argue against vector immunity giving rise to poor immune responses to encoded antigens within MVA.

10. Confirmation of immunogenicity of MVA-BN

The CHMP requested two additional Phase III studies to provide confirmation in terms of immunogenicity, one study will provide this information against placebo and the second study will provide comparative information against the active comparator currently available. These studies are considered key for the benefit risk of the product.

2.5.4. Conclusions on the clinical efficacy

Taking into consideration the current scientific knowledge, the particularities of this condition and the non-feasibility to perform efficacy studies as it would be contrary to medical ethics, it is not possible to conclude that MVA-BN will provide protection against smallpox that is comparable to that afforded by replication-competent vaccines nor is it possible to determine how long after vaccination some degree of protection will occur and how long it will persist. Therefore it is not possible to make specific recommendations regarding booster doses.

Nevertheless, taking into account the non-clinical data and the safety profile of MVA-BN, the CHMP concluded that it was not appropriate to restrict the use of MVA-BN to persons at high risk of life-threatening complications of vaccination with a replication-competent vaccine. Rather, it was concluded that the actual mode of use of MVA-BN should be in accordance with individual national recommendations. The SmPC carefully documents what is known and what remains unknown.

2.6. Clinical safety

Patient exposure

A total of 3,432 subjects received Imvanex in this study program. The number of subjects that received one or more doses of MVA-BN containing 1×10^8 $TCID_{50}$ (the selected dose) were:

- 1854 vaccinia-naïve subjects received two doses 4 weeks apart
- 534 vaccinia-experienced subjects received a single dose

Included in the numbers above, there were:

- 152 subjects that received a single dose after an earlier priming regimen with MVA-BN

Table 29 Summary of Subjects who Received the Standard Imvanex Dosing Regimen

Standard IMVANEX® dosing regimen	Healthy subjects	HIV infected subjects	AD subjects	Total
	Number of subjects			
Vaccinia-naïve subjects: 2 injections of 1 × 10^8 $TCID_{50}$ IMVANEX®, 4 weeks apart	1137	355	362	1854
Vaccinia-experienced subjects: 1 booster injection of 1 × 10^8 $TCID_{50}$ IMVANEX®	464	70	0	534

Abbreviations: AD, atopic dermatitis; HIV, Human Immunodeficiency Virus; 1 × 108 TCID50, 1 × 108 tissue culture infected dose 50.

The pooled disposition of healthy subjects (from POX-MVA-005, POX-MVA-008, POX-MVA-011, POX-MVA-023, and POX-MVA-024) is summarised in Table 30, with the numbers of subjects who received the standard dosing regimens highlighted in bold.

Table 30 Pooled Disposition of Healthy Subjects (Safety Dataset)

Number of subjects (%)	HNMM (N = 537)	HEM (N = 339)	HNM (N = 197)	HEMM (N = 64)
Received 1 or 2 doses of IMVANEX® (safety population)	**537 (100)**	**339 (100)**	197 (100)	64 (100)
Completed	**528 (98.3)**	**331 (97.6)**	179 (90.9)	63 (98.4)
Early withdrawal:	**9 (1.7)**	**8 (2.4)**	18 (9.1)	1 (1.6)
AE	**0**	**1 (0.3)**	3 (1.5)	0
Withdrawal by subject	**8 (1.5)**	**1 (0.3)**	13 (6.6)	(1.6)
Other	**1 (0.2)**	**6 (1.8)**	2 (1.0)	0

N = Number of subjects per treatment group; n = number of subjects with specified variable; % = Percentage based on N.
Note: Numbers in bold represent subjects who received the standard dosing regimen for vaccinia-naïve subjects (2 standard injections of 1 × 108 TCID50 IMVANEX, 4 weeks apart) and vaccinia-experienced subjects (booster injection of 1 × 108 TCID50 IMVANEX).
Abbreviations: AE, adverse event; HNM, healthy vaccinia-naïve subjects with 1 dose IMVANEX; HNMM, healthy vaccinia-naïve subjects with 2 doses of IMVANEX; HEM, healthy vaccinia-experienced subjects with 1 dose of IMVANEX; HEMM, healthy vaccinia-experienced subjects with 2 doses of IMVANEX.

There were 3 US NIH-sponsored clinical studies of MVA-BN on-going at time of the initial MAA. Two of these NIH sponsored clinical trials have recently been completed (POX-MVA-028 and POX-MVA-029). These studies add more than 300 VV-naïve subjects vaccinated with the standard dose regimen of MVA-BN to the total safety database. The third study is ongoing (POX-MVA-030) in 24 HSCT recipients (> 2 years previously). According to the latest information received from the NIH, enrolment has been completed and all subjects have received their second dose.

These numbers bring the totals up to those shown in the tables below for subjects who received at least one dose of at least the standard dose of MVA-BN.

Subjects that received the MVA-BN standard dose and higher (≥ $1x10^8$ $TCID_{50}$) according to any schedule in BN and NIH sponsored clinical trials (i.e. at Day 0, Day 0+7, Day 0+28 or Day 0+56+112 in vaccinia-naives; at Day 0, Day 0+28 or Day 0+56+112 in vaccinia-experienced (see Tables below with Standard dose and higher (≥ 10^8 $TCID_{50}$), any regimen).

Vaccinia Naives

STUDY	Formulation*	All subjects (n)	n	Healthy naive	HIV naive	AD naive
POX-MVA-002	FD	45	45	45	0	0
POX-MVA-004	FD	55	55	55	0	0
POX-MVA-007	FD	60	60	29	0	31
POX-MVA-010	FD	60	60	30	30	0
POX-MVA-029	FD	165	165	165	0	0
HIV-NEF-004	FD	5	5	0	5	0
Subtotal freeze-dried formulation		*390*	*390*	*324*	*35*	*31*
POX-MVA-001	LF	34	34	34	0	0
POX-MVA-005	LF	364	364	364	0	0
HIV-POL-002	LF	10	10	0	10	0
POX-MVA-008	LF	632	632	282	0	350
POX-MVA-009	LF	198	198	198	0	0
POX-MVA-011	LF	439	439	88	351	0
POX-MVA-023	LF	0	0	0	0	0
POX-MVA-024	LF	0	0	0	0	0
POX-MVA-028	LF	91	91	91	0	0
POX-MVA-029	LF	167	167	167	0	0
Subtotal liquid-frozen formulation		*1935*	*1935*	*1224*	*361*	*350*
TOTAL No. of SUBJECTS		**2325**	**2325**	1548	396	381

Vaccinia Experienced

STUDY	Formulation*	All subjects (n)	n	Healthy exp.	HIV exp.	AD exp.
POX-MVA-002	FD	0	0	0	0	0
POX-MVA-004	FD	0	0	0	0	0
POX-MVA-007	FD	0	0	0	0	0
POX-MVA-010	FD	91	91	30	61	0
POX-MVA-029	FD	0	0	0	0	0
HIV-NEF-004	FD	21	21	0	21	0
Subtotal freeze-dried formulation		*112*	*112*	*30*	*82*	*0*
POX-MVA-001	LF	18	18	18	0	0
POX-MVA-005	LF	200	200	200	0	0
HIV-POL-002	LF	0	0	0	0	0
POX-MVA-008	LF	0	0	0	0	0
POX-MVA-009	LF	0	0	0	0	0
POX-MVA-011	LF	140	140	9	131	0
POX-MVA-023	LF	152	152	152	0	0
POX-MVA-024	LF	119	119	119	0	0
POX-MVA-028	LF	0	0	0	0	0
POX-MVA-029	LF	0	0	0	0	0
Subtotal liquid-frozen formulation		*629*	*629*	*498*	*131*	*0*
TOTAL No. of SUBJECTS		**741**	**741**	528	213	0

The total that received 2 doses of 1 x 10^8 $TCID_{50}$ of MVA-BN 4 weeks apart if VV- naïve or one dose of 1 x 10^8 $TCID_{50}$ if VV-experienced subjects is now 2,388. This total includes healthy subjects as well as HIV-infected subjects (N= 425) and subjects with atopic dermatitis (N = 362). The population includes subjects from 18 up to 80 years of age. A total of 3,066 subjects received at least one MVA-BN dose of 1 x 10^8 $TCID_{50}$ (or higher).

Adverse events

Solicited AEs

Typically, after each vaccination subjects were asked to document the occurrence of five general symptoms on the day of vaccination and the 7 days post-vaccination in a diary card (i.e. increased body temperature, headache, myalgia, nausea and fatigue). Additionally, the grading of severity was recorded. However, studies varied considerably in the list of solicited systemic events and the mode of capture of the information and therefore no reliable synthesis across all the studies is possible. In addition, subjects documented the occurrence of local symptoms at the

injection site (i.e. erythema, swelling, induration and pain) for each day of the 7 days post-injection. The intensity of erythema, swelling and induration was documented and graded according to size of the lesion (diameter measurement). As an example, solicited AEs for POX-MVA-005/023 are summarised. Study 005 compared one (Group 2) and two (Group 1) doses in naïve subjects with two doses of placebo (Group 3) and a single dose in VV-experienced (Group 4). Study 023 involved a single booster dose of MVA-BN at Year 2 in the original Groups 1 and 2 from study 005.

For local solicited symptoms (see Table 32) erythema (70.3%) and pain (70.9%) were reported at the injection site after at least one injection while over half reported swelling (55.5%) and induration (63.2%). Most symptoms were of mild to moderate severity. There were 20 incidences of Grade 3 symptoms in Groups 1 and 4 but rates did not exceed 3.3% and there were no Grade 4 symptoms.

Table 31 Study POX-MVA 005 Summary of Maximum Intensity of Solicited General Symptoms (7-day Follow-up Period after Vaccination) as Recorded in the Subject Diary, Safety dataset

	Group 1 (N=182)	Group 2 (N=179)	Group 3 (N=179)	Group 4 (N=200)	p-value
	Number of subjects (%)				
Body temperature increased					
Grade 0*	164 (90.1)	158 (88.3)	169 (94.4)	189 (94.5)	0.096~
Grade 1	13 (7.1)	17 (9.5)	8 (4.5)	10 (5.0)	
Grade 2	5 (2.7)	4 (2.2)	2 (1.1)	0 (0.0)	
Grade 3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Grade 4	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Missing	0	0	0	1	
Headache					
Grade 0*	122 (67.0)	95 (53.1)	130 (72.6)	147 (73.5)	0.002~
Grade 1	39 (21.4)	57 (31.8)	35 (19.6)	41 (20.5)	
Grade 2	19 (10.4)	21 (11.7)	12 (6.7)	10 (5.0)	
Grade 3	2 (1.1)	6 (3.4)	2 (1.1)	2 (1.0)	
Grade 4	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Myalgia					
Grade 0*	153 (84.1)	157 (87.7)	159 (88.8)	158 (79.0)	0.054~
Grade 1	25 (13.7)	14 (7.8)	14 (7.8)	33 (16.5)	
Grade 2	4 (2.2)	8 (4.5)	6 (3.4)	9 (4.5)	
Grade 3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Grade 4	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Nausea					
Grade 0*	165 (90.7)	157 (87.7)	166 (92.7)	182 (91.0)	0.073~
Grade 1	13 (7.1)	12 (6.7)	8 (4.5)	16 (8.0)	
Grade 2	4 (2.2)	5 (2.8)	4 (2.2)	2 (1.0)	
Grade 3	0 (0.0)	5 (2.8)	1 (0.6)	0 (0.0)	
Grade 4	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Fatigue					
Grade 0*	114 (62.6)	120 (67.0)	124 (69.3)	122 (61.0)	0.663~
Grade 1	51 (28.0)	41 (22.9)	38 (21.2)	52 (26.0)	
Grade 2	16 (8.8)	14 (7.8)	14 (7.8)	21 (10.5)	
Grade 3	1 (0.5)	4 (2.2)	3 (1.7)	5 (2.5)	
Grade 4	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	

N: Number of subjects in the specified group with subject diaries recording general symptoms
%: Percentage based on total number of subjects in each group
*Grade 0 indicates that the subject did not experience the particular symptom.
~ Chi-square test (p-values from Chi-square test to detect differences in proportions between all treatment groups)

Table 32 Study POX-MVA 005 - Summary of Maximum Intensity of Solicited Local Symptoms (7-day Follow-up Period after Vaccination) as Recorded in the Subject Diary, Safety dataset

	Group 1 (N=182)	Group 2 (N=179)	Group 3 (N=179)	Group 4 (N=200)	p-value
	Number of subjects (%)				
Injection site pain					
Grade 0*	16 (8.8)	24 (13.4)	142 (79.3)	33 (16.5)	<0.001~
Grade 1	117 (64.3)	114 (63.7)	25 (14.0)	123 (61.5)	
Grade 2	48 (26.4)	41 (22.9)	12 (6.7)	41 (20.5)	
Grade 3	1 (0.5)	0 (0.0)	0 (0.0)	3 (1.5)	
Grade 4	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Grade 0*	16 (8.8)	24 (13.4)	142 (79.3)	33 (16.5)	<0.001~
Injection site erythema					
Grade 0*	16 (8.8)	33 (18.4)	140 (78.2)	31 (15.5)	<0.001~
Grade 1	55 (30.2)	87 (48.6)	37 (20.7)	71 (35.5)	
Grade 2	105 (57.7)	59 (33.0)	2 (1.1)	93 (46.5)	
Grade 3	6 (3.3)	0 (0.0)	0 (0.0)	5 (2.5)	
Grade 4	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Injection site swelling					
Grade 0*	33 (18.1)	76 (42.5)	169 (94.4)	51 (25.5)	<0.001~
Grade 1	58 (31.9)	71 (39.7)	9 (5.0)	91 (45.5)	
Grade 2	89 (48.9)	32 (17.9)	1 (0.6)	57 (28.5)	
Grade 3	2 (1.1)	0 (0.0)	0 (0.0)	1 (0.5)	
Grade 4	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Injection site induration					
Grade 0*	20 (11.0)	33 (18.4)	174 (97.2)	45 (22.5)	<0.001~
Grade 1	86 (47.3)	111 (62.0)	4 (2.2)	97 (48.5)	
Grade 2	75 (41.2)	35 (19.6)	1 (0.6)	57 (28.5)	
Grade 3	1 (0.5)	0 (0.0)	0 (0.0)	1 (0.5)	
Grade 4	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	

N: Number of subjects in the specified group with subject diaries recording general symptoms
%: Percentage based on total number of subjects in each group
*Grade 0 indicates that the subject did not experience the particular symptom.
~ Chi-square test (p-values from Chi-square test to detect differences in proportions between all treatment groups)

Rates are not shown or described for the individual doses of MVA-BN in Group 1, i.e. it cannot be discerned if rates were higher or lower with the second vs. first dose. There were significant differences between the placebo group and subjects who received at least one dose of MVA-BN. Note that in the tables presented above Grade 0 means no symptom reported.

More subjects had an increased body temperature in Groups 1 and 2 (9.9% and 11.7%) than in Groups 3 and 4 (5.6% and 5.0%). Headache was reported most frequently in Group 2 (46.9%)

compared to other groups (26.5 – 33.0%). Myalgia was most often reported in Group 4 (21.0%) with the lowest rates in Groups 2 and 3 (12.3% and 11.2%).

In the booster study POX-MVA-023 local solicited symptoms were observed in 43.4%-82.2%. The highest rates were for erythema (82.2%) and pain (80.3%) at the injection site. There were no significant differences in the frequencies of local symptoms between Groups 1 (MM) and 2 (MP).

Table 33 Study POX-MVA 023 - Summary of maximum intensity of solicited local symptoms by subject: category Grade > 0 (or diameter > 0 mm, FAS

		Count (%)			
		Group 1 (MM) (N=75)	Group 2 (MP) (N=77)	Total (N=152)	p-value
Injection site pain					
	Grade > 0	58 (77.3)	64 (83.1)	122 (80.3)	0.419
Injection site erythema					
	Grade > 0	60 (80.0)	65 (84.4)	125 (82.2)	0.529
Injection site swelling					
	Grade > 0	51 (68.0)	48 (62.3)	99 (65.1)	0.499
Injection site induration					
	Grade > 0	58 (77.3)	58 (75.3)	116 (76.3)	0.850
Injection site pruritus					
	Grade > 0	30 (40.0)	36 (46.8)	66 (43.4)	0.418

N: Number of subjects in the specified group with patient diaries recording general symptoms
%: Percentage based on total number of subjects

Two subjects in Group 1 and four in Group 2 had at least one solicited local AE of intensity Grade 3. Severe injection site symptoms included pain (in 2.6%), erythema (1.3%), swelling (1.3%), induration (0.7%) and pruritus (0.7%). Grade 3 symptoms improved after maximal three days and in all but two subjects they had resolved by day 8.

The commonest general solicited symptoms were fatigue, headache and myalgia (23.7%). There were no significant differences in frequencies of general symptoms between the two groups. Rates of Grade 3 were low (e.g. headache 2.0%, myalgia 1.3% and fatigue 1.3%).

Table 34 Study POX-MVA 023 - Summary of maximum intensity of solicited general symptoms recorded within 8-day follow-up period by subject: category Grade > 0, FAS

		Count (%)			
		Group 1 (MM) (N=75)	Group 2 (MP) (N=77)	Total (N=152)	p-value
Fatigue	Grade > 0	22 (29.3)	27 (35.1)	49 (32.2)	0.490
Headache	Grade > 0	19 (25.3)	25 (32.5)	44 (28.9)	0.374
Myalgia	Grade > 0	17 (22.7)	19 (24.7)	36 (23.7)	0.850
Nausea	Grade > 0	6 (8.0)	12 (15.6)	18 (11.8)	0.209
Body temperature increased	Grade > 0	3 (4.0)	4 (5.2)	7 (4.6)	>0.999

N: Number of subjects in the specified group with patient diaries recording general symptoms
%: Percentage based on total number of subjects

Across other studies with MVA-BN pain, erythema and swelling at the injection sites were consistently collected but pruritus at the injection site was only assessed in POX-MVA-023 and

024 and in the NIH-sponsored studies POX-MVA-002, 009, 028 and 029. With the exception of POX-MVA-001, 008 and 011, induration was also assessed in all studies. There were some differences in the grading of solicited local and systemic reactions between the NIH and BN studies. On request, the applicant analysed the available data on solicited local reactions and standardised the grading intensities used in NIH-sponsored studies to match those applied by the applicant.

The applicant concluded that local reactions at the injection site (pain, erythema, swelling, induration and pruritus) are very common. There was a tendency for higher local reactogenicity with freeze-dried vaccine vs. the proposed commercial liquid frozen product. The lower incidence of solicited local AEs in HIV-infected subjects could be partly due to under-reporting and/or lesser reactogenicity associated with the consequences of CD4 cell depletion. Rates were not clearly higher in AD subjects. The majority of the reactions resolved completely without intervention within the first seven days.

Unsolicited AEs

Unsolicited AEs were most commonly reported in the SOCs General Disorders and Administration Site Conditions and Infections and Infestations. At the PT level, the most commonly experienced individual unsolicited AE was injection site pruritus. Other common individual AEs, excluding local injection site reactions, were nasopharyngitis, increased troponin I levels (see below), headache and respiratory tract infection.

Across the studies in healthy subjects the proportions reporting at least one unsolicited AE and at least one causally-related unsolicited AE were comparable between vaccinia-naïve subjects who received 2 doses of MVA-BN and vaccinia-experienced subjects who received one dose of MVA-BN. The distribution of SOCs and PTs for healthy subjects appeared to reflect the distribution overall. The applicant's summary states that no clear pattern emerged regarding reporting rates with first and second doses but in some individual studies slightly more subjects reported AEs following the first dose vs. the second dose.

Data from healthy subjects aged 56–80 years come only from POX-MVA-024 and all these subjects were VV-experienced. The AE reporting rates were compared with those for subjects aged 18–55 years in other studies. The distribution of SOCs and PTs for older subjects appeared to reflect the distribution observed in younger subjects and the distribution overall.
Regarding HIV-infected subjects (enrolled in four studies) the proportions reporting at least one unsolicited AE and at least one causally-related unsolicited AE were comparable for healthy subjects and HIV-infected subjects. In POX-MVA-011 an increasing incidence of unsolicited AEs was observed with decreasing CD4 count in VV-experienced subjects but there was no consistent relationship between AE rates and CD4 counts detected in the vaccinia-naïve HIV-infected groups. The distribution of SOCs and PTs for HIV-infected subjects appeared to reflect the distribution for healthy subjects.

Proportions with at least one unsolicited AE or at least one causally related AE were slightly higher for subjects with atopic dermatitis (AD) and allergic rhinitis (AR) compared with healthy

subjects. All subjects with AD and AR were vaccinia-naïve. The pattern of unsolicited AEs resembled that in the overall VV-naïve population.

The rates of unsolicited AEs were higher with an earlier liquid-frozen formulation compared with the current liquid-frozen formulation and the freeze-dried formulation. Rates for unsolicited AEs were comparable between the current liquid-frozen formulation and the freeze-dried formulation. In the dose-finding studies (POX-MVA-001, -002 and -004) a clear dose-response was seen for all AEs and for vaccine-related unsolicited AEs in 004 and 001. Proportions of subjects with AEs increased with increasing doses in vaccinia-naïve subjects in these studies. No clear pattern with regards to the most common unsolicited AEs for the different doses was evident.

Details of unsolicited AEs in POX-MVA-005, 008, 011, 023 and 024

The comparison focuses on the vaccinia-naïve subjects who received two doses of MVA-BN (HNMM) and the VV or MVA-BN-experienced subjects who received a single dose of MVA-BN (HEM). The following observations can be made:

- 470 (43.8%) HNMM subjects experienced 835 unsolicited AEs
- 164 (48.4%) HEM subjects experienced 240 unsolicited AEs
- 249 (23.2%) HNMM subjects experienced 351 causally-related unsolicited AEs
- 94 (27.7%) HEM subjects experienced 128 causally-related unsolicited AEs
- 5 (0.5%) HNMM subjects experienced 6 severe (Grade 3) causally-related unsolicited AEs
- 2 (0.6%) HEM subjects experienced 2 severe causally-related unsolicited AEs

The most common individual AEs for HNMM subjects were injection site pruritus (13.8% of vaccination periods), nasopharyngitis (5.2%), increased troponin I (4.3%) and headache (3.1%). The most common individual causally-related AEs for HNMM subjects were injection site pruritus (13.5% of vaccination periods), increased troponin I (2.2%), and injection site warmth (1.4%).

For HEM subjects, the most common individual AEs were injection site pruritus (14.5% of vaccination periods), nasopharyngitis (7.4%) and injection site warmth and headache (both 3.2%). Increased troponin was not observed in vaccinia-experienced subjects. The most common individual causally-related AEs were injection site pruritus (13.9% of vaccination periods), injection site warmth (3.2%) and injection site haematoma (1.8%).

On a per subject basis, at least one unsolicited AE was experienced by more HNMM subjects (345 [64.2%] for overall AEs and 192 [35.8%] for causally-related AEs) than HEM subjects (164 [48.4%] for overall AEs and 94 [27.7%] for causally-related AEs). For HNMM subjects the proportions of subjects with unsolicited AEs, both overall and causally-related, were largely similar after the second vs. first dose of MVA-BN (overall, 45.6% versus 41.7%; causally-related 24.4% versus 22.0% for the first and second vaccinations, respectively).

The events reported as “rash” usually did not provide information as to the extent of such eruptions. According to CTCAE version 3.0 (which was applicable during conduct of the clinical studies) a mild rash (grade 1) is defined as “macular or papular eruption or erythema without associated symptoms”, a moderate rash (grade 2) is a “macular or papular eruption or erythema with pruritus or other associated symptoms; localised desquamation or other lesions covering <50% of body surface area” while a severe rash (grade 3) would be a “severe, generalised

erythroderma or macular, papular or vesicular eruption; desquamation covering ≥ 50% of body surface area".

Following this definition, only rash with severe intensity would be regarded as generalised. Only one case of severe rash was found in the database and this was also the only case of rash rated as serious. This occurred in an HIV-infected female aged 37 years with CD4 counts in the 300s and the rash, described as herpetic, had onset 4 months after the second dose of MVA-BN. The investigator assessed the events as unrelated to the vaccination and definitely related to other conditions.

If moderate rashes are classified as generalised then 12 additional cases were found in the database. All of them were rated as non-serious.

AEs of special interest (SIAEs)

In the main study period 61 SIAEs in 53 subjects were considered possibly related to study vaccine, 4 SIAEs in 4 subjects were considered probably related and 4 SIAEs in 4 subjects were considered definitely related. All probably related SIAEs and all except 1 definitely related SIAEs were increased troponin I levels.

In the follow-up period 13 SIAEs in 13 subjects were considered possibly related to study vaccine, one SIAE in a single subject was considered probably related and 3 SIAEs in three subjects were considered definitely related. All possibly, probably, and definitely related SIAEs in the follow-up period were increased troponin I levels except for i) a possibly related SIAE of Grade 1 intensity palpitations in one diagnosed AD, vaccinia-naïve subject (MM) in POX-MVA-008 and ii) a possibly related SIAE of Grade 1 intensity ST segment elevation in one HIV-infected vaccinia-naïve subject (CD4 ≥ 501- 750/μl subgroup; MM) in POX-MVA-011.

In POX-MVA-005 and POX-MVA-024 safety laboratory tests were performed at the screening visit and 10 - 15 days after each vaccination. In POX-MVA-008 and 011 testing was at 7-14 days after each dose. The safety laboratory measurements were performed at a central laboratory. Tests included AST, ALT and troponin I.
A standard 12-lead ECG was recorded from all subjects at screening and 10-15 days after each vaccination in POX-MVA-005 and POX-MVA-024 and at 7-14 days post-dose in POX-MVA-008 and 011. In 005 ECGs were evaluated by the investigator and transmitted electronically to a central database. In the other studies ECGs were read by a central cardiologist. Abnormal and unclear ECG findings at screening or throughout the study were evaluated and were to be followed-up by an external cardiologist, including a thorough examination and performance of an echocardiogram and/or treadmill ECG.

In the booster study POX-MVA-023 post-dose ECG data were not collected except in a few subjects with pre-dose abnormalities, while safety laboratory testing was carried out post vaccination at visit 3. Further ECG testing was performed at follow-up visits only if clinically indicated.

Across studies the proportions with at least one SIAE were comparable for vaccinia-naïve healthy subjects who received 2 doses of MVA-BN. There was no clear pattern regarding the proportion of healthy subjects with SIAEs and causally-related SIAEs for vaccinia-naïve versus vaccinia-experienced subjects. Most SIAEs in healthy subjects were increased troponin I levels. Other common SIAEs were palpitations, tachycardia and ECG abnormalities. Proportions with SIAEs were comparable between healthy elderly vs. younger subjects. There was a slight increase in the number of healthy subjects with SIAEs with increasing dose in POX-MVA-002. There was no clear pattern for the most common SIAEs by dose.

Across the five pivotal studies (see table below) 56 SIAEs occurred in 52 vaccination periods (4.8%) for HNMM subjects and 10 SIAEs occurred in 9 vaccination periods (2.7%) for HEM subjects. SIAEs that were considered causally-related were experienced in 26 vaccination periods (2.4%) for HNMM subjects (27 events) and in 2 vaccination periods (0.6%) for HEM subjects (3 events). Thus, on a per dose basis, SIAEs and causally-related SIAEs were more frequently reported for HNMM subjects.

On a per subject basis, more HNMM group subjects experienced SIAEs (9.5% of subjects) and causally-related SIAEs (4.8%) than HEM group subjects (SIAEs 2.7%, causally-related SIAEs 0.6%). In HNMM subjects the most common SIAEs (4.3% of vaccination periods) were increased troponin I levels. More SIAEs (overall and related) were reported during vaccination period 2 (overall 6.1% vs. 3.5% of vaccination periods; causally-related 2.6% vs. 2.2%) but again most events were increased troponin I levels.

In HEM subjects 9 (2.7%) experienced SIAEs and only 2 had SIAEs considered causally-related to study vaccination. These SIAEs included palpitations (5) and one case each of AV block first degree, bundle branch block left, bundle branch block right, supraventricular extrasystoles and tachycardia. In the SOC of Cardiac Disorders 4 SIAEs were experienced in 4 vaccination periods (0.4%) for HNMM subjects and 10 SIAEs were experienced in 9 vaccination periods (2.7%) for HEM subjects. Three events in the HEM group were considered causally-related.

In POX-MVA-005, 023 and 024 and in supportive studies HIV-POL-002, POX-MVA-001 and HIV-NEF-004 all ECG recordings were assessed to be normal or not clinically significant (i.e. abnormal but considered to represent natural fluctuations of no concern). ECG measurements were not included in the safety evaluations in POX-MVA-004.

In POX-MVA-008 and 011 and in supportive studies POX-MVA-002, 007, 009 and 010 abnormal ECG findings were reported but were mostly considered to be not clinically significant and unrelated or unlikely to be related to study vaccination.

In the initial summary provided, numerous troponin I values between ULN (0.04 µg/l) and 2 x ULN (0.08 µg/l) were observed. These elevated values were reported for many subjects in the screening phase. Following extensive analysis of these minimally elevated values by the applicant together with DSMB members it was concluded that isolated troponin I values < 2 x ULN do not correlate with any cardiac abnormalities and therefore do not represent a relevant safety signal.

Furthermore, the applicant maintained that the troponin I assay is not intended for screening purposes in asymptomatic individuals and elevated troponin I values are only meaningful in connection with cardiac symptoms and/or ECG abnormalities.
Subjects with elevated troponin I values were confirmed to be asymptomatic and all but one subject showed no corresponding ECG abnormalities. Subjects with elevated values were to be sent for a cardiac consultation and the results of all examinations ruled out any acute cardiac conditions, particularly no cases of myo-/pericarditis were identified. Hence, with the exception of one 23-year old healthy male subject who showed an ST segment elevation in two leads (V2 & V3) at the same visit when an elevated troponin I value of 0.06 µg/l was measured, all elevated troponin I values measured to date have been isolated, clinically insignificant measurements.

Further details on troponin I measurements were requested. The response showed that throughout the clinical trials with MVA-BN numerous troponin I values between the ULN (0.04 µg/l) and 2 x ULN (0.08 µg/l) were measured. Elevated values were reported also for many subjects still in the screening phase prior to vaccination.

Most of the increased troponin I values were detected in two studies that enrolled only (POX-MVA-008) or mainly (POX-MVA-011) vaccinia-naïve subjects after the central laboratory implemented a change from a troponin I assay with an ULN < 0.08 µg/l to a more sensitive assay with a normal range < 0.04 µg/l. This more sensitive normal range meant that additional, asymptomatic cases of increased troponin I levels were detected than would be the case under normal circumstances.

A pooled analysis of the available data was conducted. All results for all studies were given in the same units of ng/mL (or equivalently in SI Units µg/L). The analysis focussed on the actual observed values rather than the number of subjects considered beyond the normal ranges. The normal ranges and severity grading for troponin I values were confirmed to vary by study and study sites/central laboratories used.

The numbers of subjects from POX-MVA-005/-008/-011/-023/-024 and HIV-POL-002 with available troponin I values at screening and after the first and second vaccination are shown in the Table 35.

Table 35 Number of subjects with troponin I values available in POX-MVA-005/-008/-011/-023/-024 and HIV-POL-002

Screening and After Vaccination 1 / Vaccination 2				
Vaccinia Status	**Indication**			
	AD	**Healthy**	**HIV**	**Total**
Experienced	0 / 0	454 / 60	113 / 108	567 / 168
Naive	259 / 242	590 / 394	327 / 305	1176 / 941
Total	259 / 242	1054 / 464	440 / 413	1743 / 1109

- Using the mid-range of the assay cut-off value for values below the cut-off (worst case scenario) there were 158/1,743 (9%) subjects with a doubling between screening and post-first vaccination and 122/1109 (11%) with doubling after the second vaccination.

- Using the assay cut-off value itself to impute the values below the cut-off value (best case scenario) there were 71 doublings (4.07%) after the first and 25 cases after the second vaccination (2.25%).

In vaccinia-naïve subjects depending on how values below the assay cut-off were handled.

- Between 139 (11.82%) and 31 (2.64%) out of 1,176 subjects had a doubling after the first vaccination
- Between 117 (12.43%) and 24 (2.55%) out of 941 had a doubling after the second vaccination

In vaccinia experienced subjects

- Between 49 (8.64%) and 31 (5.47%) of 567 had doubling after the first vaccination
- Between 5 (2.98%) and 1 (0.60%) of 168 had doublings after the second vaccination

In POX-MVA-007 and POX-MVA-010, no values (0/211) were above the assay cut-off (0.20 µg/l) at screening or after vaccination 1 while 3/118 subjects had values above the assay cut-off values after the second vaccination. All three subjects were from POX-MVA-010 with one value of 0.20 µg/l and two values of 0.24 µg/l. Hence it is unclear how much of a rise these three cases represent. However, as a conservative approach these were counted as cases of doubling.

All subjects in the NIH-sponsored studies POX-MVA-002 and POX-MVA-028 had troponin I values < 1.5 µg/l at screening and after both MVA-BN vaccinations so no cases of doubling were observed. Because of the high assay cut-off value this is the only statement that can be made. In the NIH-sponsored study POX-MVA-009, troponin I was only assessed at screening. BN presumes that the same approach applied in POX-MVA-029. Also, no data are available from POX-MVA-001 and POX-MVA-004.

The applicant concluded that numbers with a doubling of troponin I values were relatively low and that no increase in the proportion presenting with doublings was observed after the second vs. first dose. Most of the values representing a doubling were still below the upper range of normal of the reference value for the particular study.

In the listing of the cases with doublings the majority of the doublings were from < cut-off at screening to just above the cut off at one or both post-vaccination measurements.
Values recorded as grade 2 or higher (at least 0.08 µg/l) were observed only in POX-MVA-008 and POX-MVA-011. With one exception, all subjects were vaccinia-naïve and received two doses. The exception was subject 2040-012 in POX-MVA-011, who was HIV-infected and vaccinia-experienced. The subject's troponin I values increased to 0.20 µg/l after MVA-BN (0.27 µg/l on repeat testing), then decreased again back to normal values at follow-up. Subject 26-050 had a value of 1.90 µg/l recorded after the second dose only and 2022-043 had a value of 1.34 µg/l on follow-up only. For the other 7/10 with Grade 3 values two occurred after the first dose and 5 after the second dose. One of these (31-008) had a high screening value (0.82 µg/l) which remained above 0.2 µg/l throughout but the local lab values were within normal limits (i.e. < 0.02 µg/l). There were 19 other subjects listed with Grade 2 values but in a few cases these occurred at screening.

While all the subjects with abnormal troponin I were supposed to have been investigated, it was not clear if this rule was followed with rigour. In addition, not all studies captured troponin I

and/or used appropriate assays. In response to the CHMP concerns, it was finally clarified that less than a quarter (54/229) of those who had cardiac events (experienced AEs of elevated cardiac enzymes, abnormal ECGs or cardiovascular AEs which were reported during active treatment phases and follow-up phases) were actually fully assessed by a cardiologist.

A thorough cardiac assessment will be performed for all subjects enrolled in clinical trial POX-MVA-013. Before enrolment into the trial, subjects will undergo a thorough screening with a special focus on cardiologic aspects. Throughout the trial a close cardiac monitoring will be performed. However, this study has not yet been initiated. It can only be stated at this time that it remains unknown whether MVA-BN may be associated with any risk of peri/cardiomyopathy.

Serious adverse events and deaths

There was one death in POX-MVA-011 in a vaccinia-naïve HIV-infected subject who took an overdose of Xanax and developed rhabdomyolysis followed by renal failure with subsequent death. The subject had a history of bipolar disorder and suicidal thoughts.

In the overall study programme 53 SAEs were reported in 44 subjects during the main study period and 60 SAEs were reported in 47 subjects during the follow-up period. In 109/113 cases the SAEs were considered unrelated or unlikely to be related to study vaccine and none was considered to be definitely related. There was no particular pattern with regard to the nature of the individual SAEs.

Proportions of subjects who experienced at least one SAE were comparable across the studies for vaccinia-naïve healthy subjects who received 2 scheduled doses of MVA-BN and vaccinia-experienced healthy subjects who received one dose. Rates in older subjects and HIV-infected subjects were each comparable to those in younger healthy subjects. In POX-MVA-011 there was an increasing incidence of SAEs with increasing CD4 count in vaccinia-experienced subjects but not in vaccinia-naïve HIV-infected subjects. There was no evidence that the incidence of SAEs increased with viral titre in MVA-BN.

In the main study period the single SAE that was considered probably related to vaccination was a case of Grade 3 intensity extraocular muscle paresis with onset 8 days after the second vaccination in a healthy vaccinia-naïve subject in POX-MVA-008. The single SAE considered possibly related was a case of Grade 3 pneumonia after the second vaccination in a HIV-infected vaccinia-naïve subject [CD4 ≥ 200- 349/µl] in POX-MVA-011. In the follow-up period two SAEs were considered possibly related to vaccination. One was Grade 2 sarcoidosis in a healthy vaccinia-naïve subject in POX-MVA-005. The other was cardiomyopathy in a HIV-infected vaccinia-naïve subject in POX-MVA-010 with onset 133 days after the second dose. She was also participating in a GH-RH study. Laboratory data are not reported from this subject.

Across the five pivotal studies there were 7 SAEs in 6 [1.1%] HNMM subjects compared with 5 SAEs in 5 [1.5%] HEM subjects. For HNMM subjects who received two doses of MVA-BN there were 4 SAEs in 3 [0.6%] subjects after dose 2, 3 SAEs in 3 [0.6%] subjects during follow-up but none after dose 1. All but 2 SAEs were considered unrelated or unlikely to be related to study vaccination.

Laboratory findings

Haematology

In POX-MVA-011 more HIV-infected subjects than healthy subjects presented with at least one out-of-range variable at each clinic visit. One male vaccinia-naïve HIV-infected subject with a CD4 count of 349–200/µl had a Grade 4 abnormal low neutrophil value (0.43×103/µl) at Week 6 that was reported as an AE with an unlikely relationship to the study vaccination. Another male vaccinia-experienced HIV-infected subject with CD4 counts of 750–501/µl had a Grade 3 abnormal low neutrophil value (0.68×103/µl) at Week 2 that was reported as an AE with possible relationship to the study vaccination.

In POX-MVA-024 two subjects who received two doses of MVA-BN and 3 who received placebo followed by MVA-BN had clinically significant abnormal low neutrophil counts at Visit 2 after the first dose or Visit 4 after the second dose and all were reported as AEs.
In POX-MVA-010 at Week 30 haematology values below LLN that had been within the normal range at Screening were documented in ≥ 10% of subjects in any treatment group for:

- Haematocrit in both vaccinia-naïve groups
- RBC in both HIV groups
- Neutrophils in the HIV vaccinia-naïve group
- MCHC in HIV vaccinia-experienced and both healthy groups

No treatment-emergent abnormal haematology values of Grade 3 or 4 were reported.

Biochemistry

In POX-MVA-008 clinically significantly abnormal biochemistry values that fulfilled the toxicity Grade 3 criterion were reported for 17 subjects, including 7 with low potassium values, 4 with high AST and ALT, one with a high ALT and 5 with elevated troponin I values (reported as SIAEs - 4 assessed as possibly related and one not related). In POX-MVA-011 HIV-infected subjects with abnormal biochemistry values not present at Screening included three vaccinia-naïve subjects with Grade 3 troponin I values (0.49 µg/l, 0.22 µg/l, and 1.34 µg/l, respectively) and one vaccinia-experienced subject with Grade 3 increased troponin I value at Week 2 (0.2 µg/l).
In POX-MVA-024 significant abnormal biochemistry values in 6 subjects were related to concentrations of AST, ALT or CK.

Pregnancies

Thirteen pregnancies occurred during 5 of the studies. One of the pregnancies was terminated with an elective abortion and one pregnancy ended as spontaneous abortion. All other pregnancies were followed up and all women gave birth to healthy babies.

Discontinuation due to adverse events

Across the studies 28 AEs in 18 (0.6%) subjects led to withdrawal either from the study or from vaccination and all occurred within the main study periods. Although leading to withdrawal, 23/28 were considered unrelated or unlikely to be related to study vaccine with no definitely

related AEs. Four AEs in 4 subjects were considered possibly related to study vaccine and one other AE was considered probably related (Grade 2 intensity injection site dermatitis after the first vaccination in an HIV-infected vaccinia-naïve subject [CD4 ≥ 350- 500/μL] in POX-MVA-011). On a PT level, there was no particular pattern with regard to the nature of the individual AEs leading to withdrawal.

2.6.1. Discussion on clinical safety

Across the 15 reported studies the total that received 2 doses of 1 x 10^8 $TCID_{50}$ of MVA-BN 4 weeks apart if VV- naïve or one dose of 1 x 10^8 $TCID_{50}$ if VV-experienced subjects was 2,388. The population includes subjects from 18 up to 80 years of age. A total of 3,066 subjects received at least one MVA-BN dose of 1 x 10^8 $TCID_{50}$ (or higher). This total included healthy subjects as well as HIV-infected subjects (N= 609) and subjects with atopic dermatitis (N = 381). No trends for unexpected and/or serious adverse reactions were detected and no difference in the safety profile has been observed between vaccinia-naïve and vaccinia-experienced subjects receiving MVA-BN. The majority of adverse drug reactions (ADRs) are related to local, injection-site reactions of mild to moderate intensity, which were completely reversible within days. The feature that stands out is the rate of reporting raised troponin I as a laboratory AE. The applicant clarified that < 25% of subjects with abnormal troponin I and/or abnormal ECG findings were actually investigated by a cardiologist and therefore it was not possible to reach a definitive conclusion regarding lack of any effect of MVA-BN on myopericardial tissue.

The CHMP requested two additional Phase III studies to provide confirmation of the safety of MVA-BN, one study will provide this confirmation against placebo and the second study will provide comparative safety data against the active comparator currently available. These studies are considered key for the benefit risk of the product.

As a consequence of the inability to provide comprehensive clinical data on the safety under normal conditions of use of the vaccine the CHMP requested specific post-authorisation obligations to gather safety data in observational prospective non-interventional cohort studies (as detailed in the RMP). In addition, post-authorisation studies will be conducted in children in case of a declared outbreak situation to provide safety data in this population.

Furthermore, the CHMP considered essential that planned studies should provide reliable information on the effects of the vaccine by systematic collection of data from troponin I assays, ECGs and echocardiograms. The risk of myopericarditis or other adverse events of special interest will be monitored in all phase III clinical studies (as detailed in the RMP).

2.6.2. Conclusions on the clinical safety

The CHMP considered the safety data package as sufficient to support the marketing authorisation. The Product Information adequately reflects the currently known safety profile of the product.

The planned studies will provide reliable information on the effects of the vaccine by systematic collection of data from troponin I assays, ECGs and echocardiograms.

2.7. Pharmacovigilance

Detailed description of the pharmacovigilance system

The CHMP considered that the Pharmacovigilance system as described by the applicant fulfils the legislative requirements.

2.8. Risk Management Plan

The CHMP received the following PRAC Advice on the submitted Risk Management Plan:

PRAC Advice

Based on the PRAC review of the Risk Management Plan version 3.3, the PRAC considers by consensus that the risk management system for modified Vaccinia Ankara virus (Imvanex) in the treatment against smallpox infection and disease in persons 18 years of age and older is acceptable.

Advice on conditions of the marketing authorisation

Risk management Plan (RMP)

The MAH shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the Marketing Authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

If the submission of a PSUR and the update of a RMP coincide, they can be submitted at the same time.

Additional risk minimisation measures

The PRAC considers that no additional risk minimisation measures will be necessary for the safe and effective use of the medicinal product.

Obligation to conduct post-authorisation measures

The PRAC recommends that the following studies should be conditions or specific obligations of the MA:

Description	Due date
POX-MVA-038: An observational, non-interventional post-authorisation safety study for the prophylactic vaccination with IMVANEX in adults.	Status Reports updates with PSURs in the annual re-assessment application
POX-MVA-039: An observational, non-interventional post-authorisation safety and efficacy study for the prophylactic vaccination with IMVANEX following re-emergence of circulating smallpox infections	Status Reports updates with PSURs in the annual re-assessment application
POX-MVA-027: A randomized, double-blind, multicenter Phase II trial to compare the immunogenicity and safety of a liquid-frozen and a freeze-dried formulation of IMVAMUNE (MVA-BN) smallpox vaccine in vaccinia-naïve healthy subjects.	Final clinical study report Q2 2016
POX-MVA-013: A randomized, double-blind, placebo-controlled Phase III trial to evaluate immunogenicity and safety of three consecutive production lots of MVA-BN smallpox vaccine in healthy, vaccinia-naïve subjects	Final clinical study report Q4 2016
POX-MVA-006: A randomized, open-label Phase III non-inferiority trial to compare the immunogenicity of IMVAMUNE (MVA-BN) with the conventional smallpox vaccine ACAM2000 in 18-40 year old healthy vaccinia-naïve subjects	Final clinical study report Q4 2017

This advice is based on the following content of the Risk Management Plan:

- **Safety concerns**

The he following safety concerns were identified:

Summary of safety concerns	
Identified risks	None
Potential risks	Myo-/pericarditis Vaccinia rash, eczema vaccinatum Generalised vaccinia Progressive vaccinia Erythema multiforme Post-vaccinial encephalitis Incorrect route of drug administration
Additional information to be provided:	Children and adolescents (<18 years) Pregnant and lactating women Elderly subjects Individuals with organ impairment Clinically immunocompromised individuals

	Safety experience in mass vaccination due to smallpox outbreak Interactions with other vaccines and concomitantly administered immunoglobulins

- **Pharmacovigilance plans**

Pharmacovigilance measures included in the RMP:

Study / Pharmacovigilance measure Type, title and category (1-3)	**Objectives**	**Safety concerns addressed**	**Status** (planned, started)	**Date for submission of final reports**
Nonclinical Protocol: Developmental Toxicity Study of Subcutaneously Administered MVA-BN Vaccine in Early Pregnant Wistar Rats Category 3	To investigate the effect of Imvanex administration during the early phase of pregnancy	important missing information of potential relevance to use of IMVANEX in pregnant women	Planned (protocol submission planned for 30 September 2013	Final data 31 December 2014
POX-MVA-006: A randomized, open-label Phase III non-inferiority trial to compare the immunogenicity of IMVAMUNE (MVA-BN) with the conventional smallpox vaccine ACAM2000 in 18-40 year old healthy vaccinia-naïve subjects Category 1	Primary: To assess non-inferiority of Imvamune compared to a conventional smallpox vaccine Secondary: immune response, safety and reactogenicity,	myopericarditis or other AESI for Imvanex	Planned Protocol submission planned for 31 March 2014	Final data 31 December 2017
POX-MVA-013: A Randomized, Double-Blind, Placebo-Controlled Phase III Trial to Evaluate Immunogenicity and Safety of Three Consecutive Production Lots of IMVAMUNE (MVA-BN) Smallpox	Primary: To assess consistence of three consecutively produced Imvamune lots Secondary: uncommon ADRs including myo/pericarditis Collection of vaccinia specific humoral immune	myopericarditis or other AESI for Imvanex	Planned	Final CSR 31 December 2016

Vaccine in Healthy, Vaccinia-Naïve Subjects Category 1	response data			
POX-MVA-027: A randomized, double-blind, multicenter Phase II trial to compare the immunogenicity and safety of a liquid frozen and a freeze-dried formulation of IMVAMUNE (MVA-BN) smallpox vaccine in vaccinia-naïve healthy subjects Category 1	Primary: non-inferiority of immune responses induced by different formulations Secondary: safety and reactogenicity	myopericarditis or other AESI for Imvanex	Planned	Final CSR 30 June 2016
POX-MVA-035: Open-label, non-controlled, multicenter immunogenicity and safety study of MVA-BN smallpox vaccine in children from birth to less than 12 years of age Category 3	To investigate safety and immunogenicity of Imvanex in children	Missing data in children	Planned	Dependent on start of mass vaccination programs, according to an agreed protocol

POX-MVA-037: Clinical study in a selected highly immunocompromised population that explores the dose of virus per injection including a dose higher than 1 x 10^8 $TCID_{50}$/ml, the number of injections and/or the time interval between injections Category 3	To investigate safety and immunogenicity of Imvanex in an immunocompromised population	Missing data in clinically immunocompromised individuals	Planned Protocol submission no later than 28 February 2014	CSR 31 May 2016
POX-MVA-038: An observational, non-interventional post-authorisation safety study for the prophylactic vaccination with IMVANEX in adults Category 2	Primary: investigate incidence of AEs across all age groups >18 years following active surveillance of vaccinated subjects Secondary: assessment of incidence of important and potential risks in RMP	Identified and potential risks in RMP	Planned Submission of core protocol no later than 31 December 2013	Updates with PSURs in annual re-assessment application FSR within 12 months of end of data collection
POX-MVA-039: An observational post-authorisation safety and efficacy study for the prophylactic vaccination with IMVANEX following re-emergence of circulating smallpox infections Category 2	Primary: investigate incidence of AEs across all age groups >18 years following active surveillance of vaccinated subjects Secondary: assessment of incidence of important and potential risks in RMP Effectiveness of Imvanex	Identified and potential risks in RMP Missing information: children, elderly, immunocompromised, safety in mass vaccination, interactions with vaccines, immunoglobulins.	Planned Protocol submission no later than 31 December 2013	Dependent on start of mass vaccination, according to an agreed protocol

*Category 1 are imposed activities considered key to the benefit risk of the product.
Category 2 are specific obligations
Category 3 are required additional PhV activity (to address specific safety concerns or to measure effectiveness of risk minimisation measures)

The PRAC, having considered the data submitted, was of the opinion that the proposed post-authorisation PhV development plan is sufficient to identify and characterise the risks of the product.

The PRAC also considered that routine PhV is sufficient to monitor the effectiveness of the risk minimisation measures.

- **Risk minimisation measures**

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
Identified risks		
None	NA	NA
Potential risks		
Myo-/pericarditis	None (Applicant commits to updating the product information to include a warning should information become available to suggest a causal relationship between Imvanex and myo/pericarditis)	None
Vaccinia rash, eczema vaccinatum	None	None
Generalised vaccinia	None	None
Progessive vaccinia	None	None
Erythema multiforme	None	None
Post-vaccinal encephalitis	None	None
Incorrect route of administration	SmPC section 4.2 (posology and method of administration) indicates the correct route of administration	None
Additional information to be provided:		
Children and adolescents (<18 years)	SmPC section 4.1 'Therapeutic indications' indicates correct age group (persons 18 years of age and older).	None
Pregnant and lactating women	SPC Section 4.6 'Pregnancy and lactation' states that vaccinating pregnant or lactating women is not recommended	None
Elderly subjects	None	None
Patients with organ impairment	None	None
Clinically immunocompromised patients	None	None
Safety experience in mass vaccination due to smallpox outbreak	None	None
Interactions with other vaccines and concomitantly administered immunoglobulins	SPC section 4.5 states: 'No interaction studies with other vaccines or drugs have been performed. Therefore, concomitant administration with other vaccines should be avoided'	None

The CHMP endorsed this advice with changes:

Study "POX-MVA-027: A randomized, double-blind, multicenter Phase II trial to compare the immunogenicity and safety of a liquid frozen and a freeze-dried formulation of IMVAMUNE (MVA-BN) smallpox vaccine in vaccinia-naïve healthy subjects" should not be a condition of the MA as it is not considered by the CHMP as key for the benefit/risk.

2.9. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

3. Benefit-Risk Balance

Benefits

Beneficial effects

MVA-BN elicits antibody against itself and against Vaccinia Virus Western Reserve and New York City Board of Health strains. It is able to elicit an anamnestic immune response in subjects who have previously received replication competent vaccine or MVA-BN.

Uncertainty in the knowledge about the beneficial effects

Due to the current scientific knowledge, the particularities of this condition and the non-feasibility to perform challenge studies and efficacy studies as it would be contrary to medical ethics, there is inadequate evidence from non-clinical and clinical studies to determine the level of protection MVA-BN may provide against smallpox in human. The clinical data do not provide definitive support for the current posology, including the number of doses and the possible timing of sequential (booster) doses by population sub-group.

In particular, there is uncertainty regarding the posology for healthy or HIV-infected subjects, whether vaccinia-naïve or experienced. There are also doubts about doses needed in older persons and there are no data in immunocompromised persons other than HIV-infected persons.

Although the cell-mediated immune response is considered to be important in protection against and recovery from smallpox no conclusions can be drawn from available data regarding the CMI response to MVA-BN.

In light of the limitations of the submitted data supportive of the claimed indication, additional data will be generated from post-authorisation studies. In particular, confirmation on immunogenicity will be provided from the phase III trials as detailed in the Annex II of the CHMP opinion (conditions with regard to the safe and effective use of the medicinal product). Furthermore, specific procedures to monitor the use of this vaccine have been agreed and are mentioned as specific obligations.

Risks

Unfavourable effects

Vaccination has been associated with elevations in troponin I.

MVA-BN is associated with considerable local reactogenicity.

Uncertainty in the knowledge about the unfavourable effects

The data on troponin I were not collected in a comprehensive systematic fashion. Less than 25% of all subjects with abnormal troponin I and/or ECG findings were consistently investigated by a cardiologist across studies. At this point in time, it cannot be ruled out that MVA-BN may have unwanted effects on the myopericardium.

Confirmation of the safety profile will be supported by additional data to be generated from the post-authorisation studies, in particular in the phase III trials as detailed in Annex II (conditions with regard to the safe and effective use of the medicinal product). Furthermore, the requirements defined in the specific obligations will ensure the adequate safety monitoring of the vaccine.

In non-clinical studies MVA-BN did not achieve consistent suppression of viraemia whereas no viraemia was detected in the control replication-competent vaccine groups. This raises the possibility that MVA-BN could suppress clinically apparent infection in some humans but not prevent transmission.

Balance

Importance of favourable and unfavourable effects

Smallpox (variola major form) carries a high mortality and morbidity. The replication-competent vaccines were collectively shown to be effective during the global eradication campaign. However, these vaccines are not suitable for use in some subjects and their overall safety profile points to the occurrence of well-documented rare and very rare SAEs that can be life-threatening or even fatal such as eczema vaccinatum, progressive vaccinia, generalized vaccinia, and postvaccinal encephalitis.

The level and duration of protection that MVA-BN may provide against smallpox in human is unknown and the time to onset of any degree of protection cannot be determined so that its value in a deliberate release situation cannot be assessed. However, the lack of vaccine virus replication in man implies that it is less likely to be associated with the same types of SAEs observed with the replication-competent vaccines.

Benefit-risk balance

Based on the current evidence the CHMP concluded that the benefit-risk balance is favourable in the claimed indication. A substantial proportion of the population for whom a replication competent vaccine is contraindicated can benefit from the protective effect of MVA-BN. The use of the vaccine should occur in accordance with official national recommendations.

4. Recommendations

Based on the review of data on quality, safety and efficacy, the CHMP considers by majority decision that the risk-benefit balance of Imvanex for active immunisation against smallpox in adults is favourable and therefore recommends the granting of the marketing authorisation under exceptional circumstances subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to medical prescription.

Official batch release

In accordance with Article 114 Directive 2001/83/EC, the official batch release will be undertaken by a state laboratory or a laboratory designated for that purpose.

Conditions and requirements of the Marketing Authorisation

- **Periodic Safety Update Reports**

The marketing authorisation holder shall submit the first periodic safety update report for this product within six months following authorisation. Subsequently, the marketing authorisation holder shall submit periodic safety update reports for this product in accordance with the requirements set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and published on the European medicines web-portal.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

- **Risk Management Plan (RMP)**

The MAH shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the Marketing Authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

If the submission of a PSUR and the update of a RMP coincide, they can be submitted at the same time.

- **Obligation to complete post-authorisation measures**

The MAH shall complete, within the stated timeframe, the below measures:

Description	Due date
POX-MVA-013: A randomized, double-blind, placebo-controlled Phase III trial to evaluate immunogenicity and safety of three consecutive production lots of MVA-BN smallpox vaccine in healthy, vaccinia-naïve subjects.	Final clinical study report Q4 2016

Description	Due date
POX-MVA-006: A randomized, open-label Phase III non-inferiority trial to compare the immunogenicity of IMVAMUNE (MVA-BN) with the conventional smallpox vaccine ACAM2000 in 18-40 year old healthy vaccinia-naïve subjects.	Final clinical study report Q4 2017

Specific Obligation to complete post-authorisation measures for the marketing authorisation under exceptional circumstances

This being an approval under exceptional circumstances and pursuant to Article 14(8) of Regulation (EC) No 726/2004, the MAH shall conduct, within the stated timeframe, the following measures:

Description	Due date
In order to confirm the validity of the applicant's claims regarding changes in antibody titres over time, the applicant should provide status reports on additional batched serum analysis of future clinical studies	Status to be reported annually within each annual re-assessment application
To ensure adequate monitoring of safety and/or effectiveness, the applicant should perform the following studies to collect data where IMVANEX is used as a prophylactic vaccine and/or use in case of re-emergence of circulating smallpox. • POX-MVA-038: An observational, non-interventional post-authorisation safety study for the prophylactic vaccination with IMVANEX in adults • POX-MVA-039: An observational, non-interventional post-authorisation safety and efficacy study for the prophylactic vaccination with IMVANEX following re-emergence of circulating smallpox infections	Status Reports updates with PSURs in the annual re-assessment application

Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States.

Not applicable.

New Active Substance Status

Based on the CHMP review of data on the quality properties of the active substance, the CHMP considers that Modified Vaccinia Ankara - Bavarian Nordic (MVA-BN) virus is qualified as a new active substance.