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Review

The Brighton Collaboration standardized template for collection of key information for risk/benefit assessment of a Modified Vaccinia Ankara (MVA) vaccine platform

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\textbf{Abstract}

The Brighton Collaboration Viral Vector Vaccines Safety Working Group (V3SWG) was formed to evaluate the safety and characteristics of live, recombinant viral vector vaccines. The Modified Vaccinia Ankara (MVA) vector system is being explored as a platform for development of multiple vaccines. This paper reviews the molecular and biological features specifically of the MVA-BN vector system, followed by a template with details on the safety and characteristics of an MVA-BN based vaccine against Zaire ebola-virus and other filovirus strains. The MVA-BN-Filo vaccine is based on a live, highly attenuated poxviral vector incapable of replicating in human cells and encodes glycoproteins of Ebola virus Zaire, Sudan virus and Marburg virus and the nucleoprotein of the Thai Forest virus. This vaccine has been approved in the European Union in July 2020 as part of a heterologous Ebola vaccination regimen. The MVA-BN vector is attenuated following over 500 serial passages in eggs, showing restricted host tropism and incompetence to replicate in human cells. MVA has six major deletions and other mutations of genes outside these deletions, which all contribute to the replication deficiency in human and other mammalian cells. Attenuation of MVA-BN was demonstrated by safe administration in immunocompromised mice and non-human primates. In multiple clinical trials with the MVA-BN backbone, more than 7800 participants have been vaccinated, demonstrating a safety profile consistent with other licensed, modern vaccines. MVA-BN has been approved as smallpox vaccine in Europe and Canada in 2013, and as smallpox and monkeypox vaccine in the US in 2019. No signal for inflammatory cardiac disorders was identified throughout the MVA-BN development program. This is in sharp contrast to the older, replicating vaccinia smallpox vaccines, which have a known risk for myocarditis and/or pericarditis in up to 1 in 200 vaccinees. MVA-BN-Filo as part of a heterologous Ebola vaccination regimen (Ad26.ZEBOV/MVA-BN-Filo) has undergone clinical testing including Phase III in West Africa and is currently in use in large scale vaccination studies in Central African countries. This paper provides a comprehensive picture of the MVA-BN vector, which has reached regulatory approvals, both as MVA-BN backbone for smallpox/monkeypox, as well as for the MVA-BN-Filo construct as part of an Ebola vaccination regimen, and therefore aims to provide solutions to prevent disease from high-consequence human pathogens.

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1. Introduction

The Brighton Collaboration (www.brightoncollaboration.org) was launched in 2000 to improve the science of vaccine safety. The Brighton Collaboration formed the Viral Vector Vaccines Safety Working Group (V3SWG) in October 2008 to improve our ability to anticipate potential safety issues and meaningfully assess or interpret safety data, thereby facilitating greater public acceptance when a viral vector vaccine is licensed. The V3SWG has developed a standardized template describing the key characteristics of a novel viral vaccine vector to facilitate the scientific discourse among key stakeholders and increase the transparency and comparability of information. This introduction and the “specific instructions” provide definitions and additional guidance for completing the template (V2.0) that follows.

Viral vector vaccines are laboratory-generated, chimeric viruses that are based upon replicating or non-replicating virus vectors into which have been spliced genes encoding antigenic proteins for a target pathogen. Consideration of safety issues associated with viral vector vaccines requires a clear understanding of the agents used for construction of the vaccine. These include (1) the wild type virus from which the vector is derived, referred to in the template as “wild type virus”; (2) the vector itself before incorporation of the foreign antigen, referred to in the template as “viral vector”; and (3) the final recombinant viral vector vaccine, referred to in the template as “vaccine”. Wild type viruses used as vectors may originate from human or non-human hosts and may have low or high pathogenic potential in humans regardless of species of origin. Viral vectors can originate from attenuated human vaccines, from attenuated human viruses, from human viruses with low pathogenic potential, from animal viruses with low human pathogenic potential, and from vectors (for the expression of proteins) which are then adapted as a viral vector (such as DNA plasmids or baculovirus vector vaccines) to be used as a vaccine in humans or animals. Thus, viral vectors usually, but not always, have properties in a human host that differ from wild type virus from which they were derived. Incorporation of a target antigen into a viral vector to create a vaccine may alter the properties of the vector such that the vaccine may have properties that differ from the vector. The Brighton Collaboration Vaccine Vector template is designed to describe vectors into which transgenes may be incorporated to create vaccines. However, pursuant to understanding the safety aspects of a given vector, consideration is given to the wild type virus from which the vector is derived (Table 1, Section 3), and the potential impact of transgene insertion to create a vaccine (Table 1, Section 5).

1.1. Modified Vaccinia Ankara (strain MVA-BN) as a platform for recombinant vaccines

1.1.1. Background

The world was declared to be free of smallpox in May 1980 and this was because of global vaccination with vaccinia virus (VACV) based vaccines [1]. Although these vaccines were very successful at preventing smallpox there were serious adverse events indicating the need for a less virulent vaccine [2,3].

Due to these often-severe post-vaccination complications associated with Vaccinia viruses, there were several attempts to generate a more attenuated, safe smallpox vaccine. Modified vaccinia Ankara (MVA) originates from the dermal Vaccinia Virus Ankara strain (Chorioallantois Vaccinia Virus Ankara, CVA) that was maintained in the Vaccination Institute Ankara for many years and used as the basis for vaccination of humans. During the period of 1960 to 1974, Prof. Anton Mayr and his colleagues (University of Munich, Germany, Institute for Microbiology and Infectious Diseases of Animals) succeeded in attenuating CVA by over 500 continuous passages in primary CEF (chicken embryo fibroblast) cells.

A reduced virulence of CVA was reported from passage 371 on CEF cells [4]. From passage 516, the attenuated CVA virus was renamed MVA to discriminate it from other attenuated Vaccinia virus strains [5–7]. In clinical trials with MVA, the pock lesions associated with vaccinia virus vaccination are not seen [7]. This attenuated MVA vaccine was used in more than 120,000 vaccinates for priming prior to administration of a conventional smallpox vaccine in a two-step protocol used in the 1970s in Europe [3,6,8].

In the last decades, multiple recombinant MVA vectors have been tested as vaccine candidates against various pathogens, such as human immunodeficiency viruses, Mycobacterium tuberculosis, Plasmodium falciparum or Middle East Respiratory Syndrome virus [8,9].

MVA-BN, that is derived from the MVA strain developed in Prof. Anton Mayr’s laboratory, is a further attenuated MVA strain, which has lost its ability to replicate in most mammalian cell types, including human cell lines and is safe in severely immune compromised animals [10,11]. The hallmark of MVA-BN is the fact that it does not productively replicate in the human keratinocyte cell line HaCat, the human cervix adenocarcinoma cell line HeLa, the human embryo kidney cell line 293 (HEK293), and the human bone osteosarcoma cell line 143B [10,12].

However, like other MVA strains, MVA-BN effectively infects mammalian cells. Infection of mammalian cells results in transcription of the viral genes, but no MVA-BN virus is released from the cells due to a genetic block in the viral assembly and egress. The infected cells eventually undergo apoptosis (programmed cell death) [13–15]. There are several deletions and other mutations in MVA that account for the change in host-range of the virus. Six major deletions mainly account for a reduction in the size of the original vaccinia genome from 204.5 kb to 178 kb for the MVA strain [12,16]. Sequencing of the genome revealed that these deletions included immune evasion genes, host interactive protein genes and some structural proteins [17].

Due to the lack of replication competence in many mammalian cells including human cells, MVA-BN can be safely administered to immunocompromised humans. This safety feature has also been confirmed in severely immunocompromised animals [10,11]. MVA-BN is now a licensed smallpox vaccine (since 2013 in EU
Table 1
Brighton Collaboration Viral Vector Vaccines Safety Working Group (V3SWG).

| 1. Authorship | Information |
|---------------|-------------|
| 1.1. Author(s) | Anna-Lise Williamson, Thomas PH Meyer, Ariane Volkmann, Heinz Weidenthaler |
| 1.2. Date completed/updated | April 2020 |

| 2. Basic vector information | Information |
|----------------------------|-------------|
| 2.1. Vector name | Modified Vaccinia Ankara (MVA-BN) |
| 2.2. Vector origin Family/Genus/Species/subtype | Poxviridae family/orthopox virus/vaccinia virus/modified vaccinia virus Ankara/MVA-BN |
| 2.3. Vector replication in humans (replicating or non-replicating) | MVA-BN is a non-replicating vector in humans. |

| 3. Characteristics of the wild type virus from which the vector is derived | Information | Comments/Concerns | Reference(s) |
|-----------------------------------------------------------------|-------------|------------------|--------------|
| 3.1. Name of wild type virus (common name; Family/Genus/Species/subtype) | Family: Poxviridae | VACV is the virus used for the replicating smallpox vaccine that was utilized during eradication and now ACAM2000. | [39] |
| Subfamily: Chordopoxviridae | Genus: Orthopoxviridae | The origin of the VACV is debated and there is some evidence that it originated from a horse poxvirus which was able to infect cows. In Brazil and in India VACV is endemic in animals, with occasional transmission to humans, and is thought to originate from smallpox vaccine campaigns. | [39–43] |
| Species: Vaccinia virus (VACV) | | There is evidence that shedding of VACV from the vaccination lesion of healthy primary vaccinees occurs from about the third day to the end of the third week after vaccination. There are rare reports of transmission of VACV. | [44] |

| 3.2. What is the natural host for the wild type virus? | The original host is unknown, but VACV can replicate in a range of animals including primates, rodents, lagomorphs and ungulates as well as humans. | | |

| 3.3. How is the wild type virus normally transmitted? | The typical manifestation of the wildtype virus infection are vesiculopustular lesions or dermal vesicles (pox lesions). These lesions contain infectious virus particles. Transmission can occur by close contact with infected area. There is no evidence that VACV is transmitted via airborne infection. | | |

| 3.4. Does the wild type virus establish a latent or persistent infection? | No, the infections are acute | | |
| 3.5. Does the wild type virus replicate in the nucleus? | No. Poxviruses replicate in the cytoplasm. | | |

(continued on next page)
3.6. What is the risk of integration into the human genome?

Poxviral vectors are considered non-integrating according to the EMA ‘Guideline on nonclinical testing for inadvertent germline transmission of gene transfer vectors’, because they lack the machinery to actively integrate their genome into the host chromosomes.\(^1\) MVA, as well as other members of the Poxviridae family, is unusual among deoxyribonucleic acid (DNA) viruses in that they replicate in the cytoplasmic compartment of the cell. Compared to other DNA viruses, the possibility for integration of their genetic material into the host chromosome is therefore extremely low\(^{[47]}\).

In addition, vaccinia infection results in cell death.

3.7. List any disease manifestations caused by the wild type virus, the strength of evidence, severity, and duration of disease for the following categories:

- **In the healthy natural host**
  - There are reports of occurrence of vaccinia infection in dairy cattle, particularly in Brazil. The manifestation consists of painful vesiculopustular lesions\(^{[42,43]}\).

- **In healthy human host**
  - Most common AE is generalized vaccinia.
  - Association reported between the US vaccinia strain and myocarditis and/or pericarditis in up to 1 in 200 vaccinees.
  - Eczema vaccinatum, progressive vaccinia, and neurological and cardiac complications.
  - Death rate 1-5/million.
  - These rates are rather for the previously observed complication rates with replicating vaccinia virus smallpox vaccines, such as ACAM2000 or Dryvax. There is no natural occurrence of vaccinia virus infections in human hosts.

- **In immunocompromised humans**
  - Can be fatal and so vaccination with VACV is contraindicated.

- **In human neonates, infants, children**
  - Children <12 months of age have an increased rate of the complications listed above for healthy human host\(^{[54,55]}\).

- **During pregnancy and in the unborn in humans**
  - Live vaccinia virus vaccines can cause fetal harm when administered to a pregnant woman. Congenital infection, principally occurring during the first trimester, has been observed after vaccination with live vaccinia smallpox vaccines, although the risk may be low. Generalized vaccinia of the fetus, early delivery of a stillborn infant, or a high risk of perinatal death has been reported. (Source: ACAM2000 prescribing information)
  - Pregnant women can be given Vaccinia hyperimmune globulin if at risk of transmission to fetus.

- **In any other special populations?**
  - Live vaccinia virus vaccines are contraindicated for subjects with atopic dermatitis (eczema), allergies to vaccine components and immunosuppression.

This also includes treatments that cause immunodeficiency or immunosuppression, including radiation therapy, antimitabolites, alkylating agents, corticosteroids, chemotherapy agents, and organ transplant medications. Patients should not be vaccinated with live vaccinia virus until they, or their household contacts, have been off immunosuppressive treatment for three months.

Inflammatory eye diseases, including eye surgery and subsequent use of...
3.8. What cell types are infected and what receptors are used in the natural host and in humans? A wide range of cells can be infected with VACV. Four proteins participate in attachment to glycosaminoglycans and laminin. A complex of 11 proteins mediate the hemifusion and entry steps.

3.9. What is known about the mechanisms of immunity to the wild type virus? Antibody and T cell responses associated with protection. People with severe T cell abnormalities developed generalized VACV infection after vaccination. The same was not seen with in people with agammaglobulinemia. This indicated that cell medicated immunity was important in controlling the primary VACV infection. Vaccinia immune globulin (VIG) is recommended as first line therapy for adverse event after VACV vaccination.

3.10 Has disease enhancement been demonstrated with the wild type virus: No

3.11 Is DE a possible contributor to the pathogenesis of wild type disease No

3.12 What is the background prevalence of natural immunity to the virus? Low – natural VACV infections are relatively rare. “Natural” infections are rather accidents of lab workers. Background immunity in the population is based on previous vaccination programs. There are only isolated reports of natural infections with “wild type” VACV.

3.13 Is there any vaccine available for the wild-type virus? If yes, The parent virus is the vaccine strain of VACV, so this is the vaccine. The second-line therapy, Tecovirimat (TPOXX) is approved for treatment of smallpox but can be reasonably expected to show effectiveness also for other orthopoxvirus infections.

3.14 Is there treatment available for the disease caused by the wild type virus?
Vaccinia immune globulin (VIG) is as the first-line therapy, and Brincidofovir, the second-line therapy. Tecovirimat (TPOXX) Prescribing Information

4. Characteristics of the vector from which vaccine(s) may be derived

| Information | Comments/Concerns |
|-------------|-------------------|
| MVA was derived from the vaccine strain of the smallpox vaccine, vaccinia virus strain Ankara by passage on the chorioallantoic membrane of chicken eggs. MVA-BN was derived from MVA by further passaging and plaque purification, see Section 2.2. | | [4,5,7] |
| After over 500 passages in eggs MVA was shown to have restricted host tropism and did not complete replication in human cells. MVA has six major deletions which account for a reduction in the size of the original vaccinia genome from 208 kb to 177 kb for the MVA strain, and other mutations of genes outside these deletions, which all contribute to the replication deficiency in human and other mammalian cells. The deletions included immune evasion genes, host interactive protein genes and some structural proteins. | | [15,17,4,5–7,12,16,65] |
4.3. What is known about the replication, transmission and pathogenicity of the vector in humans in the following categories:

- **in healthy people**
  - Non-productive infection
  - Non-replicating in human cell lines, no egress of infectious virus particles after first infectious cycle.

- **in immunocompromised people**
  - Non-productive infection
  - Non-replicating in human cell lines, clinical trials in HIV positive subjects showed safety profile equivalent to healthy populations.

- **in neonates, infants, children**
  - Non-productive infection
  - Non-replicating in human cell lines. Clinical trials with a Measles construct and with a Filo construct in pediatric population showed a safety profile equivalent to healthy adults.

- **during pregnancy and in the unborn**
  - Non-productive infection
  - Limited experience in MVA-BN clinical trial program, in total 29 pregnancies reported and documented. No congenital abnormalities, complication rate in line with expected background rates.

- **in gene therapy experiments**
  - Non-productive infection
  - Cancer vaccines: CV301 (reference [69])

- **in any other special populations**
  - Non-productive infection.
  - Non-replicating in human cell lines
  - Brachyury (references [35,70])
  - Atopic dermatitis: references [71,72]
  - Stem cell transplant: reference [73]
  - Replicating in CEF (chicken embryo fibroblast) cells. Replicating in BHK (baby hamster kidney) cells, and some other cell lines, but not in live mammals including rabbits, rats, immunosuppressed NHP (non-human primates), immunocompromised mice

4.4. Is the vector replication-competent in non-human species?

- It replicates in chicken embryo fibroblasts, baby hamster kidney cells; no replication has been described in vivo

4.5. What is the risk of reversion to virulence or recombination with wild type virus or other agents?

- No documented incidence of reversion which appears extremely unlikely. Recombination with standard vaccinia virus strains or other Orthopoxviruses can occur

4.6. Is the vector genetically stable in vitro and/or in vivo?

- Yes

4.7. What is the potential for shedding and transmission to humans or other species?

- Negligible

4.8. Does the vector establish a latent or persistent infection?

- No

4.9. Does the vector replicate in the nucleus?

- No

4.10. What is the risk of integration into the human genome?

- Extremely low as replication takes place in cytoplasm and infection results in cell death

4.11. Is there any previous human experience with this or a similar vector (safety and immunogenicity records)?

- Yes

Excellent safety record. Reference to BN's clinical trial program and approved product texts. MVA-BN has been developed by BN under two Investigational New Drug (IND) applications in the US: IND 11596 for the LF formulation, and IND 15316 for a freeze-dried (FD) formulation. MVA-BN (LF formulation) has been approved as smallpox and monkeypox vaccine (tradename JYNNEOS) by the FDA on September 24, 2019. From the time that clinical development of MVA-BN was initiated in 1999, a total of 7,871 subjects have been vaccinated with MVA-BN in 22 completed clinical studies; 16 trials were sponsored by BN (10 under IND 11596, one under IND 15316) and 6 were sponsored by the NIH/DMID (under IND 11229). These trials were designed to identify an optimal dose and vaccination regimen; to generate data indicating the protective efficacy of MVA-BN by
comparison to replicating smallpox vaccines (Dryvax and ACAM2000); to assess the safety and immunogenicity of MVA-BN in subjects 18-80 years of age, including healthy as well as at-risk populations with contraindications to receive traditional smallpox vaccines; and to compare the FD to the LF formulation of MVA-BN. In all these trials and all populations studied, MVA-BN has demonstrated a favorable safety profile and consistently demonstrated the ability to induce a rapid and strong vaccinia-specific immune response, i.e. neutralizing antibodies measured by plaque reduction neutralization test (PRNT) and total antibodies measured by enzyme-linked immunosorbent assay (ELISA).

### 4.12. What cell types are infected and what receptors are used in humans?
Can potentially infect all cell types, multiple receptors

### 4.13. What is known about the mechanisms of immunity to the vector?
Immune responses to the vector are directed to multiple antigens and are based on antibodies as well as T cell responses

### 4.14. Has disease enhancement been demonstrated with the vector?
- **in vitro?** No
- **in animal hosts?** No
- **in humans?** No

### 4.15. Is there antiviral treatment available for disease manifestations caused by the vector?
There are no disease manifestations reported as vector does not complete replication.

### 4.16. Can the vector accommodate multigenic inserts or will several vectors be required for multigenic vaccines?
Yes, several foreign genes can be inserted into a single MVA-BN construct

### 5. Characteristics of vector-based vaccine(s)

| 5.1. What is the target pathogen? | Ebola virus |
|---------------------------------|-------------|
| 5.2. What is identity and source of the transgene? | The protein sequences for glycoproteins (GP) from Ebola Virus (EBOV) Zaire (Mayinga; GenBank: ABX75367.1), Sudan Virus (SUDV) (Gulu; GenBank: AAU43887.1) and Marburg Virus (MARV) (Musoke; GenBank: ABA87127.1) and NP from Tai-Forest Virus (TAFV) (GenBank: ACJ28629.1) were used. The corresponding DNA sequences were optimized for human cell expression, homologies between the different GP were reduced without affecting the amino acid sequence to circumvent homologous recombination, and genes were synthesized by GeneArt (Regensburg, Germany). |
| 5.3. Is the transgene likely to induce immunity to all strains/genotypes of the target pathogen? | Yes, the vaccine induces immunity against Ebola virus Zaire (target indication), subtype Mayinga (glycoprotein encoded in the vaccine) and other subtypes of EBOV, e.g. Kikwit |
| 5.4. Where in the vector genome is the transgene inserted? | Two transgenes each (GP SUDV & NP TAFV and GP EBOV & GP MARV) with their own promoters are inserted in two MVA-BN non-coding regions (intergenic regions) in a single MVA-BN viral vector |

| Information | Comments/ Concerns | Reference(s) |
|-------------|--------------------|--------------|
| Ebola virus | MVA-BN Filo contains inserts of the following viruses: Ebola virus Zaire, Sudan virus, Marburg virus, Tai Forest virus; Protection against EBOV Kikwit challenge in NHP demonstrated | [96] |
| Examples for multiple inserts in a single MVA-BN vector are: MVA-BN Filo: | [89–91] |
| MVA-BN RSV: | [30,92–97] |
| MVA-BN CV301: | [33] |
| MVA-BN Brachyury: | [69] |
| MVA-BN CV301: | [35] |

(continued on next page)
### 5.5. Does the insertion of the transgene involve deletion or other rearrangement of any vector genome sequences?

No

### 5.6. How is the transgene expression controlled (transcriptional promoters, etc.)?

Three different poxviral promoters (synthetic and native) with early and late elements

### 5.7. Does insertion or expression of the transgene affect the pathogenicity or phenotype of the vector?

Humans: no replication; this was tested in multiple human cell lines. Other species: replication in chicken embryo fibroblasts (CEF) may suggest replication in birds (not tested).

### 5.8. Is the vaccine replication-competent in humans or other species?

For vector only; see Section 4.5.

### 5.9. What is the risk of reversion to virulence or recombination with wild type or other agents?

For vector only: see Section 4.5. For insert: no risk, only single proteins contained.

### 5.10. Is the vaccine genetically stable in vitro and/or in vivo?

Yes

Recombinant product analyzed through seven production passages.

### 5.11. What is the potential for shedding and transmission to humans or other species?

Negligible

See Section 4.7 (same as for vector).

### 5.12. Does the vaccine establish a latent or persistent infection?

No

See Section 4.8 (same as for vector).

### 5.13. Does the vaccine replicate in the nucleus?

Extremely low

See Section 4.10 (same as for vector).

### 5.14. List any disease manifestations caused by the vaccine in humans, the strength of evidence, severity, and duration of disease for the following categories:

- In healthy people
- In immunocompromised people
- In neonates, infants, children
- During pregnancy and in the unborn
- In any other special populations

See Section 3.7 (same as for vector/wild type virus).

### 5.15. What cell types are infected and what receptors are used in humans?

Antibody and T cell responses are induced upon vaccination.

### 5.16. What is known about the mechanisms of immunity to the vaccine?

See Section 3.8.

### 5.17. Has disease enhancement been demonstrated with the vaccine:

- in vitro?
- in animal models?
- in human hosts?

No

Low effect of pre-existing immunity; the MVA-BN Filo vaccine is intended for single-dose and it is non-replicating, i.e. not dependent on several infection cycles; as a poxvirus, it is also not dependent on a single receptor for entry but uses multiple proteins.

### 5.18. Are there antiviral or other treatments available for disease manifestations caused by the vaccine?

No disease manifestations

### 5.19. Is the vaccine transmissible in humans or other species (including arthropods) and/or stable in the environment?

No, not transmissible due to non-replicating properties (therefore handling of MVA-BN under BSL1 conditions).

Environmentally stable

As with all poxviruses, MVA shows high environmental stability with high resistance to drying up to 39 weeks at 6.7% moisture at 4°C and increased temperature tolerance compared to other viruses.

### 5.20. Target populations for the vaccine (e.g. pediatric, maternal, adult, elderly, etc.)

Individuals ≥1 year of age

### 5.21. Does the vaccine involve deletion or other rearrangement of any vector genome sequences?

No

### 5.22. How is the transgene expression controlled (transcriptional promoters, etc.)?

Human clinical trial data as well as animal toxicology studies with recombinant MVA-BN based vaccines, including MVA-BN Filo, have shown a comparable safety profile as the MVA-BN vector.

### 5.23. Does insertion or expression of the transgene affect the pathogenicity or phenotype of the vector?

Replicating in CEF. Replicating in BHK, and some other cell lines, but not in live mammals including rabbits, rats, immunosuppressed NHP, immune-compromised mice.

### 5.24. Is the vaccine replication-competent in humans or other species?

Humans: no replication; this was tested in multiple human cell lines. Other species: replication in chicken embryo fibroblasts (CEF) may suggest replication in birds (not tested).

### 5.25. Does insertion or expression of the transgene affect the pathogenicity or phenotype of the vector?

For vector only; see Section 4.5.

### 5.26. What is the risk of reversion to virulence or recombination with wild type or other agents?

For vector only: see Section 4.5. For insert: no risk, only single proteins contained.

### 5.27. Is the vaccine genetically stable in vitro and/or in vivo?

Yes

Recombinant product analyzed through seven production passages.

### 5.28. What is the potential for shedding and transmission to humans or other species?

Negligible

See Section 4.7 (same as for vector).

### 5.29. Does the vaccine establish a latent or persistent infection?

No

See Section 4.8 (same as for vector).

### 5.30. Does the vaccine replicate in the nucleus?

Extremely low

See Section 4.10 (same as for vector).

### 5.31. List any disease manifestations caused by the vaccine in humans, the strength of evidence, severity, and duration of disease for the following categories:

- In healthy people
- In immunocompromised people
- In neonates, infants, children
- During pregnancy and in the unborn
- In any other special populations

See Section 3.7 (same as for vector/wild type virus).

### 5.32. What cell types are infected and what receptors are used in humans?

Antibody and T cell responses are induced upon vaccination.

### 5.33. What is known about the mechanisms of immunity to the vaccine?

See Section 3.8.

### 5.34. Has disease enhancement been demonstrated with the vaccine:

- in vitro?
- in animal models?
- in human hosts?

No

Low effect of pre-existing immunity; the MVA-BN Filo vaccine is intended for single-dose and it is non-replicating, i.e. not dependent on several infection cycles; as a poxvirus, it is also not dependent on a single receptor for entry but uses multiple proteins.

### 5.35. Are there antiviral or other treatments available for disease manifestations caused by the vaccine?

No disease manifestations

### 5.36. Is the vaccine transmissible in humans or other species (including arthropods) and/or stable in the environment?

No, not transmissible due to non-replicating properties (therefore handling of MVA-BN under BSL1 conditions).

Environmentally stable

As with all poxviruses, MVA shows high environmental stability with high resistance to drying up to 39 weeks at 6.7% moisture at 4°C and increased temperature tolerance compared to other viruses.

### 5.37. Target populations for the vaccine (e.g. pediatric, maternal, adult, elderly, etc.)

Individuals ≥1 year of age
### 6. Toxicology and potency (Pharmacology) of the vector

| Information | Comments/Concerns | Reference(s) |
|-------------|-------------------|---------------|
| **6.1.** What is known about the replication, transmission and pathogenicity of the vector in and between animals? | Non replicating vector so no transmission | |
| **6.2.** For replicating vectors, has a comparative virulence and viral kinetic study been conducted in permissive and susceptible species? (yes/no) If not, what species would be used for such a study? Is it feasible to conduct such a study? | N/A | |
| **6.3.** Does an animal model relevant to assess attenuation exist? | Attenuation of the MVA-BN backbone was demonstrated in immunocompromised mice and NHP | [10,11] |
| **6.4.** Does an animal model for safety including immuno-compromised animals exist? | Yes | |
| **6.5.** Does an animal model for reproductive toxicity exist? | Yes | |
| **6.6.** Does an animal model for immunogenicity and efficacy exist? | Yes | |
| **6.7.** Does an animal model for antibody enhanced disease or immune complex disease exist? | N/A | |
| **6.8.** What is known about biodistribution in animal models or in humans? | Two biodistribution studies in rabbits (not published) showed the highest number of vaccinia positive tissues within the first 48 hours following IM or SC injection of MVA-BN and confirmed that expression is mostly limited to the injection site. | |
| **6.9.** What is the evidence that vector derived vaccines will generate a beneficial immune response in: | | |
| • Small animal models? | Yes | RSV immunogenicity in mice and cotton rats (manuscript in preparation) | YF in hamster: [104]  Alphavirus: [105]  Mouse MVA-Her: [106]  [96,97] |
| • Nonhuman primates (NHP)? | Yes | Clinical trials with recombinant MVA-BN vaccines assessing immune responses, e.g. HIV or RSV | HIV: [34]  RSV: MVA-BN-RSV: [33] |
| • Human? | Yes | | |
| **6.10.** Have challenge or efficacy studies been conducted in subjects with: | | |
| • HIV? | No | MVA has been tested in HIV positive ART treated individuals with no serious AE.  BN comment: BN studies (using MVA-BN or recombinant vaccine) with HIV positive subjects were no challenge or efficacy studies. | [34,107] |
| • Other diseases? | Yes. No evidence for interaction/interference. | No studies performed using different MVA-based recombinant vaccines.  But studies performed with heterologous prime – boost regimen (oncology, CV301, Ebola,..) | HIV: [108]  CV301: [69]  Ebola:[30,92,94,95]  Additionally: [109–111] |

### 7. Adverse Event (AE) Assessment of the Vector:

| Information | Comments/Concerns | Reference(s) |
|-------------|-------------------|---------------|
| **7.1.** Approximately how many humans have received this viral vector vaccine to date? If variants of the vector, please list separately. | 7,871 in 22 completed clinical trials  Approx. 2,700 more in ongoing clinical trials, not yet analyzed | Same references as Section 4.11 |
| **7.2.** Method(s) used for safety monitoring: | No, all mentioned data from clinical trial sources. However spontaneous reporting is ongoing for post-authorization sources, but the product was not yet used extensively | |
| • Spontaneous reports/passive surveillance | If yes, describe method: | |
| • Diary | Yes | If yes, number of days: 8 days (vaccination day plus 7 subsequent days) | Same as Section 4.11 |

(continued on next page)
- Other active surveillance: Yes

If yes, describe method and list the AE's solicited:
- Routine AE/SAE reporting in clinical trial setting.
- Active cardiac monitoring using ECG, Troponin I and targeted physical exams in most of the clinical trials.

7.3. What criteria was used for grading the AE's?
- 2007 US FDA Guidance for Industry
Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials
- If no or other, please describe:
  Other: protocol specific definitions of toxicity grades, in particular for assessment of laboratory abnormalities.

7.4. List and provide frequency of any related or possibly related serious AE's observed:
Across all studies, a causal relationship to MVA-BN (JYNNEOS) could not be excluded for 4 SAEs, all non-fatal, which included Crohn's disease, sarcoidosis, extraocular muscle paresis and throat tightness.

MVA empty backbone vector (MVA-BN) was approved as the vaccine JYNNEOS for smallpox/monkeypox by the FDA on September 24, 2019. The SAE information is from the US Prescribing Information.
The integrated analyses of serious adverse events (SAEs) pooled safety data across 22 studies, which included a total of 7,093 smallpox vaccine-naive subjects and 766 smallpox vaccine experienced subjects who received at least 1 dose of JYNNEOS and 1,206 smallpox vaccine-naive subjects who received placebo only. SAEs were monitored from the day of the first study vaccination through at least 6 months after the last study vaccination. A causal relationship to JYNNEOS could not be excluded for 4 SAEs (0.05%), all non-fatal, which included Crohn's disease, sarcoidosis, extraocular muscle paresis and throat tightness.

7.5. List and provide frequency of any serious, unexpected AE:
Among smallpox vaccine-naive subjects, SAEs were reported for 1.5% of JYNNEOS recipients and 1.1% of placebo recipients. Among the smallpox vaccine-experienced subjects enrolled in studies without a placebo comparator, SAEs were reported for 2.3% of JYNNEOS recipients.

7.6. List and provide frequency of any serious, unexpected statistically significantly increased AE or lab abnormality in vaccinee vs. control group:
There were no statistically significant differences in serious AEs or lab abnormalities.

Number of SAEs too small and evenly distributed across groups for any statistically significant imbalance. For Troponin I an imbalance was observed although not evaluated statistically. The imbalance in Troponin values is described in Section 7.7, as those were defined as AESIs.

7.7. List and provide frequency of Adverse Events of Special Interest:
Cardiac AESIs were reported to occur in 1.3% (95/7,093) of JYNNEOS recipients and 0.2% (3/1,206) of placebo recipients who were smallpox vaccine-naive.
Cardiac AESIs were reported to occur in 2.1% (16/766) of JYNNEOS recipients who were smallpox vaccine-experienced. The higher proportion of JYNNEOS recipients who experienced cardiac AESIs was driven by 28 cases of asymptomatic post-vaccination elevation of troponin-I. The clinical significance of these asymptomatic post-vaccination elevations of troponin-I is unknown.

Myopericarditis is a known risk of previously approved vaccinia smallpox vaccines. Evaluation of cardiac adverse events of special interest (AESIs) included any cardiac signs or symptoms, ECG changes determined to be clinically significant, or troponin-I elevated above 2 times the upper limit of normal. In the 22 studies performed with MVA-BN, subjects were monitored for cardiac-related signs or symptoms through at least 6 months after the last vaccination. No signal for inflammatory cardiac disorders was identified throughout the MVA-BN development program.

Control groups included placebo and the smallpox vaccine ACAM2000.

Data of MVA-BN vs. placebo is published in [31]
Data of MVA-BN vs. ACAM2000: [32]
[31,87]
Prescribing Information of JYNNEOS (www.jynneos.com) [112]
and Canada and since 2019 in the US, where it is also licensed as a monkeypox vaccine after undergoing a clinical development program involving >7800 trial participants in completed clinical trials.

1.1.2. Poxvirus as vaccine vectors

Poxviruses make excellent vaccine delivery vehicles since their genomes allow large insertions of foreign DNA [18,19]. Conventionally, foreign genes are inserted into poxviruses by homologous recombination into non-essential genes or into intergenic regions [20]. The genes are under the control of a poxvirus promoter and may have a reporter gene or selection marker to aid selection of recombinants [21–24]. The foreign genes are usually modified to remove the poxvirus early transcription termination signals (TTTTTNT) [25] and must be devoid of introns. Recently a Horsepox virus genome has been made by chemical synthesis and rescued by coinfection with Shope fibroma virus [26] demonstrating that this strategy can potentially be used in the future to synthesize other poxviruses. One of the most successful poxvirus vectored vaccines is the VACV vectored rabies vaccine distributed in oral baits for foxes, which has almost completely eradicated terrestrial rabies in parts of Europe [27,28]. Host restricted poxviruses, such as the canary poxvirus, ALVAC, have been registered as commercial vaccine vectors for a number of veterinary diseases including equine influenza, canine distemper, rabies, feline leukemia and West-Nile fever [29].

This publication presents the properties of MVA-BN as a vaccine vector and specifically focuses on MVA-BN-Filo as a component of

### 8. Overall Risk Assessment of the Vector

| Information | Comments/ Concerns | Reference(s) |
|-------------|--------------------|--------------|
| None serious safety concerns identified, safety profile is consistent with other licensed, modern vaccines. Mostly local and systemic reactogenicity, rare cases of allergy/hypersensitivity | None identified safety concerns in overall development program in 7,871 vaccinated subjects in completed clinical trials. Overall exposure is currently >10,500 subjects including ongoing trials, confirming the lack of safety concerns. | [49,50,112] |
| Investigated in HIV positive subjects | | [66–68] |
| No clinical trials performed with the empty backbone vector (smallpox vaccine), but some data with recombinant constructs, such as a measles vaccine and the MVA-BN Filo construct. No signals towards differences in safety profile between adults and pediatric populations were detected. | Experience with a predecessor MVA strain in children in the 1970s, no safety concerns. | [114] |
| No safety signal in 29 pregnancies observed during the clinical development program. Rate of spontaneous abortions in line with published background experience. Data basis is insufficient for a relevant assessment. | | |
| Specifically tested in this population, who cannot receive traditional, replicating smallpox vaccines. | | [71,72] |
| Negligible | | |

See Section 4.11 and Prescribing Information of JYNNEOS (www.jynneos.com) [112,113]
an Ebola virus two-dose regimen (Ad26.ZEBOV/MVA-BN-Filo) which was granted Marketing Authorization by the European Commission on July 1, 2020 [30] (Table 1).

2. Disclaimer

The findings, opinions, conclusions, and assertions contained in this consensus document are those of the individual members of the Working Group. They do not necessarily represent the official positions of any participant’s organization (e.g., government, university, or corporations) and should not be construed to represent any Agency determination or policy.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: The authors Ariane Vollmann, Heinz Weidenthaler, and Thomas Meyer are employees of Bavarian Biotec.

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