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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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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 ebolavirus 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] See Acknowledgement for other V3SWG members; the new name of V3SWG is Benefit-Risk Assessment of VAccines by TechnolOgy (BRAVATO).

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Contents

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 completely 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 570 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 vaccinees 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 Poxviruses are large, enveloped virus particles with a rounded brick shape of approximately 350 x 250 x 250 nm. They are surrounded by 1-2 membranes containing protein projections that are discernible by electron microscopy. The dumbbell shaped viral core surrounded by the core wall is mainly composed of proteins and contains the viral genome. The poxvirus genome consists of a linear double-stranded DNA of 130 - 230 kb with closed hairpin ends. The genome of **MVA** is a linear, double-stranded DNA molecule with covalently closed ends (hairpins) comprising 177,923 nucleotides [17]. Inverted terminal repeats (ITRs) of 9.8 kb are located at the ends of the genome. These ITRs consist of 3 major parts: The terminal hairpin loops comprising 165 nucleotides, 6 kb of DNA mainly consisting of tandem repeats and 3.7 kb of non-repeat region coding for 4 polypeptides. In total, the genome contains 193 open reading frames (ORFs) coding for polypeptides of more than 62 amino acids. In comparison to the parental Vaccinia Virus Ankara strain (Chorioallantois Vaccinia Virus Ankara, CVA) the genome of MVA has lost around 26,600 nucleotides. Six major deletions totaling more than 25 kb and deleting or truncating 31 ORFs have occurred in the genomic DNA of MVA compared to CVA. In addition, a multitude of shorter deletions and insertions as well as point mutations have occurred in the MVA genome, resulting in gene fragmentation, truncation, short internal deletions, and amino acid exchanges. **MVA-BN** is derived from the MVA strain developed by Professor Anton Mayr. In 1998, one vial of the MVA virus (at passage 582) was transferred from the Institute of Molecular Virology, a section of the Research Center for Environment and Health (GSF, Munich), to Bavarian Nordic GmbH, Martinsried, Germany. The isolate was further passaged by Bavarian Nordic and has been named MVA-BN. Documented virus stocks i.e. the original Master Virus Bank (MVB, passage 584) and the newly rederived MVB (passage 597) have been prepared at BN which were used as primary virus stocks for GMP production. The preparation of the re-derived primary virus stock consisted of 5 rounds of plaque purification by limiting dilutions. Selection and further amplification of the final clone resulted in a new re-derived primary virus stock MVA-BN MVB corresponding to passage number 597. The complete coding DNA-sequence was determined and the re-derived MVB was confirmed to be genetically and phenotypically identical to the original MVA-BN MVB. MVA-BN was shown to be more attenuated compared to two other MVA isolates, namely MVA-572 and MVA-I721, and even fails to replicate in immune compromised animals [10,12]. Despite its high attenuation and reduced virulence, MVA-BN has been shown to elicit both humoral and cellular immune responses to Vaccinia virus and foreign genes cloned into the MVA-BN genome [31–36]. MVA is a potent inducer of type I interferon (IFN) in human cells. MVA expresses a soluble interleukin-1 receptor, which has been implicated as an anti-virulence factor for certain Poxviruses. MVA does not express soluble receptors for IFN-γ, IFN-α/-β, tumor necrosis factor and CC chemokines [37]. Neurovirulence assessment of vaccinia virus based smallpox vaccines demonstrated the inability of MVA to replicate in suckling mouse brains following intracranial inoculation of 10-100 plaque forming units (pfu) of virus, while all other vaccinia virus strains tested (Dryvax®, Lister, Copenhagen, IHD-J, WR) replicated to peak titers of 10^7 to 10^8 pfu per gram tissue. Moreover, none of the doses of MVA tested, i.e. up to 10^5 pfu administered intracranially, induced death in the suckling mice. In contrast, mortality induced by Dryvax® started at a dose of 10 pfu; Lister, Copenhagen, IHD-J and WR induced death in some mice at 1 pfu and injection of 10^3 pfu was 100% lethal confirming neurovirulence reported for these strains [38]. MVA-BN replicates extensively and rapidly in CEF cells and also in certain other avian cell lines.
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* Subfamily: *Chordopoxviridae* Genus: *Orthopoxviridae* Species: Vaccinia virus (VACV)	VACV is the virus used for the replicating smallpox vaccine that was utilized during eradication and now ACAM2000.	[39]
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.	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]
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.	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]
3.4. Does the wild type virus establish a latent or persistent infection?	No, the infections are acute		[45]
3.5. Does the wild type virus replicate in the nucleus?	No. Poxviruses replicate in the cytoplasm.		[46]

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3.6. What is the risk of integration into the human genome?	Very low 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		1. Guideline on nonclinical testing for inadvertent germline transmission of gene transfer vectors' EMEA/273974/2005. Available at https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-non-clinical-testing-inadvertent-germline-transmission-gene-transfer-vectors_en.pdf; Accessed 28 February 2019. [47–50]
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].		[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.	[51,52] Reference for myocarditis and/or pericarditis frequency: [53]
• In immunocompromised humans	Can be fatal and so vaccination with VACV is contraindicated.	Applicable for the replicating vaccinia-based smallpox vaccines	[54,55]
• In human neonates, infants, children	Children <12 months of age have an increased rate of the complications listed above for healthy human host		[55,56]
• 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. Reference [57] is from 1975. The data on pregnancy is from uncontrolled sources referring to the years 1932 to 1975. In contrast, the prescribing information for ACAM2000 has a clear and prominent warning [58].	[57,58]
• 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, antimetabolites, 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		[55,59]

	steroid eye drops. These patients should not be vaccinated with live vaccinia virus until the ocular condition is well controlled and they have not been using steroid eye drops for at least two weeks.		
3.8. What cell types are infected and what receptors are used in the natural host and in humans?	Wide range of cells can be infected with VACV.	VACV enters cells in a multistep process. Four proteins participate in attachment to glycosaminoglycans and laminin. A complex of 11 proteins mediate the hemifusion and entry steps.	[60]
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.	[61,62]
3.10 Has disease enhancement been demonstrated with the wild type virus:	No		
• in vitro?	No		
• in animal models?	No		
• in human hosts?	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.	[42]
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.		
• What populations are immunized?	Military personnel, health care workers and laboratory workers at risk of infection with virulent poxviruses		[63,64]
• What is the background prevalence of artificial immunity?	Low in people born after 1980.	Smallpox was declared eradicated in 1980 and so with the exception of selected groups people born after 1980 were not vaccinated.	[1]
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) is approved for treatment of smallpox but can be reasonably expected to show effectiveness also for other orthopoxvirus infections.		[62] (TPOXX (Tecovirimat) Prescribing Information)

4. Characteristics of the vector from which vaccine(s) may be derived	**Information**	**Comments/ Concerns**	**Reference(s)**
4.1 Describe the source of the vector (e.g. isolation, synthesis)	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]
4.2. What is the basis of attenuation/ inactivation of the wild type virus to create the vector?	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]

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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.	[10,14]
• 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.	[66–68]
• 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.	[31]
• in gene therapy experiments	Non-productive infection	Cancer vaccines: CV301 (reference [69]) Brachyury (references [35,70])	[69,35,70]
• in any other special populations	Non-productive infection. Non-replicating in human cell lines	Atopic dermatitis: references [71,72] Stem cell transplant: reference [73]	[71–73]
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*	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	[13,48,10,11,14]
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	Even *in vivo* in immunosuppressed mice, MVA-BN remains stable, while replicating variants become apparent after administration of other MVA strains	[13,10]
4.7. What is the potential for shedding and transmission to humans or other species?	Negligible	Non-replicating, therefore, basically no risk following s.c. or i.m. injection.	
4.8. Does the vector establish a latent or persistent infection?	No	Non-replicating, see biodistribution section (Section 6.8)	
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	[30,74–88,81,73,66,72,79,67,31,71,32,68]

		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	see above section on wildtype virus (Section 3.8)	[60]
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	see above section on wildtype virus (Section 3.9)	[61,62]
4.14 Has disease enhancement been demonstrated with the vector:	No		
• in vitro?	No		
• in animal models?	No		
• in human hosts?	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	Examples for multiple inserts in a single MVA-BN vector are: MVA-BN-Filo MVA-BN-RSV MVA-BN-CV301 MVA-BN-Brachyury	[89–91] MVA-BN-Filo: [30,92–97] MVA-BN-RSV: [33] MVA-BN-CV301: [69] MVA-BN-Brachyury: [35]

5. Characteristics of vector-based vaccine(s)	**Information**	**Comments/ Concerns**	**Reference(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: ACI28629.1) 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	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]
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 promotors are inserted in two MVA-BN non-coding regions (intergenic regions) in a single MVA-BN viral vector		[20]

(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 promotors (synthetic and native) with early and late elements		[21–24]
5.7. Does insertion or expression of the transgene affect the pathogenicity or phenotype of the vector?	No	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.	[30,92–94,98]
5.8. Is the vaccine replication-competent in humans or other species?	Humans: no replication; this was tested in in multiple human cell lines Other species: replication in chicken embryo fibroblasts (CEF) may suggest replication in birds (not tested)	Replicating in CEF. Replicating in BHK, and some other cell lines, but not in live mammals including rabbits, rats, immunosuppressed NHP, immune-compromised mice	[10]
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?	No		
5.14. What is the risk of integration into the human genome?	Extremely low	See Section 4.10 (same as for vector)	
5.15. 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)		For references see Section 3.7 (same as for vector)
5.16. What cell types are infected and what receptors are used in humans?	See Section 3.8		[60]
5.17. What is known about the mechanisms of immunity to the vaccine?	Antibody and T cell responses are induced upon vaccination		[30]
5.18 Has disease enhancement been demonstrated with the vaccine:	No		
• in vitro?	No		
• in animal models?	No		
• in human hosts?	No		
5.19 What is known about the effect of pre-existing immunity, including both natural immunity and repeat administration of the vector or the vaccine, on 'take', safety or efficacy in any animal model or human studies using this vector?	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	Recombinant MVA vaccines induce immune responses in people previously vaccinated against smallpox and in mice previously vaccinated with VACV or MVA.For recombinant MVA-BN based vaccines safety and immunogenicity following multiple administrations could be demonstrated [4,98]	[34,99,98]
5.20. 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	[48,49,100]
5.21. Are there antiviral or other treatments available for disease manifestations caused by the vaccine?	No disease manifestations		
5.22. Vaccine formulation	Tris buffer saline		
5.23. Proposed route of vaccine administration	Intramuscular		
5.24 Target populations for the vaccine (e.g. pediatric, maternal, adult, elderly, etc.)	Individuals $\geq$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, suitable animal models are immunocompromised mice and NHP		
6.5. Does an animal model for reproductive toxicity exist?	Yes	Rat and rabbit, not published.	
6.6. Does an animal model for immunogenicity and efficacy exists?	Yes	Mice and NHP	[101–103] More manuscripts to be published.
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]
• Nonhuman primates (NHP)?	Yes		[96,97]
• Human?	Yes	Clinical trials with recombinant MVA-BN vaccines assessing immune responses, e.g. HIV or RSV	HIV: [34] RSV: MVA-BN-RSV: [33]
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?			
6.11 Have studies been done simultaneously or sequentially administering more than one vector with different transgenes? Is there evidence for interaction/interference?	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:[30,92,93,94,95] 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:			
• Spontaneous reports/passive surveillance	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	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.	Same as Section 4.11, for cardiac refer in particular to [87]
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	Yes		
• 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-naïve subjects and 766 smallpox vaccine experienced subjects who received at least 1 dose of JYNNEOS and 1,206 smallpox vaccine-naïve 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	Prescribing Information of JYNNEOS (www.jynneos.com) [112]
7.5. List and provide frequency of any serious, unexpected AE:	Among smallpox vaccine-naïve 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.	
• Describe the control group:	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]
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-naïve. 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	Prescribing Information of JYNNEOS (www.jynneos.com) [112]

7.8. Did Data Safety Monitoring Board (DSMB) or its equivalent oversee the study?	Yes	DSMB across the whole BN sponsored development program	
• Did it identify any safety issue of concern?	No	No safety concerns in BN sponsored trials. One temporary halt in an NIH sponsored trial	
• If so describe:		POX-MVA-036 (DMID 11-0021): One case of throat tightness (angioedema), responsive to epinephrine treatment, with short delay after vaccine administration. Finally assessed as allergic/anaphylactoid reaction. Trial resumed after an investigation of product quality of the particular batch had not identified any issues.	[88]

8. Overall Risk Assessment of the Vector	**Information**	**Comments/ Concerns**	**Reference(s)**
8.1. Please summarize key safety issues of concern identified to date, if any:	None serious safety concerns identified, safety profile is consistent with other licensed, modern vaccines. Mostly local and systemic reactogenicity, rare cases of allergy/hypersensitivity		[49,50,112]
• how should they be addressed going forward:	N/A		
8.2. What is the potential for causing serious unwanted effects and toxicities in:	**Describe the toxicities**	**Please rate risk as: none, minimal, low, moderate, high, or unknown**	
• healthy humans?	minimal	No 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.	See Section 4.11 and Prescribing Information of JYNNEOS (www.jynneos.com) [112,113]
• immunocompromised humans?	minimal	Investigated in HIV positive subjects	[66–68]
• Human neonates, infants, children?	Minimal or unknown	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]
• pregnancy and in the unborn in humans?	unknown	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.	
• in any other special populations.	Minimal in Atopic Dermatitis subjects	Specifically tested in this population, who cannot receive traditional, replicating smallpox vaccines.	[71,72]
8.3. What is the potential for shedding and transmission in risk groups?	Negligible	See Section 4.7	

and Canada and since 2019 in the US, where it is also licensed as 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 an Ebola vaccine 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 Volkmann, Heinz Weidenthaler, and Thomas Meyer are employees of Bavarian Nordic.

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