**A Brighton Collaboration standardized template with key considerations for a benefit/risk assessment for a viral vector vaccine based on a non-replicating Modified Vaccinia virus Ankara viral vector**

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For the Benefit-Risk Assessment of VAccines by TechnolOgy Working Group (BRAVATO; ex-V3SWG)1

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**Keywords:**

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**Abstract:**

The Brighton Collaboration Benefit-Risk Assessment of VAccines by TechnolOgy (BRAVATO) was formed to evaluate the safety and other key features of new platform technology vaccines. This manuscript reviews key features of MVA-vectored vaccines.

Infectious diseases continue to be a major cause of human death worldwide. In this context, emerging zoonotic infectious diseases pose a particular challenge for public health systems. In the last two decades, three different respiratory coronaviruses, including the Middle East respiratory syndrome Coronavirus (MERS-CoV) have emerged. For many years, safe and efficacious vaccines have been a major tool to combat infectious diseases.

Here, we report on a promising candidate vaccine (MVA-MERS-S) against MERS-CoV based on Modified Vaccinia virus Ankara (MVA). We demonstrate that the MVA vector platform is a viable and effective tool for producing safe, immunogenic, and efficient vaccines against emerging infectious diseases.

1. **Introduction**

The Brighton Collaboration ([www.brightoncollaboration.org](http://www.brightoncollaboration.org)) was launched in 2000 to improve the knowledge of vaccine safety [1]. The Brighton Viral Vector Vaccine Safety Working Group (V3SWG) was formed in 2008 in recognition of the increasing importance of viral vectors for the development of new vaccines and the need to understand their associated safety issues. To better meet the needs of many other platform technologies used to develop vaccines to prevent COVID-19 beyond just vaccines using viral vectors, the V3SWG was renamed to Benefit-Risk Assessment of VAccines by TechnolOgy (BRAVATO) Working Group in July 2020. BRAVATO uses a standardized template to describe the key characteristics of novel vaccine vectors, compiled from the latest research, to facilitate scientific discourse among key stakeholders [2].

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.

1. **Background**

**2.1 Middle East respiratory syndrome Coronavirus (MERS-CoV), a highly pathogenic respiratory betacoronavirus.**

Middle East respiratory syndrome Coronavirus (MERS-CoV) is one of three beta-coronaviruses that have emerged as new infectious agents in the last two decades [3]. All three viruses are currently classified as priority pathogens by WHO with a high potential to cause global pandemics. MERS-CoV was first described in 2012, and as of April 2024, a total of 2622 laboratory-confirmed cases and 950 associated deaths have been reported to WHO, representing a mortality rate of 36%. Most MERS-CoV infections in humans have been reported in the Middle East, in particular from Saudi Arabia. However, travelers infected with MERS-CoV in the Middle East have exported the virus to more than 27 countries [4], including Qatar, Austria, Bangladesh, and Indonesia.

An interesting aspect of the MERS-CoV epidemiology was observed during a MERS outbreak in South Korea in 2015 [5], [6]. A 68-year-old man who got contracted with MERS-CoV in Saudi-Arabia, traveled back to South Korea where he ended up in the hospital with severe disease symptoms, infecting also other patients and health care workers with MERS-CoV. In the end, 186 people were infected, and 36 patients died as a result of a severe MERS disease outcome. This epidemic in South Korea highlights that MERS-CoV can be efficiently transmitted from human-to-human and can also spread rapidly to other countries not yet affected by this virus [5], [7], [8]. Although most detected human cases of MERS are due to human-to-human transmission in health care settings, dromedary camels are believed to be the primary animal reservoir transmitting the virus to humans [9], [10]. That is of particular interest in the Arabic area, as dromedary camels and humans live close to each other, and due to that, the possibility of being infected with MERS-CoV is high. Infections with MERS-CoV are mainly characterized by cold-like disease symptoms, including cough, fever, or shortness of breath. However, elderly or immunocompromised individuals, and individuals with chronic diseases such as cancer, diabetes, chronic lung disease, and kidney disease, are at high risk. In these patients, an infection with MERS-CoV can lead to serious complications and even death [11].

The use of vaccines is considered the best option to halt the spread of respiratory viruses such as MERS-CoV. Although several attempts have been made, no licensed vaccine is currently available on the market. However, at least three candidate vaccines against MERS-CoV, all using different approaches, have completed phase 1 trials [12], [13], [14]. One of these candidate vaccines is MVA-MERS-S, which is based on the MVA vector platform. Relevant target groups for vaccination with an MVA-MERS-S candidate vaccine are health care workers, all individuals with close contact to dromedary camels and dromedary camels, which all are likely to acquire and pass on MERS-CoV infection.

**2.2 Modified Vaccinia virus Ankara (MVA), a promising vaccine vector platform against emerging infectious diseases**

Developing vaccines against emerging infectious diseases can be quite challenging and a suitable vaccine platform should fulfill several prerequisites. The vaccines may be used in emergency outbreak situations and must have a high standard of safety and quality for human use. Immunogenicity and efficacy must be demonstrated in preclinical and clinical studies. In addition, it is advantageous if vaccines can be industrially produced in advance and readily stored at an appropriate scale for prophylactic use or until an outbreak occurs. Finally, it is beneficial if the vaccines are stable and do not require a strict cold chain, so that they can be used in remote areas.

Modified Vaccinia virus Ankara (MVA), a highly attenuated vaccinia virus, combines all these desirable requirements. Vaccinia virus, the prototype vaccine used to eradicate human smallpox, is the best studied member of the poxvirus family with regard to its biology and its use as viral vector. The highly attenuated and replication deficient vaccine strain MVA has a high-level profile in clinical safety established in many studies as next generation smallpox vaccine and as recombinant vaccine against various infectious diseases (for review see [5]). The MVA vector presented here is derived from the clonal virus isolate F6 of an MVA seed virus which was provided to the Bavarian State Vaccine Institute in 1972 and served to obtain the first marketing authorization for an MVA vaccine in Germany in 1977 [15]. Restriction mapping demonstrated characteristic alterations in the MVA genome that are the likely genetic basis for its high-level attenuation [16]. Comparison to the genome sequence of other vaccinia virus strains revealed that the MVA genome harbors major deletions and mutations affecting many genes with regulatory functions in virus-host interaction [17].

MVA is no longer able to complete its replication cycle in most mammalian cells, but is still able to efficiently express heterologous DNA, that was inserted into the MVA genome and placed under transcriptional control of a vaccinia-virus specific promoter, making it an ideal and safe candidate vector vaccine platform [18]. Furthermore, like all poxvirus vectors, MVA does not exhibit viral persistence in the host, shows efficacy as a vaccine, and is suitable for industrial large scale vaccine production [5].

In summary, MVA is an excellent vector vaccine platform against emerging infectious diseases. Preclinical studies in various animal models and data from clinical trials (e.g., Ebola [19], West Nile virus [20], SARS-CoV-2 [21]) also confirm that recombinant MVA viruses are safe and efficacious. In addition, the often-discussed role of vector-specific immunity appears to play a minor role in MVA compared to other vector systems [22].

**2.3 MVA-MERS-S, a promising candidate vaccine against MERS-CoV**

Shortly after the appearance of MERS-CoV in 2012, a recombinant MVA candidate vaccine, expressing the full-length coding sequence of the MERS spike protein (MVA-MERS-S), was generated [23]. In preclinical testing, Volz et al. demonstrated the induction of neutralizing antibodies and epitope-specific CD8+ T cells against MERS-S in BALB/c mice using different routes of application and doses of the MVA-MERS-S candidate vaccine [24]. Since dromedary camels are considered a relevant reservoir of MERS-CoV, additional preclinical testing was performed in this animal model. Haagmans et al. were able to confirm the immunogenicity and efficacy of the MVA-MERS-S candidate vaccine also in dromedary camels under experimental conditions. Following MERS-CoV challenge infection, camels immunized with MVA-MERS-S showed a significant reduction of viral RNA and infectious virus shedding compared to control animals [9].

The promising MVA-MERS-S candidate vaccine has already been tested successfully in a phase Ia clinical study which further resulted in the approval of a follow-up phase Ib clinical trial. In the phase Ia clinical trial, the safety, tolerability and immunogenicity of MVA-MERS-S could be shown in humans for the first time [14]. In this study, participants received intramuscularly either a low dose (1x107 plaque-forming units (PFU)), or high dose (1x108 PFU) of MVA-MERS-S in a prime-boost regimen . After prime-boost immunization, both cellular and humoral immune responses specific for MERS-S antigen could be confirmed. All participants in the high dose group and three quarters of the low dose group showed seroconversion with the appearance of MERS-CoV neutralizing antibodies [14]. In a follow-up study, a subgroup of individuals (10 participants, 3 low dose and 7 high dose) received one additional MVA-MERS-S immunization after one year to evaluate the safety and immunogenicity of a third vaccination. Again, this booster vaccination proved to be safe. In addition, persistence of MERS-CoV-specific B cells and a significant increase of MERS-CoV-specific binding and neutralizing antibody titers was observed (mean titers 12-fold higher than maximum titers one year earlier) [25], [22]. Of note, neutralizing antibodies were detectable in all participants, including those in the low dose group who had not formed detectable levels of antibodies after the first booster vaccination one year earlier.

With these promising results, the MVA-MERS-S candidate vaccine reached the next stage of clinical development. A double-blinded, randomized, placebo-controlled phase 1b study (NCT04119440) with more participants, different doses (1x107 and 1x108 PFU), and two prime-boost intervals (28 and 56 days), with an additional booster vaccination after one year, was conducted between July 2021 and November 2022. The evaluation of this study is currently ongoing, but first results published by Raadsen et al. demonstrated that the intramuscular application of MVA-MERS-S was well tolerated and highly immunogenic, by inducing strong cellular and humoral immune responses [26] against both the integrated MERS-S antigen and the viral MVA vector.

1. **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 participants organization (e.g., government, university, or corporations) and should not be construed to represent any Agency determination or policy.

1. **Declaration of Competing Interest**

The BRAVATO authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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| Brighton Collaboration Benefit-Risk Assessment of Vaccines by Technology (BRAVATO) Working Group  Standardized Template v3.0 for Collection of Key Information for Risk Assessment of Viral Vaccine Vector Candidates | | | |
| 1. Authorship and Affiliation | Information | | |
| 1.1. Author(s) and affiliation | LMU Munich, Department of Veterinary Sciences, Division of Virology: Dres. Ellen K. Link, Alina Tscherne, Gerd Sutter  University of Veterinary Medicine Hannover, Institute of Virology, Dr. Asisa Volz  Brighton Collaboration, a program of the Task Force for Global Health, Decatur: Emily R. Smith, Marc Gurwith, Robert T. Chen | | |
| 1.2. Date completed/updated | 15-07-2024 | | |
| Part I: Viral Vector (Sections 2-7) | | | |
| 2. Basic vector information | Information | Comments/Concerns | |
| 2.1 Vector name | Modified Vaccinia virus Ankara (MVA F6 LMU SF) |  | |
| 2.2. Vector origin Family/Genus/Species/subtype | *Poxviridae, Orthopoxvirus*, *Vaccinia virus* (VACV), Chorioallantois Vaccinia virus Ankara (CVA) |  | |
| 2.3. Vector replication in humans (replicating or non-replicating) | Non-replicating |  | |
| 3. Characteristics of the wild type virus from which the vector is derived | Information | Comments/Concerns | |
| 3.1 Name of wild type virus (common name; Family/Genus/Species/subtype) | Chorioallantois Vaccinia virus Ankara (CVA); *Poxviridae/ Orthopoxvirus*/ *Vaccinia virus* (VACV) [27]. |  | |
| 3.2 What is the natural host for the wild type virus? | The natural host of VACV is unknown. In India and in Brazil VACV has been repeatedly isolated from animals (Asian water buffalo, cattle, horses) with reports of zoonotic transmission to humans. The introduction of VACV in the animal populations is believed to originate from worldwide smallpox vaccine campaigns. According to genomic data, VACV has the highest homology to horsepox virus (99.7%). CVA is derived from the Vaccinia virus strain Ankara which was originally propagated on the skin of calves and donkeys at the Turkish Vaccine Institute in Ankara for the production of smallpox vaccine. In 1953, the virus strain was brought to Munich, cultured on the chorioallantois membranes (CAM) of embryonated chicken eggs and renamed Chorioallantois Vaccinia virus Ankara (CVA) [5], [27], [28], [29]. |  | |
| 3.3. How is the wild type virus normally transmitted? | Direct contact with pock lesions (crusts), infectious fluids or contaminated objects. Accidental infection after contact with pock lesion after vaccination (“inadvertent inoculation”) [30] [31] | [*in vitro* Data only] | |
| 3.4. Does the wild type virus establish a latent or persistent infection? | No [32]. |  | |
| 3.5. Does the wild type virus replicate in the nucleus? | No. It is replicating in the cytoplasm [30], [33], [34]. |  | |
| 3.6. What is the risk of integration into the human genome? | No risk of genome integration. |  | |
| 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 | The natural host of VACV is not known. Exanthematous lesions are described for VACV infected domesticated animals (water buffalo, cattle, horses on the teats of dairy cows) in India and Brazil. The disease horsepox is extremely rare and it may be extinct today [35] [36]. |  | |
| * In laboratory hosts (specify species) | * Mouse strains (e.g. BALB/c, C57BL/6): pneumonia, encephalitis, skin lesions depending on dose, route of inoculation, virus strain [37], [38], [39], [40]. * Wild type (SD rats) and immunodeficient rats (Rag2-/- rats): hemorrhage in the lung, spleen and kidney, hyperplasia of connective tissues in lung and heart [41]. * Rabbits (New Zealand White rabbits): hemorrhagic nasal discharge, ocular secretion, dark diarrhea, progressive apathy. |  | |
| * In healthy human host | Mild reactions, which disappear without treatment include fever, enlarged lymph nodes, fatigue and painful puncture site [42]. Serious adverse reactions occurred after vaccination with VACV, including major local reactions, eczema vaccinatum, vaccinia generalisata, vaccinia secundaria, or postvaccinal encephalitis [43]. In addition, vaccination [44] [45] [46] [42]with VACV was associated with myo-/pericarditis, including heart and chest pain, ST-segment elevation, cardiomyopathy, shortness of breath, and night sweats. |  | |
| * In immunocompromised humans | In immunocompromised individuals, serious side effects occur much more frequently, so vaccination with VACV is not recommended These include conditions and treatments associated with immunosuppression or immunodeficiency (HIV/AIDS, Leukemia, Lymphoma, Generalized malignancy, solid organ transplant, hematopoietic stem cell transplant recipients, persons undergoing therapy with alkylating agents, antimetabolites, radiation, tumor necrosis factor (TNF) inhibitors, high-dose corticosteroids) [47]. [48]. |  | |
| * In breast milk, human neonates, infants, children | First-time vaccinations of children over 2 years of age showed an increased incidence of the vaccination side effects listed above. VACV is not recommended for children under 1 year of age [49]. |  | |
| * During pregnancy and in the unborn in humans | A rare but serious complication of smallpox vaccination is fetal vaccinia. This often results in fetal or neonatal death or premature birth. Fetal vaccinia, which probably follows maternal viremia, manifests as skin lesions and involvement of internal organs. Smallpox vaccination during pregnancy and while breastfeeding is contraindicated because of the risk of fetal vaccinia and possible other unknown risks to the fetus [50]. |  | |
| * In any other special populations? | VACV vaccines are not recommended for individuals with atopic dermatitis or other active exfoliative skin conditions (eczema, burns, impetigo, varicella zoster virus infection, herpes simplex virus infection, severe acne, severe diaper dermatitis with extensive areas of denuded skin, psoriasis, keratosis follicularis)  The contraindication to live vaccine administration is individuals with known heart disease (e.g., coronary artery disease or cardiomyopathy) and three or more known cardiac risk factors, including hypertension, diabetes, hypercholesterolemia, heart disease at age 50 in a first-degree relative, and smoking [48]. |  | |
| 3.8. What cell types are infected and what receptors are used in the natural host and in humans? | VACV possess complex mechanisms involving many different proteins to invade a variety of different cells. Attachment of mature VACV virions occurs through four viral proteins that bind glycosaminoglycans and laminin on the cell surface. Cell entry occurs via plasma membrane fusion or actin-dependent macropinocytosis. Entry of the enveloped virions into the cell occurs via an entry fusion complex consisting of 12 proteins. The receptors for the specific proteins have not yet been determined [51], [52], [53]. |  | |
| 3.9. What is known about the mechanisms of immunity to the wild type virus? | Both antibody and T-cell responses are associated with protection against VACV [33]. |  | |
| 3.10 Has disease enhancement (including antibody dependent enhancement (ADE), vaccine associated enhanced respiratory disease (VAERD)) been demonstrated with the wild type virus: | No | In preclinical models VACV vaccination very efficiently  protects against disease induced by respiratory  challenge infections [40], [54]. | |
| ● in vitro? | No |  | |
| ● in animal models? | No |  | |
| ● in human hosts? | No |  | |
| 3.11 Is disease enhancement (including antibody dependent enhancement (ADE), vaccine associated enhanced respiratory disease (VAERD)) a possible vaccine-induced contributor to the pathogenesis of wild type disease | No |  | |
| 3.12 What is the background prevalence of natural immunity to the virus? | Low |  | |
| 3.13 Is there any vaccine available for the wild-type virus? If yes, | No, the wild type virus itself was used as smallpox vaccine for a long time |  | |
| ● What populations are immunized? | All persons at risk of infection with virulent orthopox viruses (variola virus, monkeypox virus). These include laboratory workers [55], militarypersonnel [56], and healthcare workers [57]. |  | |
| ● What is the background prevalence of artificial immunity? | This prevalence can be considered low because the nationwide smallpox vaccination program has been completed and persons born after 1980 are vaccinated only in very few and specially selected groups of persons (see above). |  | |
| 3.14 Is there treatment available for the disease caused by the wild type virus | Vaccinia immunoglobulin (VIG) has long been the only FDA-approved [58] treatment for complications following VACV vaccination and can be therapeutically effective for systemic orthopoxvirus infections. Tecovirimat (TPOXX) and brincidofovir (TEMBEXA) are now also approved for smallpox treatment starting in 2018 and 2021, respectively [59]. |  | |
| 4. Characteristics of the vector from which vaccine(s) may be derived | Information | Comments/ Concerns | |
| 4.1 Describe the source of the vector (e.g. isolation, synthesis) | MVA originated from the VACV strain Ankara, which was used as a smallpox vaccine in Turkey and was produced as dermal vaccine on the skin of calves and donkeys. VACV was brought to Munich and further passaged in primary chicken embryo fibroblasts (CEF) leading to the name Chorioallantois Vaccinia virus Ankara (CVA). Long-term serial passaging on CEF cells resulted in an attenuated virus that had lost virulence and the ability to efficiently replicate in cells of human or other mammalian origin. To distinguish this highly attenuated strain of virus from other attenuated VACV strains, it was renamed Modified Vaccinia virus Ankara (MVA) after CEF passage number 516 and transferred as seed virus (e.g. preparation 572nd CEF passage) to the Bavarian State Vaccine Institute for vaccine production. The Bavarian State Vaccine Institute in Munich obtained the first marketing authorization for MVA as primary pre-vaccine against smallpox in Germany in 1977 (Paul-Ehrlich-Institut, 31.01.1977) [5], [16],, [18], [60], [61], [62], [63], [17]  Our vector virus is based on the clonal isolate MVA F6 LMU [17]which was generated by repetitive rounds of limiting dilution plaque isolations starting with the MVA vaccine seed virus preparation at the 572nd CEF passage. CEF passages 580, 582, and 584 served as primary, secondary and tertiary stock virus preparations of MVA F6 LMU. The stock virus 580th CEF of MVA F6 LMU served also to generate the GMP-compatible seed virus stock MVA-F6-sfMR using CEF prepared from specified pathogen free (SPF) chicken eggs under serum-free conditions. |  | |
| 4.2. What is the basis of attenuation/inactivation of the wild type virus to create the vector? | In general, MVA is characterized by its exceptional safety profile based on its lack of replication in human and most other mammalian cells. MVA fails to produce infectious mature virions in mammalian cells. However, unlike other attenuated VACV, this does not adversely affect viral early, intermediate and late gene expression, allowing unimpaired production of MVA proteins and foreign antigens. Restriction mapping of the MVA F6 LMU genome demonstrated alterations in the MVA genome that are the genetic basis for attenuation and growth restriction. Comparison to the genome maps of CVA ancestor viruses revealed that the MVA genome harbors large deletions and mutations affecting many genes with functions in virulence and immune evasion. This may explain the MVA ability to activate the innate immune system early leading to versatile innate immune responses and efficient induction of adaptive immunity in the absence of productive virus replication [16] [18] [17] [63] [14] [64] [65] [66] [67]. |  | |
| 4.3. What is known about the replication, transmission and pathogenicity of the vector in humans in the following categories: |  |  | |
| * in healthy people | Non-replicating | Strong safety profile [5] [15] [68] | |
| * in immunocompromised people | Non-replicating [69], [70], [71] [15]. |  | |
| * in breast milk, neonates, infants, children | Non-replicating [72] [73] [74] |  | |
| * during pregnancy and in the fetus | Non-replicating [75] [76] |  | |
| * in gene therapy experiments | Non-replicating |  | |
| * in any other special populations | Non-replicating |  | |
| 4.4. Is the vector replication-competent in non-human species? | MVA is productively replicating on the chorioallantois membrane of embryonated chicken eggs, in primary chicken embryo fibroblasts (CEF) or avian cell lines (e.g. DF-1) and in Syrian baby hamster kidney cells (BHK-21).  Productive replication of MVA in animals has not been described [21], [77], [78], [64], [79]. |  | |
| 4.5. What is the risk of reversion to virulence, recombination or reassortment with wild type virus or other agents? | To date, no reversion to virulence has been documented and is considered very unlikely to occur. Genetic recombination or reassortment with genomes of VACV or other Orthopoxviruses may occur in cells co-infected with MVA. This feature is well known for poxviruses [80], [81].  Reversion of MVA to a replication-competent phenotype is highly unlikely, because the attenuation and replication restriction is based on multiple missing/mutated and dysfunctional gene sequences. | Since infections of humans with VACV or other orthopoxviruses are extremely rare, the likeliness for in vivo recombination and reassortment of MVA and other orthopoxvirus genomes is also considered very low [80], [81], [82]. | |
| 4.6 Is the vector genetically stable in vitro and/or in vivo? | Yes |  | |
| 4.7. What is the potential for shedding and transmission, including arthropod borne transmission, to humans or other species? | The risk of virus spread and transmission to humans or other species can be considered negligible. | MVA does not replicate and survive in infected human host cells. Therefore, it can be assumed that full clearance of virus particles and recombinant DNA occurs within days (48 hpv) after vaccine administration [83]. | |
| 4.8. Does the vector establish a latent or persistent infection? | No |  | |
| 4.9. Does the vector replicate in the nucleus? | No | The replication cycle of poxviruses, unlike other DNA viruses,  is restricted to the cytoplasm of infected host cells [84], [85],  [86]. | |
| 4.10. What is the risk of integration into the human genome? | Very low | A characteristic of poxviruses is that gene expression  occurs under tight control of virus-specific transcription  systems without integration of viral DNA into the host  genome. [87] | |
| 4.11. Is there any previous human experience with this or a similar vector (safety and immunogenicity records)? | MVA is a replication-deficient and safety-tested VACV, which serves as an advanced viral vector platform for the development of new vaccines against various infectious diseases and cancer [5] [15] [70] [88] [89] [90] [91] [92] [93] [94] [95] [96] [97]   1. The basic vector MVA alone was already successfully tested in the final phase of the smallpox vaccination campaign. There, over 100,000 people, including immunocompromised individuals, were vaccinated with MVA without any significant side effects. In addition, several phase I and II clinical trials have confirmed the safety of MVA in immunocompromised persons for whom conventional smallpox vaccination poses a high risk. In these, MVA was shown to be well tolerated and immunogenic in individuals with atopic dermatitis and HIV infection. Currently, MVA is licensed in the European Union and Canada as a stand-alone third-generation smallpox vaccine for active immunization of adults, including high-risk individuals, demonstrating its high safety. 2. To date, there are already numerous different recombinant MVA vaccine candidates that have entered Phase I/II clinical trials, having previously undergone successful preclinical testing in various animal models and been shown to be safe and immunogenic:   MVA-H5-sfMR [63]: Candidate vaccine against Influenza was proven to be immunogenic (induction of high titers of H5-specific antibodies and T cells)  MVA-NP-M1 [98]: Another influenza vaccine candidate targeting the T-cell antigens NP and M1 was found to be well tolerated, immunogenic, and protective, including fewer symptoms and reduced viral shedding. This was the case both in healthy adult subjects and at comparable levels in the elderly (>50 years).  MVA-MERS-S [14], [23]: Candidate vaccine against MERS, which was found to be well tolerated and immunogenic in animals and humans. Vaccinated mice showed high levels of neutralizing antibodies. Vaccinated humans showed strong induction of CD8+ T cells and elevated levels of neutralizing and binding antibodies in a Phase Ia and Ib clinical trial.  MVA-SARS-2-S [99]: Candidate vaccine against COVID-19 was found to be well tolerated and partially immunogenic (induction of S2-specific antibodies)  In addition, several MVA-based vaccines targeting [100] [101] [102] [103] [104] human immunodeficiency virus 1 (HIV-1) were tested in clinical studies and were found to be well tolerated and partially immunogenic. |  | |
| 4.12. What cell types are infected and what receptors are used in humans? | As well known for VACV also MVA can readily enter various targets cells. In cells of mammalian origin MVA cannot produce new infectious progeny but activates its molecular life cycle allowing for expression of all classes of viral and recombinant genes.  As early as 1994, a first recombinant MVA vaccine expressing the influenza antigens HA and NP confirmed induced both high antigen-specific cellular and humoral immune responses. The efficacious induction of cellular immune responses is supported by the fact that upon infection MVA appears to accumulate in professional antigen-presenting cells which are necessary for the priming, activation, and amplification of antigen-specific T cells. This cellular tropism of MVA has been noted in several studies using a recombinant MVA expressing reporter genes such as her2neu or green fluorescent protein (gfp) [5], [18], [83], [105]. |  | |
| 4.13. What is known about the mechanisms of immunity to the vector? | MVA induces orthopoxvirus-specific antibody and T cell immunity, and by now serves as a licensed next generation vaccine for prevention of orthopoxvirus infections (against variola virus and MPOX in the US, Europe and Canada).  Correspondingly vector-specific antibodies and T cells are also elicited upon vaccination with recombinant MVA vaccines. Anti-vector immunity is known to hamper an efficient vaccine take with replication-competent VACV following a low-dose intradermal vaccine inoculation. MVA vaccines are usually applied by high dose injections mostly by the intramuscular route of application. Thus, anti-MVA immunity may have a lesser impact on the induction and amplification of recombinant antigen-specific immune responses elicited by MVA vector vaccines. Indeed, several studies have shown that antigen-specific immune responses can be repeatedly enhanced by multiple booster administrations of recombinant MVA. [63], [106], [107]. | [68] | |
| 4.14 Has disease enhancement (including antibody dependent enhancement (ADE), vaccine associated enhanced respiratory disease (VAERD)) been demonstrated with the vector? |  |  | |
| ● 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? | No disease manifestations occur because the vector is not capable of replication in human cells. |  | |
| 4.16. Can the vector accommodate multigenic inserts or will several vectors be required for multigenic vaccines? | MVA, as an attenuated member of the orthopoxviruses, has a large genome and can accommodate large amounts of heterologous gene sequences encoding for even multiple foreign antigens into its genome. Therefore, multiple vectors are not necessarily needed for the development of multigenic / multivalent vaccines [5]. |  | |
| 5. Toxicology and Potency (Pharmacology) of the Vector | Information | Comments/ Concerns | |
| 5.1. What is known about the replication,  transmission and pathogenicity of the vector in and between animals? | MVA does not productively replicate in animals and in most cells / cell lines of mammalian origin. Therefore, transmission and pathogenicity of the vector in and between animals has not been described and is expected very unlikely to occur. |  | |
| 5.2. 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? | MVA is a non-replicating vector. |  | |
| 5.3. Does an animal model relevant to assess attenuation exist? | Yes [16], [71], [108], [109]. |  | |
| 5.4. Does an animal model for safety including immuno-compromised animals exist? | Yes. The excellent safety profile of MVA has been confirmed in vivo in various animal models, including immunodeficient mice and immunocompromised NHPs. No clinical or pathological signs were observed even when high doses of MVA were used for inoculation [71], [109]. |  | |
| 5.5. Does an animal model for reproductive toxicity exist? | Yes, the rabbit and rat models can serve as established models to test reproductive toxicity of MVA vector vaccines. |  | |
| 5.6. Does an animal model for immunogenicity and efficacy exist? | Yes, e.g., recently several studies have confirmed the immunogenicity and efficacy of recombinant MVA vaccine candidates against infections with the betacoronaviruses MERS-CoV [9], and SARS-CoV-2 [21], [99], [110]. Many other preclinical studies are published targeting various diseases in established animal models (for review see [5]). |  | |
| 5.7 Does an animal model for antibody enhanced disease (including antibody dependent enhancement (ADE), vaccine associated enhanced respiratory disease (VAERD)) or immune complex disease exist? | No signs of ADE or VAERD in preclinical studies of MVA vector vaccines against infections with MERS-CoV and SARS-CoV-2. |  | |
| 5.8. What is known about biodistribution in animal models or in humans, including neurovirulence and/or neuroinvasion? | Inoculation of lethally immune suppressed macaques with high doses of MVA was not associated with clinical, hematological or pathological abnormalities related to MVA immunization. With regard to biodistribution the presence of MVA genomic DNA was demonstrated by PCR in all macaques during a 13-day observation period, however from none of the animals infectious MVA could be reisolated [71], [77].  The in vivo distribution of a single i.m. dose of the vaccine candidate MVA-MERS-S in mice was studied with respect to biodistribution. Inflammation at the injection site and hyperplasia of the draining lymph nodes were observed, the extent of which may be consistent with immunological responses to vaccine inoculation. MVA DNA was detected primarily at the injection site and in the draining lymph nodes. Data during the observation period indicate that there is continuous clearance of the vaccine candidate. No MVA DNA was found in the excretory organs. Therefore, the risk of excretion of MVA and spillover of the GM organism to the environment is considered negligible. |  | |
| 5.9 What is the evidence that vector derived vaccines will generate a beneficial immune response in: | For review see reference [111] [112] [5] |  | |
| * Small animal models? | There are multiple established animal models that confirm the immunogenicity and efficacy of recombinant MVA vaccine candidates against various infectious [21], [23], [24], [78] , [99] diseases and cancer. |  | |
| * Nonhuman primates (NHP)? | Immunogenicity and protective capacity could [113], [114], [115] be shown in non-human primates. |  | |
| * Human? | Safety, immunogenicity, tolerability and efficacy could be shown in human clinical trials [14], [26], [63], [90], [99]. |  | |
| 5.10. Have challenge or efficacy studies been conducted in subjects with: |  |  | |
| * Immunocompromised conditions including HIV? | No. There are no challenge or efficacy studies, but MVA has been tested in studies with HIV-positive individuals treated with ART. No serious side effects occurred there [70], [116], [117], [92]. |  | |
| * Other diseases? |  |  | |
| 5.11 Have studies been done simultaneously or sequentially administering more than one vector with different transgenes? Is there evidence for interaction/interference? | Yes, several clinical studies were conducted using adenoviral based and MVA-based vaccines in a heterologous prime-boost vaccination schedule:  Zabdeno (Ad26.ZEBOV) and Mvabea (MVA-BN-Filo). Administration induced strong immunity against Ebola [118].  ChAd63 ME-TRAP and MVA ME-TRAP. Administration induced strong immunity against Malaria. [119]  ChAdOx1-HBV and MVA-HBV. Administration induced strong immunity against Hepatitis B virus (HBV). [120] |  | |
| 6. Adverse Event (AE) Assessment of the Vector (\*see Instructions): | Information | Comments/ Concerns | |
| 6.1. Approximately how many humans have received any vaccine using this viral vector to date? If variants of the vector, please list separately. | Over 100,000 people, including those at high risk for conventional smallpox vaccination, received MVA as a pre-vaccine as part of the smallpox immunization during the eradication program (licensed by the Paul-Ehrlich-Institut in Germany 1977) [15], [69], [70], [88], [89].  Multiple studies sponsored by Bavarian Nordic using MVA-BN (7,811 people in 12 completed studies.  Multiple studies sponsored by Jenner Institute, Oxford, UK [90], [92], [121], [122], [123], [124], [125], [126] [123] [95]. |  | |
| 6.2. Method(s) used for safety monitoring: | Several MVA-based vaccine candidates are currently being tested in numerous clinical trials. As an example, two vaccine candidates, MVA-MERS-S and MVA-H5-sfMR, [14], [22], [63] tested in a double-blind phase 1/2a clinical trial are described in more detail. The main objectives of these two studies were safety, tolerability and immunogenicity. |  | |
| * Spontaneous reports/passive surveillance | No | \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ | |
| * Diary | MVA-MERS-S (phase Ia): A total of 26 participants received the following vaccine regime intramuscularly:   * 14 participants were immunized with 10e7 plaque-forming units (low dose) on days 0 and 28 * 12 participants were immunized with 10e8 plaque-forming units (high dose) on days 0 and 28.   Safety, tolerability and immunogenicity was controlled throughout the study. Heart rate, blood pressure and body temperature were recorded 2, 4, 6, 8, 12 and 24 h after each vaccination. Participants were monitored for 180 days, including study visits at day -1 (1 day before vaccination), day 0 (day of vaccination) and days 1, 3, 7, 14, 27, 28, 29, 35, 42, 56, 84 and 180 after prime immunization. Clinical and laboratory evaluations (complete blood counts, CRP, troponin, liver function and creatinine) were performed during each visit.  MVA-H5-sfMR: A total of 79 volunteers were enrolled and completed the study divided into 8 groups (control and vaccinee group). Participants were immunized intramuscularly with one or two doses with the candidate vaccine. Two different doses (low dose: 10e7, high dose: 10e8 PFU) were tested. The main objectives were tolerability and immunogenicity. Participants were monitored before vaccination, 1 h after immunization, 4 weeks after immunization, 4 weeks after second immunization and 20 weeks after second immunization. | (<https://www.trialregister.nl/trial/3249>) | |
| * Other active surveillance | No |  | |
| 6.3. What criteria were 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 | MVA-MERS-S: Beside the FDA’s 2007 voluntary guidance, also the Common Terminology Criteria for Adverse Events (CTCAE v4) was used for grading the AEs (more detailed information can be found under <https://clinicaltrials.gov/ProvidedDocs/11/NCT03615911/Prot_000.pdf>) | |
| * If no criteria were used for grading, or if other metrics were employed, please describe: | - |  | |
| 6.4. List and provide frequency of any related or possibly related serious\* AE’s as well as any severe, expected or unexpected AE observed: (\*see Instructions): | MVA-MERS-S and MVA-H5-sfMR : No severe or serious adverse events were observed |  | |
| 6.5. List and provide frequency of any serious, unexpected significantly increased AE or lab abnormality in vaccinee vs. control group: | MVA-MERS-S and MVA-H5-sfMR: No serious, unexpected significantly increased AE or lab abnormality in vaccine was observed. |  | |
| * Describe the control group: \_\_\_\_\_\_\_\_\_\_. | MVA-MERS-S: 6 healthy individuals who did not get any injection  MVA-H5-sfMR: As a control, individuals were immunized with MVA-F6-sfMR (empty vector) vaccine |  | |
| 6.6. List and provide frequency of Adverse Events of Special Interest | MVA-MERS-S:   * 67 vaccine-related adverse events were reported in 10/14 (71%) participants in the low dose group * 111 vaccine-related adverse events were reported in 10/12 (83%) participants in the high dose group   Solicited local reactions were the most common adverse events:   * Pain (65%, 7/14 in low dose group, 10/12 in high dose group) * Swelling (38%, 2/14 in low dose group, 8/12 in high dose group * Headaches (7/14 in high dose group, 9/12 in high dose group)   MVA-H5-sfMR:  Most participants had one or more local (redness, swelling) or systemic reactions (headache, myalgia, chills, and fatigue). Individuals who were immunized with the lower dose had fewer systemic reactions.  11 Participants had adverse events recorded during the study:  1 individual: headache and lightheadedness  5 individuals: respiratory illness (accompanied by influenza-like symptoms)  1 individual: erythema nodosum  1 individual: injection-site reaction  3 individuals: other adverse events that were unlikely to have a causal relation with the vaccination |  | |
| 6.7. Did a Data Safety Monitoring Board (DSMB) or its equivalent oversee the study? | Yes | MVA-MERS-S: A Local Safety Board oversaw the study, checking for relationship of AE/SAE to vaccine or relationship of AE/SAE to vaccine dose | |
| * Did it identify any safety issue of concern? | No |  | |
| * If so describe: |  |  | |
| 7. Overall Risk Assessment of the Vector | Information | Comments/ Concerns | |
| 7.1. Please summarize key safety issues of concern identified to date, if any: |  |  | |
| * how should they be addressed going forward: | Definition of appropriate inclusion and exclusion criteria in clinical studies |  | |
| 7.2. 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? | Mild or moderate AEs based on expected local and systemic reactions to vaccination; no severe or unexpected significantly increased AEs or serious reactions/change in laboratory values [14], [63] , [89]. | Low risk based on data from multiple clinical studies. | |
| * immunocompromised humans? | No severe or unexpected significantly increased AEs reported in HIV-infected individuals or melanoma patients under therapy [70], [89], [116], [117], [127]. | Low risk based on data from multiple clinical studies. | |
| * Breast milk, human neonates, infants, children? | Strong safety data from vaccination of children and infants with no severe or serious AEs [128], [129], [130], [131], [132]. | Low risk based on data from clinical studies. | |
| * pregnancy and in the fetus in humans? | No data or very limited data from humans [133], [134] | Low risk based on safety data from preclinical studies in NHPs. | |
| * elderly | Mild or moderate AEs with expected local and systemic reactions to vaccination; no serious or unexpected AEs [135], [136]. | Low risk based on data from several clinical studies. | |
| * in any other special populations (e.g., institutionalized population, individuals with associated chronic comorbidity)? | No severe or unexpected significantly increased AEs reported in individuals with cancer under therapy [89], [106], [137] [138] [139]. | Low risk based on data from several clinical studies. | |
| 7.3. What is the potential for shedding and transmission in risk groups? | None. | Minimal risk for shedding based on MVA biology and its use as non-replicating vector virus. Data on MVA replication-deficiency in cells of human origin based on the loss of multiple gene functions (for review see [5], [84] ). Data from preclinical studies demonstrating lack of productive MVA replication in NHPs under immunosuppression [71], and very limited in vivo biodistribution of the vector transiently detectable at the site of vaccine administration and draining lymphnodes [77]. | |
| Part II: Vaccine (Sections 8-12) | | | |
| 8. Target Pathogen and Population for the vaccine | Information | | Comments/Concerns |
| 8.1 What is the target pathogen for the vaccine? | Middle East respiratory syndrome coronavirus (MERS-CoV), a novel pathogen first described in 2012 | |  |
| 8.2 What are the disease manifestations caused by the target pathogen in humans, for the following categories: |  | |  |
| * In healthy people | MERS-CoV infections range from showing no symptoms (asymptomatic) or mild respiratory symptoms to severe acute respiratory disease and death. These include fever, cough, shortness of breath, sore throat and myalgia.  The most common extrapulmonary symptoms are gastrointestinal tract symptoms including vomiting, diarrhea, nausea, and abdominal pain [140], [141]. | | WHO |
| * In immunocompromised people | More severe disease than in healthy people. | |  |
| * In neonates, infants, children | Neonates: neurological symptoms (hyperspasmia, poor reactivity, delirium), hyponatremia  Children: severe respiratory symptoms, multi-organ dysfunction, nephrotic syndrome [142], [143], [144]. | |  |
| * During pregnancy and in the fetus | fetal distress, fetal growth restriction, preterm birth, fetal demise [145], [146] [147]. | |  |
| * In elderly | Elderly people have an increased risk of serious illness with a fatal outcome. | |  |
| * In any other special populations | MERS-CoV patients with comorbidities, such as diabetes, immunosuppression, cancer, chronic lung and kidney disease, are at significantly higher risk for hospitalizations associated with severe illness and possible fatal outcomes [147]. | |  |
| 8.3 Briefly, what are the key epidemiologic characteristics of the disease caused by the target pathogen (e.g. incubation period, communicable period*,* route/s of transmission,case fatality rate, transmissibility characteristics such as basic reproductive ratio *(*R0*)*, and intrinsic mutation)? | The incubation period of MERS-CoV is between two and 14 days. The communicable period is unknown. Individuals suffering from MERS should be isolated until 10 days after fever has resolved. MERS-CoV requires DPP4 as an entry receptor and is transmitted via aerosols. This receptor is highly expressed in the upper respiratory tract in camels, but only in the lower respiratory tract in humans. This could explain the efficient camel-to-camel viral shedding and transmission, but the only sporadic human-to-human transmission of MERS-CoV. Many of the first human cases resulted from direct contact with dromedary camels, the primary animal reservoir of MERS-CoV. Other outbreaks of MERS have been caused by nosocomial transmission in health care facilities.  The mortality rate is approximately 35%, which may be overestimated because the mortality rate is currently counted only in laboratory-confirmed cases.  Basic reproductive ratio: 2-3. Intrinsic mutations may occur. Studies revealed mutations in various genes, including ORF1ab, N protein, S protein, ORF4b [148], [149], [150], [151], [152], [153], [154], [155]. | | WHO |
| 8.4 What sections of the population are most affected by the target pathogen (e.g. pediatric, pregnant, lactating women (breast feeding), adult, elderly) | The highest incidence of severe disease is observed in elderly and immunocompromised individuals. However, the majority of patients (73.2%) with confirmed MERS-S CoV were between 21 and 60 years of age. This also fits with the assumption that there is a general risk of infection for health care workers and persons in close contact with dromedary camels. These groups are therefore considered relevant target groups for prophylactic vaccination against MERS-CoV infection and prevention of MERS. | | WHO |
| 8.5 What is known about the immune responses, duration, and potential correlates of protective immunity to the target pathogen or to the disease? | In coronaviruses, the S and N proteins are the most important immunogenic target antigens. Neutralizing antibodies are mainly directed against epitopes within the RBD region of the S protein.  Payne and co-workers described detectable amounts of neutralizing antibodies for <34 months post infection. Choe and co-workers demonstrated that patients surviving severe MERS, showed detectable amounts of neutralizing antibodies for 1 year. The levels of neutralizing antibodies in patients with a mild disease had waning over time. Alshukairi and co-worked detected neutralizing antibodies in patient with a severe disease (100%) and a mild disease (50%) more than 6 years after infection. Virus-neutralizing antibodies directed against the spike glycoprotein are known to generally correlate with protective immunity against coronavirus infection. Virus clearance and recovery from MERS disease are also associated with T cell responses [156], [157], [158], [159], [160], [161], [162], [163], [164]. | |  |
| 8.6 Please describe any other key information about the target pathogen or population that may inform benefit-risk | Asymptomatic individuals are thought to play an important role in virus transmission. This is supported by the fact that a high percentage of camel workers have a positive MERS-CoV-specific immune response without showing symptoms of severe disease. These camel workers with mild or subclinical MERS infections are believed to be the origin of the first severe MERS cases in the population [152], [153]. | |  |
| 9. Characteristics of the Vaccine | Information | | Comments/ Concerns |
| 9.1 Vaccine name | MVA-MERS-S | |  |
| 9.2. What is the identity and source of the transgene? | MERS-CoV-S gene sequence derived from the complete sequence of human betacoronavirus 2c EMC/2012 (GenBank Asseccion no. JX869059), including three silent mutations to remove the termination signals of vaccinia virus translation (TTTTTTNT). The modified gene was synthesized by Invitrogen Life Technology, Regensburg, Germany [23]. | |  |
| 9.3. Is the transgene likely to induce immunity to all strains/genotypes of the target pathogen? | To date, there are no strains/genotypes of MERS-CoV with notable changes in the transgene. However, there are MERS variants with mutations in the S protein that may lead to differences in virus uptake via the DPPT4 receptor and partial resistance to antibody-mediated neutralization. [165] [154] | |  |
| 9.4. Where in the vector genome is the transgene inserted? | MVA-MERS-S was generated by homologous recombination of the complete MERS S gene into the existing deletion site III of the MVA genome [23]. | |  |
| 9.5. Does the insertion of the transgene involve deletion or other rearrangement of any vector genome sequences? | No | | The genetic integrity of MVA-MERS-S was confirmed by PCR analysis of genomic viral DNA using standard methods [23]. NGS was performed (IDT). |
| 9.6. How is the transgene expression controlled (transcriptional promoters, etc.)? | MERS S is under the transcriptional control of the vaccinia virus early/late promotor PmH5 [166]. | |  |
| 9.7. Does insertion or expression of the transgene affect the pathogenicity or phenotype of the vector? | No | | MVA-MERS-S still has the desired replication profile as the MVA vector |
| 9.8. Is the vaccine replication-competent in humans or other species? | MVA-MERS-S is replication deficient in human cell lines (HeLa and HaCat) but replicated efficiently in CEF cells (primary avian cells). This was confirmed by multiple-step growth analysis [23]. | |  |
| 9.9. What is the risk of reversion to virulence, recombination or reassortment with wild type virus or other agents? | See section 4.5 | |  |
| 9.10. Is the vaccine genetically stable in vitro and/or in vivo? | Yes | |  |
| 9.11. What is the potential for shedding and transmission to humans or other species? | The risk of virus spread and transmission to humans or other species can be considered negligible. | | MVA does not survive in infected host cells. Therefore, it can be assumed that full clearance of MVA-MERS-S and recombinant DNA occurs within days after vaccine administration. |
| 9.12. Does the vaccine establish a latent or persistent infection? | No | | MVA-MERS-S has no changes in host cell restriction compared to its vector MVA F6 LMU SF |
| 9.13. Does the vaccine replicate in the nucleus? | No | | Poxviruses replicate exclusively in the cytoplasm. |
| 9.14. What is the risk of integration into the human genome? | Very low | | The risk of integration into the human genome is generally negligible for poxviruses. |
| 9.15. 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 | No disease manifestation | |  |
| * In immunocompromised people | No disease manifestation | |  |
| * In breast milk, neonates, infants, children | No disease manifestation | |  |
| * During pregnancy and in the fetus | No disease manifestation | |  |
| * In any other special populations | No disease manifestation | |  |
| 9.16. What cell types are infected and what receptors are used in humans? | A wide range of different cell types can be infected with poxviruses by plasma membrane fusion or endocytosis. Poxviruses encode a large number of proteins that mediate virus entry. Attachment occurs by binding to glycosaminoglycans or laminin on the cell surface. A variety of other proteins are involved in cell entry [52], [167], [168]. | |  |
| 9.17. What is known about the mechanisms of immunity to the vaccine? | Both MERS-S-specific antibody and T-cell responses are elicited by vaccination and provide protection upon MERS-CoV challenge infection in mice. In addition, the vaccine was shown to significantly reduce the amount of infectious virus in the excretions of MVA-MERS-S-immunized dromedary camels following MERS-CoV Challenge infection [9], [24]. | |  |
| 9.18 Has disease enhancement (including antibody dependent enhancement (ADE), vaccine associated enhanced respiratory disease (VAERD)) been demonstrated with the vaccine? |  | |  |
| ● in vitro? | No | |  |
| ● in animal models? | No | |  |
| ● in human hosts? | No | |  |
| 9.19 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? | The effect of pre-existing immunity may be considered low in individuals born after 1980. Repeated application of the vector or vaccine has no negative effect on specific immunity to the transgene. Several studies have shown that strong antigen-specific immune responses could even be enhanced by repeated administration of recombinant MVA and this despite the fact that MVA neutralizing antibodies were detectable after immunization [63], [106], [107]. | | Vaccination program against Smallpox ended 1980, when Smallpox was declared officially eradicated by the WHO. |
| 9.20. Is the vaccine transmissible in humans or other species (including arthropods) and/or stable in the environment? | No, because the vaccine is not capable of replication. | |  |
| 9.21. Are there antiviral or other treatments available for disease manifestations caused by the vaccine? | The vaccine does not cause disease manifestations. | |  |
| 9.22. Vaccine formulation | Formulation in saline (IDT Biologika) | |  |
| 9.23. Proposed route and method of vaccine delivery (e.g. oral, intramuscular injection microneedles, skin patch, intranasal, mucosal) | Intramuscular injection | |  |
| 9.24. Target populations for the vaccine (e.g. pediatric, maternal, adult, elderly etc.) | There is a general risk of infection for health care workers and persons in close contact with dromedary camels. These groups are therefore considered relevant target groups for prophylactic vaccination against MERS-CoV infection and prevention of MERS. | |  |
| 10. Toxicology and Potency (Pharmacology) of the Vaccine | Information | | Comments/ Concerns |
| 10.1. What is known about the replication,  transmission and pathogenicity of the vaccine in and between animals? | No differences concerning these aspects compared to the vector MVA (See Section 5.1) | |  |
| 10.2. 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? | Not applicable | |  |
| 10.3. Does an animal model relevant to assess attenuation exist? | Yes. See section 5.3 | |  |
| 10.4. Does an animal model for safety including immuno-compromised animals exist? | Yes. See Section 5.4 | |  |
| 10.5. Does an animal model for reproductive toxicity exist? | Yes, inoculations of rats or rabbits can serve as models. | |  |
| 10.6. Does an animal model for immunogenicity and efficacy exists? | Yes [9], [24]. | |  |
| 10.7 Does an animal model for antibody enhanced disease (including antibody dependent enhancement (ADE), vaccine associated enhanced respiratory disease (VAERD)) or immune complex disease exist? | No indication of ADE or VAERD in preclinical studies. | |  |
| 10.8. What is known about biodistribution in animal models or in humans, including neurovirulence and/or neuroinvasion? | See Section 5.8 [77]. | |  |
| 10.9 What is the evidence that vector derived vaccines will generate a beneficial immune response in: |  | |  |
| * Small animal models? | Immunogenicity and protective efficacy in dromedary camels and in the mouse model [9], [24]. | |  |
| * Nonhuman primates (NHP)? |  | |  |
| * Human? | B-cell and T cell immune responses in humans [14] [22]. | |  |
| 10.10. Have challenge or efficacy studies been conducted in subjects with: |  | |  |
| * Immunocompromised conditions including HIV? | No | |  |
| * Other diseases? | No | |  |
| 10.11 Have studies been done simultaneously or sequentially administering more than one vector with different transgenes? Is there evidence for interaction/interference? | No – yet from similar candidate vaccines there is evidence for the likeliness of favorable interactions when using sequential administration e.g. of adenovirus and MVA vector vaccines (e.g. Ad26 ZEBOZ-GP and MVA-BN-Filo as licensed prime-boost Ebola vaccines Zabdeno und Mvabea) | | EMA/298997/2020 EMEA/H/C/005337 |
| 11. Adverse Event (AE) Assessment of the Vaccine (\*see Instructions): | Information | | Comments/ Concerns |
| 11.1. Approximately how many humans have received this viral vector vaccine to date? If variants of the vector, please list separately. \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ | > 120 subjects (tested in phase Ia and Ib clinical trial) [14], [26] | |  |
| 11.2. Method(s) used for safety monitoring: |  | |  |
| * Spontaneous reports/passive surveillance | No | | If yes, describe method: |
| * Diary | Yes | | If yes, number of days: 180 |
| * Other active surveillance | No | | If yes, describe method and list the AE’s solicited: |
| 11.3. What criteria were 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 | | MVA-MERS-S: Beside the FDA’s 2007 voluntary guidance, also the Common Terminology Criteria for Adverse Events (CTCAE v4) was used for grading the AEs (more detailed information can be found under <https://clinicaltrials.gov/ProvidedDocs/11/NCT03615911/Prot_000.pdf>) |
| * If no criteria were used for grading, or if other metrics were employed, please describe: | - | |  |
| 11.4. List and provide frequency of any related or possibly related serious\* AE’s as well as any severe, expected or unexpected AE observed: (\*see Instructions): | No severe or serious adverse events were observed | |  |
| 11.5. List and provide frequency of any serious, unexpected significantly increased AE or lab abnormality in vaccinee vs. control group: | No serious, unexpected significantly increased AE or lab abnormality were observed | |  |
| * Describe the control group: \_\_\_\_\_\_\_\_\_\_. | 6 healthy individuals who did not get any injection | |  |
| 11.6. List and provide frequency of Adverse Events of Special Interest | MVA-MERS-S (phase Ia clinical trial) [14]:   * 67 vaccine-related adverse events were reported in 10/14 (71%) participants in the low dose group * 111 vaccine-related adverse events were reported in 10/12 (83%) participants in the high dose group   Solicited local reactions were the most common adverse events:   * Pain (65%, 7/14 in low dose group, 10/12 in high dose group) * Swelling (38%, 2/14 in low dose group, 8/12 in high dose group * Headaches (7/14 in high dose group, 9/12 in high dose group) | |  |
| 11.7. Did a Data Safety Monitoring Board (DSMB) or its equivalent oversee the study? | Yes | | MVA-MERS-S: A Local Safety Board oversaw the study, checking for relationship of AE/SAE to vaccine or relationship of AE/SAE to vaccine dose |
| * Did it identify any safety issue of concern? | No | |  |
| * If so describe: |  | |  |
| 12. Overall Risk Assessment of the Vaccine | Information | | Comments/ Concerns |
| 12.1. Please summarize key safety issues of concern identified to date, if any: |  | |  |
| * how should they be addressed going forward: | See Section 7.1 | |  |
| 12.2. 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? | See Section 7.2 | | Low risk based on data from Koch 2020 and data from multiple clinical studies testing similar MVA vector vaccines [14]. |
| * immunocompromised humans? | See Section 7.2 | | Low risk based on data from multiple clinical studies testing similar MVA vector vaccines. |
| * Breast milk, human neonates, infants, children? | See Section 7.2 | | Low risk based on data from Koch 2020 and data from multiple clinical studies testing similar MVA vector vaccines [14]. |
| * pregnancy and in the fetus in humans? | See Section 7.2 | | Low risk based on data from preclinical studies / preclinical reproductive toxicology testing of similar MVA vector vaccines. |
| * elderly | See Section 7.2 | | Low risk based on data from clinical studies of similar MVA vector vaccines. |
| * in any other special populations (e.g., institutionalized population, individuals with associated chronic comorbidity)? | See Section 7.2 | | Low risk based on data from clinical studies of similar MVA vector vaccines. |
| 12.3. What is the potential for shedding and transmission in risk groups? | See Section 7.3 | | Minimal risk [77]. |

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