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| DATE | 24 March 2021 |
| REFERENCE | CGM/210324-01 |
| SUBJECT | Advice generic environmental risk assessment for clinical applications with MVA vectors |

Geachte mevrouw Van Nieuwenhuizen,

Further to a request for advice on a broad licence application for vaccine trials using MVA vectors, COGEM has prepared a generic environmental risk assessment for clinical applications with MVA vectors for the purpose of streamlining the licensing procedures for these trials.

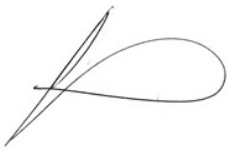
**Summary:**
The modified vaccinia virus Ankara (MVA) is a biologically contained virus with a history of safe use as a vaccine against smallpox. Vectors derived from MVA are used in various clinical applications (such as vaccination), in particular because of their high packaging capacity and high immunogenicity, and because the vector genome does not integrate into the DNA of the host cell.

COGEM has issued advice on clinical trials with MVA vectors on a number of previous occasions. Further to a recent request for advice on a broad licence application for clinical vaccine trials using MVA vectors, COGEM has prepared a generic environmental risk assessment for clinical applications with MVA vectors.

MVA vectors are contained such that they are apathogenic and unable to replicate in humans, which makes further transmission in the environment impossible. Given the properties of the MVA vectors, COGEM is of the opinion that the risks to human health and the environment from clinical trials with these vectors are negligible, provided a number of conditions are met. This generic environmental risk assessment can simplify and streamline the authorisation process, because it can provide the basis for drawing up a set of standard licence conditions (*vergunning onder vaste voorwaarden* – VoV) for these applications.

The attached report contains COGEM's advice and a discussion of the underlying reasoning.

Hoogachtend,



Prof. dr. ing. Sybe Schaap
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# Generic environmental risk assessment of clinical trials with MVA vectors

## COGEM Advisory Report CGM/210324-01

### 1. Introduction

Modified vaccinia virus Ankara (MVA) is an attenuated virus with a history of safe use as a vaccine against smallpox (caused by the variola virus). Much research has been conducted in recent years to exploit the properties of this attenuated virus and develop vectors derived from MVA that can be used in a clinical application, for example as a vaccine against infectious diseases.

#### *1.1 Generic environmental risk assessment*

For various reasons, MVA vectors are considered to be good candidates for use as vector systems in clinical applications (e.g. vaccination), in particular because of their high packaging capacity, their high immunogenicity as a vaccine and the fact that these vectors can be produced relatively easily. Moreover, replication of the MVA vector genome takes place in the cytoplasm of the cell and integration of the vector genome into the host genome has never been reported.[1,2,3]

On various occasions in the past COGEM has issued advice on clinical trials with MVA vectors. This present generic environmental risk assessment was drawn up in response to an application for a broad licence application for the use of MVA vectors in clinical vaccination trials (on their own or in combination with replication-deficient adenoviral vectors derived from human adenovirus 26). The assessment can form the basis for drawing up a simplified authorisation procedure with a set of standard licence conditions (*vergunning onder vaste voorwaarden* – VoV) for clinical trials with MVA-derived vectors. The properties of the vectors and other factors and information relevant to the environmental risk assessment are discussed further below.

### 2. Poxviruses

The *Poxviridae* family is divided into two subfamilies, the *Chordopoxvirinae* and the *Entomopoxvirinae*. The subfamily *Entomopoxvirinae* contains four genera and the *Chordopoxvirinae* subfamily contains 18 genera. Modified vaccinia virus Ankara (MVA) is an attenuated variant of the chorioallantois vaccinia virus Ankara (CVA), a poxvirus from the *Vaccinia virus* species. *Vaccinia virus* is the type species from the genus *Orthopoxvirus* (subfamily *Chordopoxvirinae*), which includes a further 11 poxvirus species, such as *Monkeypox virus* and *Cowpox virus*.[4] Another well-known species in this genus is the (now eradicated) *Variola virus*, the causative agent of smallpox, with flu-like symptoms and fever followed by the development of papules, vesicles and pustules on the skin. Infections with *Variola virus* were fatal in 30% of cases. The virus was responsible for many casualties in 18$^{th}$ century Europe.

Under natural conditions some poxviruses are very host-specific. For example, *Variola virus* only infected humans and *Camelpox virus* only infects camels, but some other poxviruses have a broader tropism and can infect several hosts (different animal species and humans). For example, the genus *Orthopoxvirus*

contains several poxvirus species that have an animal host but have also been reported to infect humans: *Cowpox virus, Monkeypox virus, Akhemeta virus*[5,6] and *Abatino macacapox virus*.[7]

The main route of infection with a zoonotic poxvirus is by direct contact with damaged skin, via an animal bite or via infected objects or materials (fomites).[8] The primary means of dissemination of the *Vaccinia virus* outside the body is by direct contact.[9] The *Variola virus* was also able to spread via respiratory droplets.[10]

Poxviruses are very stable outside the body: they are able to remain infectious for long periods (many months) and are highly resistant to desiccation in the material in which they are present and are shed (such as scabs, serum, blood residues or other excretions).[1,11] However, they are sensitive to common disinfectants.[11]

### *2.1 History of modified vaccinia virus Ankara (MVA)*

At the end of the 18th century the English physician Edward Jenner demonstrated that material obtained from the lesions of sick cows (it is assumed this was *Cowpox virus* or possibly the now extinct horsepox virus)[12] could be used to protect people against smallpox caused by the *Variola virus*. Later, in the 19th century, the *Vaccinia virus* became the preferred vaccine virus for controlling the *Variola virus*,[13] but the origin or background of *Vaccinia virus* is not known. No natural hosts are known for this virus.[14] In 1996 the World Health Organization (WHO) began a vaccination campaign to eradicate the *Variola virus* worldwide using various *Vaccinia virus* strains as a vaccine.[15] The *Variola virus* was officially declared eradicated in 1980. Vaccination with *Vaccinia virus* could in rare cases lead to severe complications, such as progressive vaccinia (often fatal), generalised vaccinia (which is similar to infection with *Variola virus* but often self-limiting), neurological complications or myocarditis. *Vaccinia virus* vaccination was contra-indicated for people with skin conditions, such as eczema, because in these people vaccination could induce a severe infection which can spread to the entire body.[16]

MVA is derived from the chorioallantois vaccinia virus Ankara (CVA), which was developed by culturing the Ankara strain of the *Vaccinia virus* on the chorioallantoic membrane of fertilised chicken eggs. In the past, CVA was also used as a vaccine against smallpox, but secondary lesions sometimes appeared as a side-effect of the vaccination. Much research has been done on the properties of CVA, including its evolution and stability, by repeatedly passaging the virus in chicken embryo fibroblast (CEF) cells.[17,18] After about 570 passages in CEF cells several mutations had arisen in the genome that were associated with phenotypic changes, such as the inability to form cytopathic effects or plaques in different cell lines and the absence of skin lesions after skin infection. This modified and highly attenuated form of CVA was renamed MVA in 1968.[18] MVA is not pathogenic in animals, including irradiated mice,[19] rabbits[20] and immunocompromised monkeys.[21]

MVA has been widely studied, has been used since the 1970s as a human vaccine against smallpox and has been proven safe in more than 120,000 vaccinated individuals without any serious side effects, even

amongst individuals with skin diseases and children.[22,23] The safety of MVA has also been demonstrated in HIV-infected individuals and cancer patients.[24,25,26,27]

In 2003 COGEM classified MVA as apathogenic and assigned it to pathogenicity class 1, because the virus had been shown to be harmless when used in applications in humans and animals.[28,29] The MVA Bavarian Nordic (BN) strain, created by subjecting the virus to additional passages in CEF cells, was approved for use on the European market under the name IMVANEX® as a vaccine against smallpox (caused by the *Variola virus*).[30] MVA-BN has also proven to be safe in individuals with a contra-indication for the regular smallpox vaccine (*Vaccinia virus*).[31,32,33]

### *2.2 Genome organisation of MVA*

MVA virus particles contain one or two membranes. The viral linear double-stranded DNA genome of approx. 178 kbp[34,35] is enclosed within a viral core. After more than 500 passages of parental strain CVA in CEF cells, six major deletions as well as several small deletions, insertions and point mutations were made in the MVA genome. As a result, about 15% of the genome (approx. 30 kbp) was lost in comparison with CVA.[1,36,37] These deletions occurred in different regions of the genome.[36] Compared with parental strain CVA, MVA contains 71 orthologous open reading frames (ORFs) which code for identical protein sequences, and 124 ORFs whose gene products contain amino acid alterations, insertions or deletions.[17] The various insertions, deletions and mutations that occurred during the development of MVA have, among other things, disrupted or removed genes that are involved in replication in different mammalian cells, in evading the host immune system and in determining the virulence of the virus.[1,18,38,39]

### *2.3 Infection, DNA replication and life cycle of poxviruses*

To adhere to and fuse with host cells, poxviruses are known to require the presence of glycosaminoglycans or extracellular matrix components, which are found on almost all cells. Poxviruses are therefore likely to be able to infect a wide range of mammalian cells, but their ability to productively replicate (i.e. form new virus particles after infection) is dependent on host-cell-specific intracellular factors.[40]

Poxviruses (*Poxviridae*) replicate in the cytoplasm of the host cell. This makes the virus dependent on its own transcription machinery, which is enclosed in the viral core where the viral DNA is also located. For translation the virus is dependent on protein synthesis by the cell.[41] To infect the host cell, the virus attaches itself to the surface of the host cell and the viral membrane fuses with the host membrane, mediated by a viral entry/fusion complex consisting of at least 12 viral proteins; the core then enters the cytoplasm.[42,43] After some time the core uncoats, releasing the genome. It is assumed that core uncoating occurs in the vicinity of the endoplasmic reticulum (ER) to form a replication complex surrounded by the membrane of the raw ER, called a 'virus factory'.[44]

The life cycle of poxviruses has three phases: early, intermediate and late. Early transcription takes place in the core of the virion by DNA-dependent RNA polymerase to produce viral mRNA that codes for proteins involved in DNA replication, in the expression of intermediate genes and in the modulation of the antiviral response by the host. Because the entire transcription machinery is present in the viral core,

transcription of the viral genome can proceed rapidly following infection. After expression of the early genes, the core falls apart, allowing DNA replication to take place, followed by transcription of the intermediate and late genes.[45] Replication of the DNA can be detected within two hours of infection. During the second or intermediate phase, it is mainly proteins needed for the expression of the late genes that are produced. In the third or late phase, the structural proteins of the virus are expressed and the proteins needed for the expression of the early genes (such as DNA-dependent RNA polymerase, the capping enzyme and polyA polymerase) are produced and enclosed in the core of the new virus particle to facilitate the next infection cycle.[46] *Vaccinia virus* is known to have more than 100 proteins packed into the virus particle.[44]

The resulting virus particle has two infectious forms: the intracellular mature virion (IMV) and the extracellular enveloped mature virion (EEV). First the viral DNA is packaged into the host cell in intracellular immature virions (IV) which mature into infectious IMV. The IMVs are released when the cell lyses as a result of the infection. Some of the IMVs can be packaged in a double membrane from the *trans* Golgi network; these are called intracellular enveloped or wrapped virions (IEV or WV). The IEVs are transported via microtubules to the cell membrane where the outer viral membrane can fuse with the plasma membrane of the host cell. This form is called the cell-associated enveloped virion (CEV). These CEVs can be detached from the cell membrane directly or indirectly by induction of actin polymers, the released virus being known as EEV.[40,47,48,49]

The IMVs and EEVs have different numbers of surrounding membranes and different antigens/glycoproteins on the membrane surface. When IMVs infect a cell the single membrane fuses and the viral core can then enter the cell cytoplasm directly. When EEVs infect a cell the outer membrane is first disrupted by proteins present on the cell and virus membrane at the site of attachment to the host cell, allowing the inner membrane to fuse with the host cell.[16]

### *2.4 Replication of MVA in different host cells*

Productive replication of MVA is possible in a limited number of cell lines, the main cell line being CEF cells. Replication is also possible in other cell lines of embryonal avian origin, such as chickens, ducks and quail. However, the deletions in the MVA genome prevent the virus from productively replicating (non-permissive cell lines) in most mammalian cells, including human cells.[37,50] For example, in HeLa cells derived from humans, only IV particles have been detected, but no IMVs.[37,51] In some specific mammalian cells, such as baby hamster kidney (BHK-21) cells and IEC-6 cells derived from rats, MVA is capable of replication (permissive cell lines).[1,50,52,53] There are also some cell lines in which some limited productive replication can take place (semi-permissive cell lines), such as MA104, BS-C-1 and CV-1 cells (derived from the grivet) and MDCK cells (derived from the dog).[52] It should be noted that the potential for productive infection in vitro can be different, and often broader, than in vivo.[40] The blockage in the replication cycle in non-permissive cell lines takes place at a later stage of the replication cycle.[52] In human cells the virus is still able to express the early, intermediate and late genes, as well as any recombinant genes, but in these cell lines the final assembly of the virus particle is blocked.[37,51]

***Host range genes***
Research into the host range genes responsible for the reduced replication capacity of MVA has been ongoing for 30 years. It is now clear that regulation of host range is multifactorial and several genes have been identified that may have a part to play in this process. Repair of a host range gene and expression of the corresponding protein product often leads to partial recovery of replication capacity, or replication is only productive when specific cell lines are used. Recently, the new host range gene C16L/B22R has been identified in the genome of *Vaccinia virus* and it appears to be involved in replication in several human host cells.[38,54] This gene (present in the genome of most orthopoxviruses in two copies, left and right) is inactivated in MVA by multiple deletions. When this gene is repaired or expressed in a human cell line, MVA is able to replicate productively in certain human cell lines. In addition, when a second host range gene (C12L) that is missing in MVA – and which was earlier thought to act as a host range gene – is also repaired, replication capacity can be restored in more human cell lines.[38]

Not all MVA strains have the same phenotypic characteristics. For example, some MVA strains (MVA-572 and MVA-1721) are able to replicate productively in cells of human origin and in immunocompromised mice.[55] MVA-572 and MVA-1721 are assumed to be heterogeneous mixes of MVA variants, some of which have a different genotype and other phenotypic characteristics, but are present below the PCR detection limit.[55] Sequence analysis of the coding regions has shown that these strains are 100% identical to the MVA reference genome.[35] Further analysis using CVA primers instead of MVA primers has shown that some variants of MVA-1721 lack deletion sites. Two variants of MVA-572 isolated from immunocompromised mice were found to have mutations in genes of unknown function. This study of the phenotypic heterogeneity of these strains and MVA-BN states that MVA-BN cannot replicate in human cell lines and that it is a homogeneous and stable MVA strain.[1,55]

### *2.5 MVA as an expression vector*
Although MVA cannot productively replicate in most mammalian cells, the virus can infect these cells and viral genes and any recombinant genes are expressed.[37] The degree to which MVA proteins are produced in HeLa cells (non-permissive for MVA) is similar to that of the wild-type *Vaccinia virus* Ankara strain in the same cells.[37] Because of the efficient gene expression of MVA and its inability to produce infectious virus particles after infection, much research is being conducted into MVA as a candidate for the development of recombinant vectors for clinical applications. The possibility of improving recombinant gene expression in MVA vectors is also being investigated, for example by using different poxvirus promoters.[56] Promoters from related poxviruses are interchangeable, but may achieve different levels of gene expression. Research indicates that use of an early promoter, which expresses transgenes during the early phase, can provide a better cellular immune reaction.[56]

#### 2.5.1 MVA vector production systems
The commonest technique for developing recombinant MVA vectors is by means of homologous recombination in MVA-infected cells. For this a transfer plasmid is used, containing a transgene and a virus-specific promoter, flanked by the homologous MVA sequences required for integration at a specific

site in the MVA genome. The transgene expression cassette can be inserted in regions where the major deletions have occurred or in non-overlapping regions between essential genes, called intergenic regions (IGRs).[18,57,58] Insertion in IGRs can improve the stability of the recombinant MVA vectors.[58,59] It is also possible to make multiple insertions in the same MVA genome. Sometimes the transfer plasmids contain selection markers that enable them to select transformed MVA vectors; these marker genes can be removed after the initial selection by a second recombination event.[61] An alternative method of obtaining recombinant MVA is by cloning the MVA genome as a bacterial artificial chromosome (BAC) which can be modified in *Escherichia coli* by homologous recombination.[18]

**3. MVA vectors in clinical trials**

Vectors derived from MVA are considered good candidates for therapeutic purposes for several reasons. Poxvirus vectors have a large packaging capacity (at least 25 kb), which allows large transgene fragments to be inserted into the genome.[47,60] In addition, the genome replication takes place in the cytoplasm and integration into the host genome has never been observed. Finally, immunogenicity as a vaccine is high (possibly due to loss or disruption of immunomodulatory genes)[53] and these vectors can be produced relatively easily.

Since the 1990s, many different preclinical studies and clinical trials have been conducted with MVA vectors as a prophylaxis or vaccine against various viral, bacterial and parasitic infectious diseases, including HIV, influenza, malaria, smallpox and tuberculosis, or as treatment for cancer.[15,18,47,61,62,63] MVA vectors are also used as a boosting agent in vaccine studies, in which the MVA vector is often introduced some time after the primary vaccination, often with a DNA vaccine or adenoviral vectors. This is also referred to as a heterologous prime-boost vaccine strategy and serves to enhance the immune response and thus the development of immunity.

MVA vectors under investigation for their potential as a cancer immunotherapy often contain tumour-associated antigens that are designed to induce or heighten an immune response to the tumour. The results of preclinical studies are predominantly positive and the MVA vector is also generally well tolerated in clinical trials.[47,64]

**4. Previous COGEM advice**

In the past COGEM has advised classifying MVA as apathogenic because the virus had been shown to be harmless when used in applications in humans and animals.[28,29] On various occasions COGEM has also issued advice on clinical trials with MVA vectors. For example, in 2003 COGEM advised on a phase 1 clinical trial with an MVA-based vaccine to which a number of genes of HIV-1 subtype A had been added.[65] The MVA vector was administered intramuscularly, subcutaneously or intradermally. COGEM considered the risks of this trial to human health and the environment to be negligible, on condition that not only the bed linen, but also the clothing is disinfected, given that both may be contaminated if the injection site is not taped off properly.

In 2006 COGEM again provided advice on a phase 1 clinical trial with a GM MVA containing sequences of the HIV-1 subtype B as a vaccine against HIV.[66] The vaccine was administered in two consecutive intramuscular injections. COGEM was of the opinion that the addition of the HIV genes does not alter the non-pathogenic nature of MVA and considered the risks to human health and the environment of this trial to be negligible. In addition, COGEM advised using water-resistant plasters to cover the injection site and instructing participants on how to remove the plaster hygienically in order to minimise spread of the GM virus. This advice was given on the basis of research showing that following injection of a *Vaccinia virus* vaccine, the virus can sometimes be detected on the plaster used to cover the injection site or on the subject's hands after removing the plaster.[67] COGEM noted in addition that even if some dissemination of the virus occurs, it considers the chance of the vaccine virus infecting non-test-subjects to be negligible and that any infection will die out because the virus cannot replicate in human cells.

In 2012 COGEM advised on a phase 1 clinical trial with GM MVA containing the sequence of the hemagglutinin (HA) gene in the *Influenza A virus*.[68] For this, 16 different GM MVA-HA vectors were developed for 16 *Influenza A virus* subtypes. The vaccines were not combined and were administered in three consecutive intramuscular injections. Despite the expectation that the risks of the clinical trial, when conducted in accordance with the proposed working instructions, would be negligible, COGEM could not provide a final judgement due to lack of information on the molecular characterisation. The applicant subsequently provided experimental data to support the molecular characterisation of one of the MVA-HA vectors. For this variant, COGEM considered the molecular characterisation to be sufficient and considered that the same analysis would be needed to come to the same judgement about the other 15 variants.[69]

In 2020, COGEM advised on a clinical trial with a GM MVA variant in which the spike protein of the Middle East respiratory syndrome-related coronavirus (MERS-CoV) was added. The vaccine was administered by intramuscular injection, and hygiene measures were applied during and after vaccination. COGEM was of the opinion that the environmental risks associated with this clinical trial with MVA MERS-S_DFI were negligible.[70]

At the end of May 2020, under an accelerated assessment procedure, the European Medicines Agency (EMA) approved the MVA-based Ebola vaccine MVA-BN-Filo for placing on the European market.[71] In 2019, COGEM issued a confidential[a] positive advice on this marketing authorisation.[72] This multivalent vaccine is based on MVA-BN into which transgenes have been inserted to express the glycoproteins of various Ebola viruses and the Marburg virus.

**5. Considerations concerning the environmental risk assessment**

Environmental risk assessments of clinical trials focus primarily on whether or not people or animals not taking part in the trial ('third parties') could be infected with the MVA vector or sequences derived from it and suffer adverse effects as a consequence. The potential environmental risks are related to the

[a] This concerns advice on an application for marketing authorisation of a medical application in the EU. Such cases and licence applications are always declared confidential by the EMA. Under EMA guidelines, COGEM may not publish its advice or make it publicly available in any way.

pathogenicity of the vector, the spread of the vector in the environment and the possible formation of recombinant viruses through recombination in the patient. To support the rationale for this advice, these aspects are discussed in more detail below.

### *5.1 Pathogenicity of an MVA vector*

COGEM has classified MVA as a non-pathogenic virus and assigned it to pathogenicity class 1.[28,29]

#### 5.1.1 Possible adverse effects

In general, MVA vectors are tolerated well in clinical trials and most reported adverse events are categorised as mild, short-lived and predictable. The method of administration can influence the reported adverse effects. Compared with the intradermal route, the intramuscular route of administration leads to significantly fewer local adverse effects (itching, swelling, heat and redness).[73] Severe adverse effects are more frequently reported in clinical trials in which high doses (more than $10^8$ plaque forming units) of MVA vectors are administered, but these effects (malaise, nausea or vomiting, and chills) are not considered life-threatening.[73,74]

Besides the properties of the vector itself, the risk assessment of recombinant MVA vectors should also take into account possible adverse effects of the inserted gene product, such as those of transgenes associated with virulence, toxins or cytotoxins and the restoration of lost functions. COGEM notes in this respect that any adverse effects of MVA vectors first and foremost present a risk to the recipient. Whether or not these effects can also occur in third parties depends on the degree of spread and third-party exposure to the MVA vectors (see section 5.2).

#### 5.1.2 Integration of the vector genome into the host genome

Replication of the MVA vector genome takes place in the cytoplasm of the cell and MVA does not possess sequences that enable integration into the host genome.[75] No evidence has yet come to light to indicate that the MVA vector genome can integrate into the host genome.[1,2]

### *5.2 Replication, shedding and distribution*

#### 5.2.1 Replication

When used for human applications, MVA is replication-incompetent.[b] Productive replication in which new virus particles can be formed is limited to specific cell lines, mainly of avian origin. When administered in human tissue, MVA vectors are biologically contained.

#### 5.2.2 Dissemination in the host (biodistribution)

Dissemination in the host of poxviruses, especially *Vaccinia virus*, can take place in several ways: direct dissemination from cell to cell through the use of actin tails (microvilli induced by the virus which contain actin),[76] as free virus particles, by infection of leukocytes or by virus-induced movement of the cell.[16,47]

[b] A few specific strains are capable of productive replication in human cells in vitro, as described in section 2.4, but it is not known whether these strains are capable of productive replication in vivo and, if so, whether this could be pathogenic.

Studies in mice have shown that after initial inoculation of the peritoneum with an MVA vector containing a bioluminescent transgene (luciferase), the vector was able to spread further in the abdomen. When administered intramuscularly, the luciferase signal was mainly confined to the site of administration.[77] The MVA vector remains for a limited time only, and in mice it was no longer detectable two days after intraperitoneal administration; even when administered intramuscularly the amount of vector decreased rapidly and was very limited 72 to 96 hours after administration.[77,47] In another study with an MVA-MERS-S vector in mice, very limited biodistribution was shown in some of the mice and no MVA DNA was detected in kidneys, rectum, faeces, bladder and urine.[78] Studies have also been done on dissemination in macaques following different administration routes (intradermal, intranasal and intramuscular). No MVA could be isolated from samples of blood, pharyngeal mucosa, lymph glands and other organs, even in immunocompromised animals. However, after a few days MVA DNA could be detected by PCR.[19] In a study using aerosol administration, the vector was mainly found in the mucosa of the lungs and other parts of the macaque respiratory system. The vector was not detectable in the brain or eyes.[47]

5.2.3 Dissemination outside the host (shedding)

There is little information available about the shedding of MVA vectors used in clinical trials. In one phase 1 study involving a recombinant MVA vector used to express the human protein MUC1 as a therapy for cancer patients, plasma and urine samples taken 1 to 4 hours or 8 days after inoculation were examined and no vector sequences or virus particles were detected.[79] It is assumed that any shedding will be limited, because the vector cannot replicate productively.[53]

When the *Vaccinia virus* vaccine is administered skin lesions often occur in which vector particles can be found. MVA (and IMVANEX®) are known not to cause skin lesions after intramuscular administration.[18,80] However, when administered subcutaneously, virus particles may still be present on the skin immediately after injection.[1] Another way in which dissemination beyond the intended patient may occur is through spillage or leakage of contaminated material, or via aerosols generated during this process.

MVA vectors shed into the environment are biologically contained and will not spread further. If the transgene or transgene product does not contribute to restoration of attenuated properties or complement the replication-incompetent property of the vector, COGEM considers the chance of further spread of MVA vectors to humans or animals as a consequence of potential shedding to be negligible. COGEM also points out that observing standard hygiene measures inside and outside the hospital – or taking additional measures when using administration methods that involve the risk of shedding – will further reduce the chance of third party exposure.

### *5.3 Recombination*

Recombination can occur when related viruses simultaneously infect the same cell, which may lead to the restoration of the lost functions in the MVA vector or introduction of the transgene sequence into a wild-type poxvirus. A requirement for this is that a related poxvirus can also infect humans. The best known poxvirus of the genus *Orthopoxvirus* that causes infections in humans and only has humans as its host is

*Variola virus*. However, this virus has been eradicated worldwide since 1980, which rules out coinfection with this virus. There are also several orthopoxviruses with zoonotic potential (see section 2), such as *Monkeypox virus* and *Camelpox virus*. Variants of *Vaccinia virus*, for which no natural hosts are known, have been demonstrated in cattle and buffalo in Brazil and India, and human infections have also been reported.[8,81] Human infections with orthopoxviruses are very rare in Europe[8,53] and known cases have been sporadic. Three cases of infection with *Monkeypox virus*, probably contracted in Nigeria, were reported in September 2018 and December 2019.[82,83] Infection with *Cowpox virus* is very rare and has been reported mainly in people who have been in direct contact with an infected animal.[84]

Vaccinia virus has been associated with the phenomenon of 'superinfection exclusion' in which secondary infection of the same host is prevented by expression of certain early proteins.[85,86] The formation of 'virus factories' (see section 2.3), in which DNA is replicated, can also hinder recombination.[87] It still possible for viruses to simultaneously infect the same cell.[86] Recombination between MVA vectors and related orthopoxviruses has been demonstrated in vitro by coinfection with an MVA vector and cowpox virus.[88,89] The resulting hybrid viruses lose the transgenes after a few passages (depending on the cell lines used). In some cases the plaque phenotypes in the hybrid viruses were similar to the parent viruses, but could also differ from both parental viruses.[88,89] Besides in vitro recombination between a recombinant MVA vector and a cowpox virus variant following coinfection, recombination after superinfection in Vero cells (semi-permissive) has also been described (2 to 6 hours after primary infection with a multiplicity of infection of 5), in which 0.4% to 7.1% of the plaques involved recombinant viruses with the transgene. Again, different plaque phenotypes were described for hybrid viruses with and without the transgene.[74]

Recombination between an MVA vector and circulating orthopoxviruses is possible. However, COGEM considers it highly unlikely that a patient administered with an MVA vector will be coinfected with a related orthopoxvirus, because zoonotic orthopoxviruses do not circulate in Europe and infection with these orthopoxviruses is therefore very rare. In addition, MVA-infected cells can only survive for a short time before they are cleared by the immune system, because after administration only a single round of infection can take place and no new infectious virus particles are formed that can infect other cells.

In addition to the genus *Orthopoxvirus*, there are other genera within the family *Poxviridae* that contain virus species capable of infecting humans, such as *Molluscum contagiosum virus* from the genus *Molluscipoxvirus*, which only infects humans.[40] *Bovine popular stomatitis virus*, *Orf virus*, *Pseudocowpox virus* and *Grey sealpox virus* from the genus *Parapoxvirus* and *Tanapox virus* and *Yaba monkey tumour virus* from the genus *Tatapoxvirus* all have zoonotic potential and can also infect humans. Tatapoxviruses are only found in Africa. COGEM considers the chance of recombination between MVA vectors and poxviruses from another genus than *Orthopoxvirus* to be negligible, because in the past this has never been reported during or after large-scale vaccinations with *Vaccinia virus* variants to prevent infection with the *Variola virus*.

Taking all of the above into consideration, COGEM concludes that the risk of recombination is negligible. Furthermore, to the best of COGEM's knowledge, there are no indications that recombination between

MVA vectors and other poxviruses has occurred at the time of vaccination with MVA or when using this vector in clinical trials.

5.3.1 Reversion to wild type

MVA vectors are biologically contained. They lack 15% of the genome of the parental strain CVA as a result of various major and minor deletions and mutations.[2] The possibility of maintaining productive replication is probably attributable to several missing or malfunctioning gene products. COGEM therefore considers the chance of spontaneous complete reversion of the genome resulting in a fully replication-competent phenotype to be negligible.

### *5.4 Sub-conclusions*

Based on the above analysis, COGEM concludes that:

- Incidentally observed adverse effects following administration of MVA vectors mainly affect the recipient. Whether or not these effects can also occur in third parties depends on the degree of spread and third party exposure to these vectors.
- MVA vectors are attenuated and biologically contained, and because they are unable to productively replicate in humans they cannot spread in the environment.
- It is not clear whether or not MVA vectors can be shed, but they can be present on the skin after administration. Their biological containment prevents further spread.
- Infection with wild-type poxvirus is very rare. Coinfection or recombination with a wild-type poxvirus following vaccination has not been described, despite a long history of use as a vaccine and application in clinical trials.

## 6. Other points of significance for the environmental risk assessment

### *6.1 Molecular characterisation*

In 2013 COGEM issued a generic advice on the genetic characterisation of GMOs for clinical applications.[90] COGEM points out that in addition to MVA-BN, other MVA strains exist, of which some are heterogeneous mixes of MVA variants and that can replicate in human cell lines. It is therefore necessary, in COGEM's opinion, that before MVA vectors are used in clinical applications it should be demonstrated that only pure MVA clones will be used that are incapable of productive replication in human cells and in most mammalian cells. In addition, COGEM considers that a complete sequence and bioinformatic analysis must be made of the MVA vector with insert to rule out the possibility of using a heterogeneous mix and to confirm that the sequence of the MVA vector corresponds to the intended sequence. COGEM is of the opinion that if these conditions are met a sufficient molecular characterisation of the MVA vector will be obtained.

## 7. Advice

Based on the above, COGEM concludes that it is possible to draw up a generic environmental risk assessment for clinical applications involving MVA vectors. Because of the apathogenic nature of these vectors and the fact that replication of these vectors in human cells is not possible, COGEM is of the opinion

that the risks to human health and the environment of clinical trials with MVA vectors are negligible, provided a number of conditions are met. In such trials COGEM advises adhering to the following conditions:

- The MVA vector has been derived from a pure MVA clone that is incapable of productive replication in human cells and most mammalian cells.
- The MVA vector has been fully sequenced and analysed to confirm that the vector sequence corresponds to the intended sequence.
- The transgene used does not code for a gene product that contributes to restoration of attenuated properties or complements the replication-incompetent property of the vector.

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