Send Orders for Reprints to reprints@benthamscience.net

Current Gene Therapy, **2013,** *13,* **413-420**

Environmental Risk Assessment of Clinical Trials Involving Modified Vaccinia Virus Ankara (MVA)-Based Vectors

Martine Goossens*, Katia Pauwels, Nicolas Willemarck and Didier Breyer

Scientific Institute of Public Health, Biosafety and Biotechnology Unit, B-1050 Brussels, Belgium

Abstract: The modified vaccinia virus Ankara (MVA) strain, which has been developed as a vaccine against smallpox, is since the nineties widely tested in clinical trials as recombinant vector for vaccination or gene therapy applications. Although MVA is renowned for its safety, several biosafety aspects need to be considered when performing the risk assessment of a recombinant MVA (rMVA). This paper presents the biosafety issues and the main lessons learned from the evaluation of the clinical trials with rMVA performed in Belgium. Factors such as the specific characteristics of the rMVA, the inserted foreign sequences/transgene, its ability for reconversion, recombination and dissemination in the population and the environment are the main points of attention. Measures to prevent or manage identified risks are also discussed.

Keywords: Biosafety, clinical trials, environmental risk assessment, gene therapy, GMO-based vaccines, MVA-based recombinant vectors.

1. INTRODUCTION

Poxviruses are considered excellent vector systems candidates for gene delivery without integration into the host genome or for vaccination. This is due to several features including (i) large packaging capacity for recombinant DNA (up to 25 kbp); (ii) precise and controllable recombinant DNA expression regulated by a strong poxviral promoter; (iii) lack of persistence or genomic integration in the host due to their cytoplasmic replication (transient expression); (iv) high immunogenicity as vaccine; and (v) ease of vector and vaccine production [1, 2]. Their main disadvantage resides in the development of neutralizing antibodies against the vector after subsequent administrations [3].

To address potential biosafety issues highly attenuated poxvirus strains have been developed, such as, in the orthopox virus (OPV) genus, the modified vaccinia virus Ankara (MVA) strain derived from the chorioallantois vaccinia virus strain Ankara (CVA). The attenuation of MVA is based on serial passages (more then 500) in primary chicken embryo fibroblasts (CEFs), resulting in a genomic loss of approximately 15% compared to the parental CVA strain, reducing its virulence and pathogenesis [4, 5]. This modified MVA is unable to propagate in human or in most mammalian cells. It remains localized in the cytoplasm [6] and there is no evidence for genomic integration. It was originally developed in the 1970s as a vaccine against smallpox and was found to be safer than other replication competent vaccinia strains [7-10]. Since the global eradication of smallpox was certified by a commission of scientists and endorsed by the World Health Organization (WHO) in 1980, this vaccine is not used anymore. However, with respect to the granting of a marketing authorisation for a new MVA vaccine called Imvanex, the European Medicines Agency (EMA) recommended in May 2013 its use for “active immunisation against smallpox in adults" under exceptional circumstances, i.e. to protect populations at risk from bioterrorism [11].

Since the nineties, MVA has been widely tested in clinical trials as recombinant vector for vaccination against various pathogens or as gene delivery vehicle for gene therapy applications. Although MVA vectors are considered safer than other vaccinia strains, several aspects should be considered carefully when performing the biological risk assessment of MVA and MVA-based vectors [5]. This includes in particular (i) the potential presence in the MVA population of variants able to replicate, (ii) the intrinsic characteristics of the transgene which may present hazardous properties or change the vector properties, (iii) recombination events with wild type OPV or homologs that could lead to the rescue of parental genes that are interrupted or deleted in MVA or the transfer of the transgene to replication competent OPV.

An increasing amount of information is becoming available on biosafety issues associated with MVA and MVA-based vectors, both from the scientific literature and from regulatory dossiers. In Belgium six clinical trials using MVA as vector systems for gene therapy or vaccination have been assessed since 1996. General or regulatory information about these trials (B-GT/11, B-GT/12, B-GT22, B-GT/24 and B-GT/26) are publicly available [12]. This paper presents the main lessons learned from the evaluation of these trials and shows how the information provided by the notifiers and the recommendations proposed by the risk evaluators can contribute to improve the risk assessment and the risk management of such trials.

*Address correspondence to this author at the Scientific Institute of Public Health, Biosafety and Biotechnology Unit, Rue J. Wytsmanstraat 14, B-1050 Brussels, Belgium; Tel: +32 2 642 52 93; Fax: +32 2 642 52 92;
E-mail: martine.goossens@wiv-isp.be

1875-5631/13 $58.00+.00

2. CRITICAL ISSUES IN THE ENVIRONMENTAL RISK ASSESSMENT OF MVA-BASED CLINICAL TRIALS

2.1. Steps in the Environmental Risk Assessment

As detailed in the lead-in article of Baldo *et al.* the environmental risk assessment (ERA) focuses on the identification of the characteristics of the genetically modified organism (GMO) and its use which have the potential to cause adverse effects on persons (non-patients) directly exposed to the gene therapy medicinal product, on animals, plants and micro-organisms. It is important to note that this assessment does not cover pure medical aspects on the efficacy of the investigational medicinal product (IMP) and its safety for the treated patient. However, very often, on a case-by-case basis, a potential adverse effect for the treated patient needs also to be considered with regards to medical personnel handling the IMP or providing patient cares. Indeed the personnel could be accidentally exposed to the IMP or contaminated material (such as dressings, sheets or waste).

The ERA is conducted following a six-step approach: (1) hazard identification or identification of GMO characteristics which may cause adverse effects, (2) hazard characterization or evaluation of the potential consequences of each hazard identified, (3) exposure assessment or determination of likelihood of occurrence for each hazard identified and characterized, (4) risk characterization which is the combination of magnitude of consequences of each hazard and the likelihood of its occurrence, (5) proposal of risk management strategies aiming at reducing risks identified in the previous step, (6) determination of overall risk and conclusions.

Several potential hazards can be identified in relation to MVA and MVA-based vectors. Their assessment and possible management are summarized in Table **1** and discussed in detail in the next sections in light of published literature and information gathered during the assessment of the six clinical trials notified in Belgium.

2.2. Hazards Related to the Molecular and Biological Characteristics of the Parental MVA Virus

According to its high attenuation profile MVA belongs to risk group 1 regrouping biological agents with no or negligible risk and for which level 1 containment is appropriate to protect human health and the environment. MVA also shows the same safety profile upon administration to immunocompromised non-human primates [23]. However, this classification is only valid if the MVA strain is genetically stable, homogeneous and characterized by (i) a high degree of attenuation in mammalian cells; (ii) a host-range restriction (inefficient propagation in mammalian cells: no viral particles are produced); and (iii) a cytoplasmic localization (no genome integration) [5].

As shown by Suter *et al.* [14], not all MVA viruses or strains show the same phenotypic properties and their safety profile can differ: some strains contain viral populations or variants that have an altered genotype compared to the original parental MVA strain and are actually able to replicate in human cell lines. To our knowledge this has never been observed in preclinical tests or in clinical trials [15] nor during the smallpox vaccination of more than 120.000 people with MVA during the 1970s [10]. Moreover, the MVA-BN® strain (the Bavarian Nordic's vaccine developed to generate the third generation smallpox vaccine like Imvanex) has been shown to be very homogeneous [14]. Information regarding the homogeneity of the MVA parental strain remains however important in order to exclude the presence of replication competent MVA particles, and should be requested if not provided by the notifier of a clinical trial. Several measures can be implemented to control and/or confirm homogeneity during the manufacturing and consists in (1) opting for a significantly homogeneous strain, (2) performing PCR analysis and sequencing to confirm the desired genotype of the chosen MVA strain, and /or (3) undertaking infection assays using immune-suppressed mice in order to check the non-presence of replicative viruses [14].

Regarding the replicability, it has been reported that MVA growth is generally restricted to a few mammalian cell lines. However, it is also recognized that only a limited number of mammalian cell lines have been evaluated for MVA multiplication [5] suggesting that it might be possible that other cell lines than those tested could support MVA replication. For instance, results from *in vitro* experiments indicated that rat intestinal epithelial IEC6 cells support efficient MVA replication [24]. However, this observation is counterbalanced by results of *in vivo* studies in mice and rhesus macaques which resulted in abortive infections [19, 23, 25, 26]. The ability to propagate in some mammalian cells could potentially have an impact on the potential for dissemination in the environment and hence infection of non-target organisms. This hazard should be carefully considered in risk assessment. General statements should be avoided in the dossiers, such as those claiming that the attenuated vaccinia virus is not able to replicate in mammalian cells other than baby hamster kidney fibroblast (BHK-21) cells. Cottingham and Carroll [16] suggest that any novel rMVA should be tested in mice not only to assess the safety of the transgene (see section 2.3) but also from the virological viewpoint. However, with regard the mitigation or prevention of identified risks, it is noted that general management measures such as the application of appropriate hand hygiene, decontamination and waste practices may be sufficient to prevent the risk by avoiding accidental dissemination into the environment.

Such as with all poxviruses, MVA shows high environmental stability with high resistance to drying up to 39 weeks at 6.7% moisture at 4°C and increased temperature tolerance compared to other viruses [21, 27]. Nevertheless there is only a limited environmental impact to be expected during unintended environmental spreading, due to the poor replicative and propagative characteristics of MVA [5]. Moreover, vaccinia virus has no natural reservoir [22].

Other attention points when assessing any modified viral vector are its potential of reconvertion to wild type, recombination with other viruses and its dispersion within the patient's body from the site of administration. These points apply also to the recombinant vector and are addressed in section 2.3.

Table 1. Potential Hazards[1 2] Associated with Clinical Trials Involving MVA-Based Vectors. Risk Assessment Considerations and Possible Management Measures[3].

Potential hazard	Risk characterization[4]		Measures for risk prevention and/or management
	Potential Consequences	Likelihood of occurrence	
Lack of homogeneity of parental MVA strain	Presence of variants, which can/may replicate in various mammalian/human cells [14]	**Low**, contrary to *in vitro* findings, it has never been observed in any preclinical or clinical trial so far [10,15]	- Use a significantly homogenous strain like MVA-BN®. - Molecular characterization of the chosen MVA strain - Infection assays to exclude presence of replicating viruses [14]
Ability to replicate in non-human mammalian cells	Potential to disseminate in the environment with potential adverse effects (e.g. virulence, disease, adverse effect of the transgene(s) on the infected organisms)	**Low**: MVA replication is restricted to a few cell lines. Uncertainty: only a limited number of mammalian cell lines have been evaluated for MVA replication [5].	- Apply appropriate working and decontamination practices to avoid accidental dissemination in the environment - Test any novel rMVA in mice for its virological activity [16]
Biological activity and recombination potential of the transgene(s)	Potential adverse effects associated with the transgene product or due to the recombination event (e.g. altered immune response, inflammatory reaction, autoimmune disease)	**High** for personnel coming accidentally into contact with the rMVA	Appropriate personal protective equipment and decontamination procedures to avoid infection
Presence of undesired sequences in the administered rMVA	Unexpected adverse effects associated with the undesired sequences	**Low**	Molecular characterisation of the GMO. GMP: appropriate quality checks performed at every step of the production process.
Lack of genetic stability and integrity of the transgene	Unexpected adverse effects associated with altered virus structure or altered expression of the transgene	**Low**	Choice of appropriate promoter [17] and insertion site for the transgene [18]. GMP: Perform appropriate stability tests.
Recombination between rMVA and naturally occurring homologs (e.g. orthopoxvirus (OPV))	- Return to virulence and spread of the vaccinia disease in animals - Transfer of the transgene into replication competent OPV and potential adverse effects of the transgene	**Negligible:** Although there is uncertainty regarding chance of co-localization [19], the risk of recombination is **unlikely** because: - MVA has lost 15% of its genome compared to the parental strain, which makes a full recovery **highly unlikely** [4]. - in human cells, no known human poxvirus is able to complement MVA to generate a replication competent virus. - the vector is short-lived (propagation defective vector)	- When administered to animals, consider host inclusion / exclusion criteria based on the hosts susceptibility to harbour homolog viruses. - Check biodistribution of MVA in function of site of administration - Personnel involved in handling rMVA must comply with protection measures to avoid e.g. accidental parenteral inoculation.
Integration vector sequences into the genome of the patient	Insertional mutagenesis and/or inadvertent regulation (activation/silencing) of neighbouring genes which may lead to e.g. oncogenetic effects.	**Negligible**: MVA has a fully cytoplasmic cycle of propagation [6]	/
Dispersion of the rMVA from the site of administration in other tissues of the treated patient	Adverse effects associated with transmission to germ cells and hence to offspring	**Negligible:** vaccinia virus remain in the cytoplasm and does not integrate its genetic material into the host chromosome	Recommend the patients, male and female, to use effective contraception during the study period and for several months after the last investigational medicinal product (IMP) administration

(Table 1) contd….

Potential hazard	Risk characterization[4]		Measures for risk prevention and/or management
	Potential Consequences	Likelihood of occurrence	
	Adverse effects for non-target infected organs	**Low** : Few biodistribution data indicate limited dissemination from the site of administration in the body of the patient (in the bloodstream). Of note, a study from Ramirez *et al.* [19] reported spread of MVA in all tissues after subcutaneous administration.	-Pre-clinical and clinical biodistribution studies to qualify the risk. -Appropriate personal protective equipment to avoid infection.
	The rMVA could be transmitted to other people by organ or blood donation resulting in potential adverse effects for organ or blood recipients especially in case of known possible side effects related to the transgene	**Uncertain** due to limited biodistribution data.	Exclusion of patients or healthy volunteers from blood or organ donation during the study and for several months after the last IMP administration.
Dissemination into the environment - shedding of rMVA (e.g. through patients excreta or skin pock lesions) -spreading through spill, aerosolisation, splashes or contaminated material.	- Adverse effects associated to the infection of personnel, people in general or animals coming into contact with the patient or contaminated surfaces or material	**Moderate to low:**- with regards to spreading through a spill, aerolisation, splashes or contaminated material, the likelihood of occurrence will depend on the amount of viral particles released. - not all patients develop skin pock lesions (often observed in vaccinia virus positive individuals [20]. - as all poxviruses MVA has a high environmental stability. The rMVA could survive (in a latent stage) for a long time up to 39 weeks at 6.7% moisture at 4°c [21]. - MVA has no natural reservoir in the environment [22] and is short-lived (propagation defective vector)	- Appropriate personal protective equipment to avoid infection and decontamination procedures. - Appropriate waste management of contaminated material - In case of subcutaneous administration clean injection site by swabbing with ethanol, apply wound dressing and instruct patient to discard the soiled dressing as biohazard waste. - If skin pock lesion develop, cover the lesion with a plaster or bandage and instruct patient to discard the soiled dressing as biohazard waste.
Inadvertent contamination of laboratory personnel, care keepers or close relatives of the patient with rMVA injected person	Risk of infection with potentially the same risk as for the treated patient	Case by case depending on the nature of the manipulation for example the threat is high for the personnel handling syringe with IMP	- Appropriate personal protective equipment to avoid infection. - Removal of needle in a hands free operation. - Avoid unnecessary manipulation of used medication vials.

[1]In line with the guidance notes of the European Commission [13] a potential hazard is defined as the characteristics of the rMVA and its use which may cause adverse effects on human health and the environment.
[2]Direct risks for the patient are not considered
[3]To scale the magnitude of the likelihood of occurrence or to characterise the risk the terminology proposed in the guidance notes of the European Commission is adopted [13]: "high", "moderate", "low", "negligible"
[4]Risk characterization: magnitude of consequences x likelihood of occurrence

2.3. Hazards Related to the Recombinant MVA Vector (rMVA)

The risk assessment of a recombinant viral vector should also take into account any potential risk associated with the transgene itself. While MVA vectors are generally classified in risk group 1 (see above), the presence of transgene(s) encoding potentially hazardous gene products (PHGPs), including cytokines, toxins or virulence factors could confer a higher risk group to the recombinant vector [28]. For instance several clinical trials notified in Belgium involved a MVA construct carrying genes coding for human mucine 1 antigen (MUC-1) and human interleukin 2 (IL-2). The presence of the MUC-1 carried by the rMVA is expected to induce a specific immune response against cancer cells carrying the MUC-1 antigen, whereas the IL-2 gene acts as an adjuvant in the immune response. However an IL2 expressing rMVA can induce a non-specific activation of the immune system possibly resulting in an inflammatory reaction or an autoimmune response. In previous trials adverse effects probably due to the expression of IL-2 were observed in treated patients. In addition potential autoimmune toxicity related to cross reactivity with natural MUC1 protein or other mucin proteins cannot be excluded. In this case, the insertion of genes coding for MUC-1 and IL-2 assigns the recombinant vector to risk class 2.

For one of the Belgian clinical trials, whereby a rMVA harbouring modified E6 and E7 genes of HPV was administered subcutaneously as a therapeutic vaccination in patients

suspected to be infected with HPV, the transgenes were assessed with more cautiousness with regard to their potential for recombination. The main concern for these rMVA treated patients was the possible recombination between the modified E6 and E7 genes of the rMVA, which had been modified to abolish their immortalizing capacities, with the wild-type E6 and E7 genes of HPV, thereby potentially recovering the oncogenic potential of E6 and E7.

Adverse effects associated with the transgene(s) represent not only a risk for the patient. The potential effect for personnel manipulating the rMVA and care keepers shall also be considered, especially in case of accidental parenteral inoculation (e.g. needle stick accident) or in case of contamination of broken skin or of mucous membranes of eye, nose or mouth. As previously mentioned, the identified risk can be kept low provided that appropriate personal protective equipment, proper hand hygiene and/or decontamination procedures are implemented to prevent accidental infection.

As described in section 2.2 it is important to investigate whether the modification of the parental MVA strain alters its virological safety profile. Issues of particular importance are the homogeneity and genetic stability of the viral preparation and potential for recombination and reconversion. The genetic stability and integrity of the transgene is one of the major concerns during the manufacturing process as viral vectors intended for clinical investigations must be amplified multiple times to reach the scale needed [18, 29]. These can be affected by several factors such as the choice of the promoter used in the construction of the recombinant vector [17]. Wyatt *et al.* also suggested that the insertion of the genes of interest between two essential vaccinia virus genes may avoid rMVA instability [18]. The genetic stability of the rMVA should therefore be confirmed by several passages (number that covers the passage from the Master Seed Virus to the Production Batch) at a low multiplicity of infection in chicken embryo fibroblast (CEF) cells and hybridisation with a DNA probe specific for the inserted gene. To further avoid unexpected adverse effects that could be associated with the presence of unintended sequences in the rMVA, sufficient information should be provided on the genetic construct, including the promoters (synthetic or not), and on the recombinant vector. This can be achieved by proper molecular characterization of the rMVA.

Because MVA is a highly attenuated strain that has lost approximately 15% of the initial vaccinia genome [4], risk of reconversion to wild-type is commonly accepted as negligible. This is due to six large deletions but also to a multitude of missing or partly functional gene products [30, 31], which cannot be rescued by naturally occurring wild-type viruses since smallpox has been eradicated and the existence of other human poxviruses is unlikely. As the repair of multiple genes is required to fully restore the ability of MVA to replicate efficiently in human cells spontaneous revertants are most unlikely [32, 33]. However, some of the disrupted or deleted genes could be rescued by recombination upon co-infection of a MVA-based vaccine and a naturally occurring homologous non-human OPV [34, 35]. Alternatively such recombination could result in the transfer of the transgene from the rMVA to a replication competent OPV. Although the probability of co-localization/co-infection of the same cells in the same host is very unlikely, the likelihood of recombination increases if epidemiological data confirm the occurrence of natural OPV in the area of administration. This risk must be evaluated in the context of the intended use of the vaccine and is of particular relevance when rMVA vaccine is developed for treatment of animals. Reconversion is less a concern in human clinical trials since no known human poxvirus are present in the human host that could complement MVA into replication competent virus. Hence potential risks associated to clinical trial activities are rather related to the escape of rMVA in the environment. Broadly speaking the risk can be mitigated by implementing appropriate measures to prevent spill, shedding and spreading of rMVA from the treated patient and by assuring proper treatment of waste.

Finally, as for each recombinant vector used in gene therapy, the potential integration of the vector's genome into patient's chromosomes is assessed since it may result in insertional mutagenesis and/or inadvertent regulation of neighbouring genes, with for example oncogenetic consequences. For MVA and recombinant variants this kind of risk is negligible as all poxviruses have a fully cytoplasmic cycle of propagation [6]. Hence, the possibility of integration of genetic material from the virus into the host chromosomes is very low. One could also mention that no increase of cancer incidence has been observed in large vaccination campaigns [7-9].

2.4. Exposure Pathways Through which the rMVA may Interact with Humans (other than Treated Patient) or the Environment

A critical step in the environmental risk assessment is evaluating pathways of exposure to the rMVA and its transgene products. There are several scenarios whereby personnel, non-patients and/or the environment may be exposed to a rMVA administered to humans enrolled in a clinical trial. Shedding, spreading, transmission to offspring, transmission to organ or blood recipients, accidental parenteral inoculation, contact with contaminated material or contact with spoiled surfaces are all means of potential exposure.

2.4.1. Dispersion to Other Tissues

Any kind of parenteral administration can potentially result in a dispersion of the recombinant vector through the whole body and can present a risk of germline transmission (with possible transmission to offspring).

Animal research performed with MVA on mice and macaques suggests that MVA is able to reach target tissues other than the site of administration. MVA is rapidly cleared from the tissues, which is consistent with the replication-defective properties of the MVA strain [19, 23, 36]. Only a few data about MVA biodistribution in humans are available in published literature and shedding studies have been rarely reported in publications on clinical trials or often have been limited to the first 2 weeks [37]. From our analysis of both published literature and application dossiers, we recommended to perform a mapping of the dissemination of the recombinant vector in different body fluids of the treated patients during early phases of each rMVA assay/clinical study. These tests need to be robust and have a reasonable limit of detection, e.g. an adapted and sensitive qPCR assay.

However, PCR assays will not allow the differentiation between intact vector and non-infectious or degraded vector. Therefore, *in vitro* cell culture assay with shed material on a permissive cell line would be complementary [38]. Ideally, these tests should be performed on a case-by-case basis, however results obtained with tests conducted with another MVA-based product administered at similar dose levels and via the same route of administration can be considered acceptable.

With regards to the possible dispersion of rMVA into the germ cells and hence transmission to offspring, it should be noted first that the occurrence of integration of genetic material from the rMVA into the host chromosome is considered negligible since MVA replicates only in cytoplasm of permissive non-human cells [25]. Moreover, transmission can be prevented by asking patients to use effective contraception during the study period and for several months after the last IMP administration. The rMVA could also be transmitted to other people by organ or blood donation with adverse consequences for the recipient depending on the characteristics of the vector and its transgene. The probability of occurrence increases when the clinical trial includes healthy volunteers. For this reason the patient or healthy volunteer should be excluded from blood or organ donation during the study period and for several months after the last IMP administration.

2.4.2. Unintentional Dissemination into the Environment

A possible dispersion of the recombinant vector through the whole body of the treated patient after parenteral inoculation should always be carefully considered since it can be a major source of (unintended) shedding of the vector (e.g. through patient's excreta such as saliva, blood or semen) with possible transmission to people in close contact with the patient

Upon subcutaneous administration some rMVA particles remain at the site of injection and are expected to be viable for a limited time since MVA is a propagation-defective vector that will induce cell death of the cells it transduces. There is increased risk of spreading when typical vaccinia skin pock lesions are formed (often observed in vaccinia virus-positive individuals) [20]. These types of dissemination can be easily prevented by decontamination (70% EtOH) of the site of administration immediately after injection and covering the administration site with a plaster or bandage. To prevent further unintentional spreading it is recommended to discard the contaminated dressing as biohazard waste. This pathway of dissemination is of less concern when inoculation of MVA is done via the intramuscular or intravenous route [15].

For personnel manipulating rMVA the primary hazards consist in droplet or aerosol exposure of mucous membrane or broken skin, and inadvertent parenteral inoculation (injury with needle stick or other sharp objects). Common to the handling of most viral vectors, the risk of cut and needle stick injury is the greatest for the personnel handling vials and or syringes during IMP reconstitutions and administration to the patient.

Accidental projection of the IMP into the eye or other mucous membrane, or unintentional contamination via close contact with MVA contaminated material are other threats for the personnel and/or care keepers. In addition, such bio-incidents could result in unintentional dissemination of the rMVA in the environment. In those cases the amount of viral particles released should be considered as well as the fact that the virus is poorly replicative, no longer able to generate infectious particles and non-integrative (see above).

The ways to prevent or manage risks associated with dissemination into the environment consist in proper hand hygiene and working practices, strict personal protective equipment, appropriate decontamination and waste procedures. This means that all potentially contaminated material needs to be properly decontaminated and disposed as biohazard material. Table **2** gives some concrete examples of good working practices which necessitates personnel trained and experienced in handling infectious material.

Table 2. Examples of Good Working Practices for Personnel Manipulating rMVA Vectors to Prevent or Manage Risks for People and/or the Environment.

- The puncture of the flask containing the vector with a needle is potentially a source of aerosolisation. The wearing of goggle and mask is mandatory unless the manipulation is carried out in a class II Biosafety Cabinet. The use of gloves is an absolute requirement to avoid any skin contamination.
- Removal of the syringe should occur by means of hands free operation (i.e. hands do not touch the needle) into a closed container.
- Skin contamination by a spill (patients or personnel) can be handled by placing an absorbent tissue on the affected area in order to absorb all viral particles. The disinfectant[1] should then directly be applied to the tissue. After removing this tissue the skin should be washed thoroughly.
- In case of contamination the eyes should be rinsed over a closed basin. Wash water should be collected for decontamination with active chlorine bleach before being released into the sewer system.
- Lab coats, goggles, patient gown and bedding or any other contaminated material should be systematically and adequately decontaminated or discarded and be disposed as biohazard material. When possible disposable material will be preferred.

[1]A list of efficient common active ingredients of disinfectants against vaccinia, their concentration and application time can be found in [27].

CONCLUSION

MVA vectors have a high safety profile. Nevertheless we have presented and discussed in this paper several biosafety issues which need to be considered carefully when performing clinical trials with rMVA, based on data published in the literature and on the evaluation of six trials notified in Belgium. Hazards related to the characteristics of the parental MVA virus or to the possible dispersion or dissemination of the rMVA should be addressed in an environmental risk assessment. Moreover, potential hazards related to the presence of the transgene warrants an assessment on a case-by-case basis. However, in most of the cases, the identified risks will be low or negligible and can be mitigated upstream by applying good manufacturing practices (GMP) and downstream by applying proper containment and workers protection meas-

ures with the primary aim of protecting people coming inadvertently or accidentally in contact with the recombinant virus.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

ACKNOWLEDGEMENTS

The authors thank Aline Baldo, Amaya Leunda and Bernadette Van Vaerenbergh (Scientific Institute of Public Health, Brussels, Belgium) for their useful contribution to this document. This work received support from the Brussels-Capital Region (IBGE-BIM), the Flemish Region (LNE) and Wallonia (DGARNE).

PATIENT CONSENT

Declared none.

REFERENCES

[1] Drexler I, Staib C, Sutter G. Modified vaccinia virus Ankara as antigen delivery system: how can we best use its potential? Curr Opin Biotechnol 2004; 15(6): 506-12.

[2] Gomez CE, Najera JL, Krupa M, Esteban M. The poxvirus vectors MVA and NYVAC as gene delivery systems for vaccination against infectious diseases and cancer. Curr Gene Ther 2008; 8(2): 97-120.

[3] Larocca C, Schlom J. Viral Vector-Based Therapeutic Cancer Vaccines. The Cancer J 2011; 17(5): 359-71.

[4] Meyer H, Sutter G, Mayr A. Mapping of deletions in the genome of the highly attenuated vaccinia virus MVA and their influence on virulence. J Gen Virol 1991; 72 (Pt 5): 1031-8.

[5] Verheust C, Goossens M, Pauwels K, Breyer D. Biosafety aspects of modified vaccinia virus Ankara (MVA)-based vectors used for gene therapy or vaccination. Vaccine 2012; 30(16): 2623-32.

[6] Moss B. Poxviridae: the virus and their repication. In: Knipe D & Howley P, Eds. Field's virology, 4. Lippincot Williams & Wilkins: Philadelphia 2001; pp. 2849-2883.

[7] Mayr A, Stickl H, Muller HK, Danner K, Singer H. The smallpox vaccination strain MVA: marker, genetic structure, experience gained with the parenteral vaccination and behavior in organisms with a debilitated defence mechanism (author's transl). Zentralbl Bakteriol B 1978; 167(5-6): 375-90.

[8] Stickl H, Hochstein-Mintzel V, Mayr A, Huber HC, Schafer H, Holzner A. MVA vaccination against smallpox: clinical tests with an attenuated live vaccinia virus strain (MVA) (author's transl). Dtsch Med Wochenschr 1974; 99(47): 2386-92.

[9] Mahnel H, Mayr A. Experiences with immunization against orthopox viruses of humans and animals using vaccine strain MVA (author's transl). Berl Munch Tierarztl Wochenschr 1994; 107(8): 253-6.

[10] Parrino J, Graham BS. Smallpox vaccines: Past, present, and future. J Allergy Clin Immunol 2006; 118(6): 1320-6.

[11] European Medicines Agency. Available at : http://www.ema.europa.eu/ema//index.jsp?curl=pages/medicines/human/medicines/002596/smops/Positive/human_smop_000518.jsp&mid=WC0b01ac058001d127. (accessed 26.07.13)

[12] Sneyers M, Goossens M. Approved gene therapy clinical trials in Belgium. Available at: http://www.biosafety.be/GT/Regulatory/Table_1.html (accessed 26.07.13)

[13] EC. Commission Decision 2002/623/EC of 24 July 2002 establishing guidance notes supplementing Annex II to Directive 2001/18/EC of the European Parliament and of the Council on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. Off J Eur Commun, L200 of 30.07.2002.

[14] Suter M, Meisinger-Henschel C, Tzatzaris M, *et al*. Modified vaccinia Ankara strains with identical coding sequences actually represent complex mixtures of viruses that determine the biological properties of each strain. Vaccine 2009; 27(52): 7442-50.

[15] Kennedy JS, Greenberg RN. IMVAMUNE: modified vaccinia Ankara strain as an attenuated smallpox vaccine. Expert Rev Vaccines 2009; 8(1): 13-24.

[16] Cottingham MG, Carroll MW. Recombinant MVA vaccines: dispelling the myths. Vaccine 2013; 4247-51

[17] Wang Z, Martinez J, Zhou W, *et al.* Modified H5 promoter improves stability of insert genes while maintaining immunogenicity during extended passage of genetically engineered MVA vaccines. Vaccine 2010; 28(6): 1547-57.

[18] Wyatt LS, Earl PL, Xiao W, *et al.* Elucidating and Minimizing the Loss by Recombinant Vaccinia Virus of Human Immunodeficiency Virus Gene Expression Resulting from Spontaneous Mutations and Positive Selection. J Virol 2009; 83(14): 7176-84.

[19] Ramirez JC, Gherardi MM, Esteban M. Biology of attenuated modified vaccinia virus Ankara recombinant vector in mice: virus fate and activation of B- and T-cell immune responses in comparison with the Western Reserve strain and advantages as a vaccine. J Virol 2000; 74(2): 923-33.

[20] Tan X, Chun S, Pablo J, Felgner P, Liang X, Davies DH. Failure of the smallpox vaccine to develop a skin lesion in vaccinia virus-naive individuals is related to differences in antibody profiles before vaccination, not after. Clin Vaccine Immunol 2012; 19(3): 418-28.

[21] Sparkes JD, Fenje P. The effect of residual moisture in lyophilized smallpox vaccine on its stability at different temperatures. Bull World Health Organ 1972; 46(6): 729-34.

[22] Drumond BP, Leite JA, da Fonseca FG, Bonjardim CA, Ferreira PC, Kroon E. Brazilian Vaccinia virus strains are genetically divergent and differ from the Lister vaccine strain. Microbes and Infection 2008; 10(2): 185-97.

[23] Stittelaar KJ, Kuiken T, de Swart RL, *et al.* Safety of modified vaccinia virus Ankara (MVA) in immune-suppressed macaques. Vaccine 2001; 19(27): 3700-9.

[24] Okeke MI, Nilssen O, Traavik T. Modified vaccinia virus Ankara multiplies in rat IEC-6 cells and limited production of mature virions occurs in other mammalian cell lines. J Gen Virol 2006; 87(Pt 1): 21-7.

[25] Hanke T, McMichael AJ, Dennis MJ, *et al.* Biodistribution and persistence of an MVA-vectored candidate HIV vaccine in SIV-infected rhesus macaques and SCID mice. Vaccine 2005; 23(12): 1507-14.

[26] Hanke T, McMichael AJ, Samuel RV, *et al.* Lack of toxicity and persistence in the mouse associated with administration of candidate DNA- and modified vaccinia virus Ankara (MVA)-based HIV vaccines for Kenya. Vaccine 2002; 21(1-2): 108-14.

[27] v.Rheinbaben F, Gebel J, Exner M, & Schmidt M. Environmental resistance, disinfection, and sterilization of poxviruses. In: A.A.Mercer, A.Schmidt, & O.Weber, Eds. Poxviruses. Birkhäuser Verlag Basel/Switzerland: 2007; pp. 397-405.

[28] Bergmans H, Logie C, Van Maanen K, Hermsen H, Meredyth M, Van der Vlugt C. Identification of potentially hazardous human gene products in GMO risk assessment. Environ Biosafety Res 2008; 71-9.

[29] Earl PL, Cotter C, Moss B, *et al.* Design and evaluation of multi-gene, multi-clade HIV-1 MVA vaccines. Vaccine 2009; 27(42): 5885-95.

[30] Meisinger-Henschel C, Schmidt M, Lukassen S, Linke B, Krause L, Konietzny S, Goesmann A, Howley P, Chaplin P, Suter M, Hausmann J. Genomic sequence of chorioallantois vaccinia virus Ankara, the ancestor of modified vaccinia virus Ankara. J Gen Virol 2007; 88(Pt 12): 3249-59.

[31] Meisinger-Henschel C, Spath M, Lukassen S, *et al.* Introduction of the six major genomic deletions of modified vaccinia virus Ankara (MVA) into the parental vaccinia virus is not sufficient to reproduce an MVA-like phenotype in cell culture and in mice. J Virol 2010; 84(19): 9907-19.

[32] Carroll MW, Moss B. Host range and cytopathogenicity of the highly attenuated MVA strain of vaccinia virus: propagation and generation of recombinant viruses in a nonhuman mammalian cell line. Virology 1997; 238(2): 198-211.

[33] Wyatt LS, Carroll MW, Czerny CP, Merchlinsky M, Sisler JR, Moss B. Marker Rescue of the Host Range Restriction Defects of Modified Vaccinia Virus Ankara. Virology 1998; 251(2): 334-42.

[34] Hansen H, Okeke MI, Nilssen O, Traavik T. Recombinant viruses obtained from co-infection *in vitro* with a live vaccinia-vectored influenza vaccine and a naturally occurring cowpox virus display different plaque phenotypes and loss of the transgene. Vaccine 2004; 23(4): 499-506.

[35] Okeke MI, Nilssen O, Moens U, Tryland M, Traavik T. *In vitro* host range, multiplication and virion forms of recombinant viruses obtained from co-infection *in vitro* with a vaccinia-vectored influenza vaccine and a naturally occurring cowpox virus isolate. Virol J 2009; 6: 55.

[36] Gomez CE, Najera JL, Domingo-Gil E, Ochoa-Callejero L, Gonzalez-Aseguinolaza G, Esteban M. Virus distribution of the attenuated MVA and NYVAC poxvirus strains in mice. J Gen Virol 2007; 88(Pt 9): 2473-8.

[37] Schenk-Braat EA, van Mierlo MM, Wagemaker G, Bangma CH, Kaptein LC. An inventory of shedding data from clinical gene therapy trials. J Gene Med 2007; 9(10): 910-21.

[38] European Medicines Agency. ICH Considerations : General principles to address virus and vector shedding. EMEA/CHMP/ ICH/449035/2009.

Received: July 30, 2013 Revised: September 18, 2013 Accepted: September 18, 2013