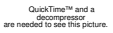


African Centre for Biosafety
Suite 3, 12 Clamart Street
Richmond, 2192 South Africa
http://www.biosafetyafrica.net
Reference: Mariam Mayet
mariammayet@mweb.co.za
083 269 4309

# INDEPENDENT EXPERT BIOSAFETY EVALUATION OF THE APPLICATION MADE FOR TRIAL RELEASE OF GENETICALLY ENGINEERED SAAVI MVA-C MULTIGENE HIV VACCCINE

## SUBMITTED BY THE ACB TO

## THE REGISTRAR: GENETICALLY MODIFIED ORGANISMS ACT

# NATIONAL DEPARTMENT OF AGRICULTULTURE, 25 MARCH 2008

## TABLE OF CONTENTS

Note on terminiology: In this document, we use the term GM and GE interchangeably, to refer to either recombinant DNA technology or the product of such technology, viz. recombinant DNA resulting from the fusion of the source DNA and the host DNA.

## 1. CONTEXT

The African Centre for Biosafety is a non-profit, activist NGO based in South Africa, focussing on biopolitics, including inter alia, biosafety, biopiracy, biofuels and challenging industrial agriculture models in Africa and the commodification of genetic resources and associated traditional knowledge.

The African Centre for Biosafety (ACB) has a long track record of engaging with a wide range of applications involving numerous GMOs for different uses or aciviities in South Africa. This engagement has principally been in the field of GMOs in food and agriculture. However, we have recently engaged with a GM poultry vaccine and during 2006, the ACB submitted a series of comprehensive comments/biosafety concerns in response to an appliation for trial release of pharmacuetical giant, Merck's GM HIV vaccine (MRKad5 HIV-1 gap/po/nef).

It is unknown to us, what in fact transpired with the numeorus biosafety concerns we raised in regard to the said GM HIV vaccine, since the office of the Registrar did not communicate with us at all in this regard. Indeed, it only recently transpired that the applicant, Professor Glenda Gray, had submitted a lengthy response to the concerns raised by us. Consequently, and after numerous persistent requests by us, only then did the office of the Register relent, and share with us, Professor Gray's response. By this time, not only had the trial been rolled out around the country, but it had already been brought to an abrupt standstill (September 2007) because similar trials conducted in the US and Astralia failed to prevent HIV infection. In other words, the GE vaccine failed to 'do its job.'

We are on record for explicitly supporting the urgency to address the HIV-AIDS pandemic. In this regard, we recognise that a range of medical interventions and research approaches must be explored. However, this does not mean that groups like us who have been

involved in the biosafety discourse for several years now, have forfeited our rights to raise biosafety concerns.

We regard it as the exercise of our democratic right, to contribute raising biosafety standards and public awareness and in so doing, ensuring that GM vaccines do not pose harm to human health and the environment. This role is clearly contemplated and encouraged by the United Nation's Cartagena Protocol on Biosafety, to which South Africa is a Party, as well as being guaranteed by our Constitution and a range of environmental policy tools.

It is in this spirit, that we submit the biosafety concerns contained in this document, in respect to the application for clinical trials of the SAAVI MVA-C multigene HIV vaccine. We trust that we will be accorded the same respect and regard that we have shown to the applicant, the office of the Registrar and a range of role players involved.

## 2. APPLICATION DETAILS

| | |
|---|---|
| Applicant: | Professor Glenda Gray, Director of the Perinatal HIV research Unit, Chris Hani Baragwanath Hospital |
| Application for: | Trial release of genetically modified organism (GMO) MVA-C multigene HIV vaccine in Johannesburg and Cape Town, South Africa. |
| Title: | A phase 1 placebo-controlled clinical trial to evaluate the safety and immunogenicity of SAAVI (South African Aids Vaccine Iniative) DNA-C2 vaccine (non-GMO) boosted by SAAVI MVA-C vaccine (GMO) in HIV uninfected, healthy, adult participants in South Africa. |
| GMO Constuct: | SAAVI MVA-C is a multigene construct, comprising of HIV-1 subtype C recombinant modified vaccinia Ankara (MVA) virus vaccine, expressing Gag-RT-Tat-Nef (grrnC) and gp150CT). |

Participants: Healthy HIV-1 uninfected vaccinia virus naïve participants (18-45 years), at low risk of HIV aqcuiistion. 'Vaccine virus naïve' is described in application (p.16) as 'have never received any small pox vaccination.' In total, 36 participants will be enrolled in South Africa, 18 in Cape Town and 18 in the Johannebug metropolitan area.

Primary objective: (a) To evaluate the safety and tolerability of IM administration of SAAVI DNA-C2 vaccine followed by SAAVI MVA-C vaccine, as a prime-booost regimen in healthy, vaccinia naïve adults. (b) To evaluate the immunogenicity of IM administration of SAAVI DNA-C2 follwed by SAAVI MVA-C vaccine, as a prime-boost regimen in healthy, vaccinia naïve adults.

Duration of trial: The trial is estimated to last between 12 and 24 months.

Safety monitoring: The trial will be monitored by the Protocol Safety Review Team and the HVTN Safety Monitoring Board (SMB). The SMB is an independent panel of experts established by the National Insitue of Allergy and Infectious Disease (NIAID). All data on safety is to be reported to the Medical Controls Council and the Institutional Review Boards.

Vaccine providers: National Institute of Allergy and Infectious Disease (NIAID); Division of AIDS (DAIDS); National Institutes of Health (NIH); Deparment of Health and Human Services (DHHS), Bethesda, Maryland, USA.

Vaccine developers: South African AIDS Vaccine initiative (SAAVI), Medical Research Council of South Africa (MRC-SA); University of Cape Town, Therion Biologics Corporation formerly of Cambridge, Ma, USA. [Therion Biologics is no longer a manufacturing facility (pp.17-18 of application), the facility closed in November 2006. Before the closure, however, all of the relevent

protocols manufacturing and testing records relating to the development, production and testing were transferred to Advanced Bioscience Laboratories (ABL) in Kensington, MD USA.]

## 3. ACCESS TO INFORMATION

In response to the ACBs' application for access to the application and risk assessment in terms of the Promotion of Access to Information Act (PAIA), we have been furnished with a non-CBI (confidential business information) version of the application for the trial release/clinial trial comprising 36 pages, including references, and a non-CBI version of the application for authorisation to import the said GM HIV vaccine from the USA, comprising 9 pages.

## 4. BACKGROUND TO THE APPLICATION

### *4.1 The GE vaccine.*

The SAAVI MVA-C vaccine is an HIV-1 subtype c recombinant MVA (modified vaccinia Ankara) vaccine. The MVA virus utilised for generating SAAVI MVA-C was derived from the Ankara vaccinia virus strain chorioallantos vaccina ankara (CVA). (p5 of application). The SAAVI MVA-C is a multigene vaccine, comprising of five different HIV genes, (gag, RT, tat, nef, env). The genes included in SAAVI MVA-C were derived from two HIV-1 subtype C strains Du151 and Du422 that were selected based on similarity to a South African concensus sequence. According to the application, the genes in SAAVI MVA-C have been modified for safety, increased expression and stability (p.6).

### *4.2 The vaccine trial*

It is propsed by the applicant that the clinical research sites in South Africa will include the folllowing:

Cape Town:

(a) Desmond Tutu HIV Cenre, Institute of Infectious Diseases & Molecular Medicine; and
(b) Desmond Tutu HIV Vaccine Centre, Emavundleni Centre for Vaccine Research

Johannesburg:

Perinatal HIV Research Unit, Chris Hani Baragwanath Hospital, Soweto

36 (18 from JHB and 18 from CT) Healthy HIV-1 uninfected participants between the ages of 18 and 45 who are vaccinia naïve will be invited to partake in the clininal trial at one of the three clinincal trial sites. According to the application (p.16), only potential participants that agree to partake via an informed consent process and that meet the enrolment criteria will join the study. The participants will receive the vaccination whilst at the clinic under the supervision of the investigator/medical officer at the relevant trial site.

The trial is estimated to last between 12 and 24 months for most recipients. According to the application, (p.2), in the event of any participant becoming infected with HIV-1 on the trial, they will be followed for a further 72 weeks after diagnosis within the trial. All persons who became HIV infected on this trial will be enrolled in a long term follow up protocol (HVTN 803) after the completion of the trial.

## 5. BIOSAFETY EVALUTION OF APPLICATION AND BIOSAFETY CONCERNS

The ACB has been assisted by an independent, world renown biosafety expert in the field, who has made a biosafety evaluation of the application, based on his own extensive biosafety research and peer reviewed publications.

At the outset, we note that if granted, this would be the first clinical trial featuring a MVA-C vaccine, involving multiple genes. Whereas a

previous MVA HIV vaccine trial (Triclinium) has been approved by the Executive Council, Genetically Modified Organisms Act, this vaccine was derived from a different clade, and only had a single gene insert. (p26 of application).

### *5.1 Recombinant poxviruses as vaccine vectors*

Poxvirus vectors are widely used for efficient expression of transgenes (Moss, 1996), and as candidate vaccines against infectious diseases and cancers (Drexler et al., 2004; Gherardi and Esteban, 2005; Moroziewicz and Kaufman, 2005; Shen and Nemunaitis, 2005). Poxvirus vectors have special advantages for use as live vaccine vectors and these include wide host range, cytoplasmic site of replication, resistance to heat/environmental stress, ability to carry large transgenes (up to 25 Kbp) and express them with appropriate post-transcriptional modification, and the ease of construction of recombinant viruses (Moss, 1996; Perkus et al., 1995; Smith and Moss, 1983). The efficiency and efficacy of poxvirus-vectored vaccines are dependent on the nature of the promoter sequence, site of transgene insertion, route of inoculation, anti-vector immunity, dosage, prime-boost regimen, and the animal model used (Coupar et al., 2000; Schneider et al., 1998; Schneider et al., 2001; Weidinger et al., 2001). This cocktail of factors complicates the interpretation of many studies and extrapolation of results obtained *in vitro* and in animal models to humans may be misleading (Bejon et al., 2006). Although several VACV-vectored (vaccinia virus vectored) candidate vaccines are currently being evaluated, only VACV-vectored rabies vaccine (RGV) has been extensively used in the field (Kieny et al., 1984). RGV was successfully used to eradicate rabies from foxes in Europe and no safety problems were reported (Boulanger et al., 1995; Pastoret et al., 1995). However, the use of replication competent VACV strains as vaccine vectors is disfavored because of safety concerns including post-vaccination complications (Casey et al., 2005; Fenner et al., 1988), and accidental or non-target human infections (Mempel et al., 2003; Rupprecht et al., 2001).

### *5.2 Genus orthopoxvirus (OPVs)*

VACV, and hence also MVA, belongs to the genus orthopoxvirus, within the family *Poxviridae.* Members of this genus are arbitrarily

divided into Old World and North American OPVs respectively (Li et al., 2007). Species within the Old World OPVs are CPXV (cowpox virus), VACV (vaccinia virus), VARV (variola virus), MPXV (monkeypox virus), ECTV (ectromelia virus), CMLV (camelpox virus), TATV (Taterapox virus) and Horsepox virus (HPXV) (www.poxvirus.org). The North American OPVs are raccoon poxvirus (RCNV), vole poxvirus (VPXV), and skunk poxvirus (SKPV). The Old World OPVs share greater than 95% nucleotide sequence identity with each other, and its sequence identity with North American OPVs is about 75% (Esposito and Fenner, 2001; Moss, 2001). Also North American OPVs are about 75% identical to each other. It may be safely assumed that a large number of OPVs have not been detected yet, since very few systematic studies have been performed anywhere in the world.

In order to assess the possibilities of poxvirus-vectored vaccines to engage in recombinations with naturally occurring OPVs, the occurrence of such viruses in any given area must be known (Sandvik et al, 1998; Traavik, 2002).

### *5.3 Attenuated poxvirus vectors*

Biosafety concerns regarding the use of replication competent VACV strains as vaccine vectors have been addressed in part by the development of highly attenuated strains. The attenuated strains including MVA, NYVAC and dVV-L are assumed not to multiply in most mammalian cells (Parrino and Graham, 2006; Pastoret and Vanderplasschen, 2003). MVA was derived from VACV Ankara (VACV-CVA), by over 570 serial passages in CEF. This resulted in the deletion of 15% of the parental VACV-CVA genome (Antoine et al., 1998; Meyer et al., 1991), and several genes encoding for immunomoderators or host range factors were either lost or fragmented (Antoine et al., 1998; Blanchard et al., 1998). MVA is highly attenuated. It has been assumed that it undergoes abortive infection in most mammalian cells (Blanchard et al., 1998; Carroll and Moss, 1997; Drexler et al., 1998), and is apathogenic even in immune deficient animals or individuals (Dorrell et al., 2007; Hanke et al., 2005). Compared to replication competent VACV strains, MVA seems to induce lower level of anti-vector immunity (Ramirez et al., 2000). Thus MVA is a very promising viral vector because of its attenuation and immunogenicity (Drexler et al., 2004). Currently, several MVA vectored vaccines against infectious diseases and malignancies are at

various phases of field and clinical trials (Drexler et al., 2004; Hanke et al., 2007). Although recombinant MVA vaccines showed promising results in animal models, they proved disappointing in many human clinical trials (Bejon et al., 2006; Bejon et al., 2007; Mwau et al., 2004; Smith et al., 2005)

### *5.4 Construction of recombinant MVA*

Recombinant MVA can be created by homologous recombination, *in vitro* ligation, recombination upon transformation into bacteria and SFV mediated recombination (Guo and Bartlett, 2004). However, homologous recombination is the method of choice used in engineering recombinant MVA (Drexler et al., 2004; Staib et al., 2004). In homologous recombination, permissive cells infected with MVA are transfected with a plasmid transfer vector carrying an expression cassette. The expression cassette consists of one or multiple transgenes placed under the control of VACV specific promoter (VV-P) and flanking MVA DNA sequences that direct recombination to the desired locus. The sites of naturally occurring deletions within the MVA genome, the thymidine kinase (*TK*) and hemagluttinin (*HA*) gene loci serve as sites for insertion of transgenes.

### *5.5 MVA-related Biosafety issues.*

Host range restriction of MVA in mammalian cells is considered the major biosafety advantage for its use as a vaccine vector (Drexler et al., 2004). MVA does assumedly not produce mature virions in mammalian cells except in BHK-21 cells. Virus assembly in studied host cells have appeared restricted to immature virus stages (Carroll and Moss, 1997). These conclusions have been based on the mammalian cell lines studied so far. But only a restricted number of mammalian cell lines and types have been evaluated for MVA multiplication and morphogenesis. It is not inconceivable that other mammalian cell lines and types will support productive MVA infection (see below). In addition, MVA host restriction in some mammalian cells may be leaky since limited increase in infectious virus titre and mature virion production have been observed in at least two human cell lines (Blanchard et al., 1998; Gallego-Gomez et al., 2003). Therefore, continued evaluation of MVA morphogenesis in many mammalian cell types is essential for understanding the extent of MVA restriction in mammalian cells. The effect of transgene inserted into an MVA

vector on the virus and the host cell need to be evaluated for all transgenic MVA vaccines. Transgenic MVAs have in some cases different phenotypic properties compared to the wild type virus (Weingartl et al., 2004; Zhang et al., 2007). Therefore, it is important to compare candidate transgenic MVA vaccines with the wild type virus. Potentially, this will help in identifying favorable or undesirable changes to the phenotypic properties of recombinant MVA.

The potential widespread use of transgenic MVA as vaccines even in immunocompromised individuals raises the possibility of recombination between transgenic MVA and naturally occurring OPVs during mixed infection. Although recombination between OPVs during dual infection is well known (Ball, 1987; Chernos et al., 1985), recombination between a candidate MVA-vectored vaccine and wild type OPV has only quite recently been reported (Hansen et al., 2004). Recombination between a transgenic MVA vaccine and a naturally circulating OPV has the potential of generating hybrid viruses with novel genetic and biological properties. The stochastic nature of poxvirus recombination in tandem with the fact that recombination is linked with transposition accentuates the chance of obtaining novel hybrid viruses. Indeed, the generation of MRV from SFV and MYXV was a coupled recombination-transposition event (Block et al., 1985). Examination of the potential for recombination between MVA-vectored vaccines and wild type OPVs, and characterizing progeny hybrid viruses will help in establishing guidelines for the risk assessment of transgenic MVA vaccines. Recombination between transgenic MVA and wild type CPXV can also result in the appearance of an atypical phenotype in the progeny viruses that is not present in the parental viruses.

### *5.6 Are Mammalian cells really non-permissive to MVA multiplication?*

The continued evaluation of MVA multiplication in several mammalian cell types is germane for increased understanding of MVA host restriction. A recent study (Okeke et al., 2006) compared the multiplication and morphogenesis of wild type and transgenic MVA strains in BHK-21 cells and 12 other mammalian cell lines. The transgenic MVA strain (MVA-HANP) contains the influenza virus hemagglutinin (HA) and nucleoprotein (NP) cDNA inserts. The MVA strains multiplied efficiently in rat small intestinal IEC-6 cells with

infectious virus titres that are similar to what was obtained in BHK-21 cells. MVA-HANP had diminished multiplication in permissive cell lines compared to the non-recombinant MVA strain. The restriction of virus morphogenesis to immature virus stages in supposedly semi or non-permissive cell lines was leaky since infectious mature virions were produced. Non-infectious dense particles that are often associated with cisternae were produced in abundance in semi or non-permissive cell lines, and to a lesser degree in permissive IEC-6 and BHK-21 cells. Collectively, the data demonstrated that rat IEC-6 cells is very permissive to MVA infection, and that infectious mature virions are produced in some mammalian cell lines, included human ones, previously considered as semi or non-permissive to MVA infection. These results are pertinent to the production and biosafety of MVA vectored vaccines.

### *5.7 Can MVA engage in recombination events with naturally occurring orthopoxviruses?*

Homologous recombination between poxvirus-vectored vaccines and wild type poxviruses during co-infection is a potential biosafety problem. A recent study (Hansen et al., 2004) examined recombination between a transgenic poxvirus and a naturally occurring relative by dual infection of cell cultures with MVA-vectored influenza vaccine (MVA-HANP), and a Norwegian cowpox virus isolate (No-H1). Progeny hybrid viruses were isolated based on the expression of the influenza virus hemagglutinin (HA) protein and the ability to form plaques in Vero cells. Three hybrid viruses with different plaque phenotypes were isolated, plaque purified and genotyped by multiplex PCR, restriction fragment length polymorphism (RFLP), and southern blotting. The data showed that recombination occurred between central and flanking parts of the genome. Apart from recombination, other gross genetic changes including duplication, transposition, addition and deletion were present in the genome of hybrid progenies. The influenza virus *HA* transgene in one of the hybrid viruses was deleted at high frequency giving rise to HA negative viruses. The loss of the transgene is significant with regard to risk assessment of poxvirus-vectored vaccines since the transgene is the genetic marker for tracking the spread and non-target transfer of transgenic poxviruses. The results emphasized the need to include potential recombination events between genetically modified and naturally occurring poxviruses in routine risk assessment.

Hence, double infection and subsequent recombination between transgenic MVA and wild type poxviruses have the potential of generating hybrid viruses with novel properties. In further studies (Okeke, 2007; Okeke et al., 2008), the same authors described in more detail the biological characteristics of recombinant viruses obtained from *in vitro* co-infection of a MVA vectored influenza vaccine (MVA-HANP) and a wild type Norwegian cowpox virus isolate (CPXV-NOH1). The tropism of progeny hybrid viruses in many mammalian cell lines is similar to CPXV-NOH1, but not MVA-HANP. MVA-HANP and one hybrid virus displayed comet formation in permissive cell lines. The loss of the influenza virus *HA* transgene in a hybrid virus gave rise to HA negative viruses with increased plaque size, multiplication and cytopathic effect (CPE) in mammalian cell lines compared to the HA positive progenitor virus strain. Serial passage of MVA-HANP and HA positive hybrid viruses in mammalian cell lines showed that the *HA* transgene or its phenotype was unstable in rat IEC-6 cells but stable in Vero cells, although there was no significant variation in virus multiplication in both cell lines. There were differences in mature virus forms produced by parental and progeny hybrid virus strains. Transgene negative virus strains were very efficient in producing enveloped virions while their transgene positive progenitor virus strain was highly inefficient in producing enwrapped virions.

## *5.8 What is the relevance of all this to the application for intentional introduction of the SAAVI MVA-C vaccine into the environment of South Africa?*

### 1. Multiplication of transgenic and non-transgenic MVA in human and other mammalian cells.

As opposed to repeated claims made in the application, it has been shown that MVA may carry out fully productive infections in a number of cell types originating from different human and mammalian organs. These investigations have not by far been performed in a complete and systematic way. Some of these cell types may be relevant to the natural portals of entry, the large mucosal membranes of the body. It has been shown that MVA may infect and initiate immunological reactions following uptake through the gastrointestinal tract. Hence, if vaccinated humans shed transgenic MVA, there are unclarified possibilities for infection of unvaccinated humans, domestic animals and wildlife mammals. Whether such events may at all take place is

dependent on the nature of contacts between vaccinated individuals and potential recipients. In these connections, no directly applicable research related to SAAVI MVA-C vaccine seems to have been performed.

**2. Stability of transgenic MVA, loss of transgene(s), monitoring, vaccine efficacy.**

As opposed to claims made in the application the vaccine transgenes of MVA may be removed after varying rounds of multiplication, and with different efficiency, in mammalian cells. Since the application names the transgene(s) as the target(s) of viral spread monitoring, such events may preclude the monitoring. Furthermore, such events may render the immunological responses to the vaccine inefficient. As far as we can see, no efforts have been done to elucidate whether such scenarios are applicable to the SAAVI MVA-C vaccine.

**3. Recombination between transgenic MVA and naturally occurring orthopoxviruses.**

This possibility is, as far as we can see, not at all considered in the application. The probabilities of such events taking place, is dependent on the occurrence of orthopoxviruses in domestic animals and wildlife in South Africa, and whether MVA-shedding, vaccinated individuals may have contact with orthopox virus reservoir animals. To our knowledge, the occurrence and distribution of naturally occurring orthopoxviruses in South Africa is totally unknown.

**4. Selection of participants in the SAAVI MVA-C trial: "Vaccinia virus naïve persons".**

If any of the participants have been earlier infected with a naturally occurring orthopoxvirus they may have antibodies cross reacting with MVA, and will hence not be "vaccinia virus naïve".

### 5. What kind of immune responses?

The application does not describe how the functional aspects of immune responses will be analyzed. The antibodies and specific T cells obtained following the prime-boost regime should be tested for *in vitro* HIV neutralizing and cytotoxic activities, respectively.

## 6. RECOMMENDATIONS

(a) Clinical trials with MVA-C vaccine should not be initiated until the risk related questions summarized in paragraph 5.8, points 1-5 have found satisfactory scientific answers.
(b) South African authorities are urged to make funding available for such studies, including funding for detection and mapping of orthopoxviruses in domestic animals and wildlife.
(c) Until questions related to issues 1-5 in the biosafety evaluation have found satisfactory research-based answers, a moratorium should be put on MVA vectored vaccines in South Africa. The moratorium may be lifted when it is accepted that satisfactory answers have been delivered.

## 7. REFERENCES

Antoine, G., Scheiflinger, F., Dorner, F. and Falkner, F.G. (1998) The complete genomic sequence of the modified vaccinia Ankara strain: comparison with other orthopoxviruses. Virology 244(2), 365-96.

Ball, L.A. (1987) High-frequency homologous recombination in vaccinia virus DNA. J Virol 61(6), 1788-95.

Bejon, P., Mwacharo, J., Kai, O., Mwangi, T., Milligan, P., Todryk, S., Keating, S., Lang, T., Lowe, B., Gikonyo, C., Molyneux, C., Fegan, G., Gilbert, S.C., Peshu, N., Marsh, K. and Hill, A.V. (2006) A Phase 2b Randomised Trial of the Candidate Malaria Vaccines FP9 ME-TRAP and MVA ME-TRAP among Children in Kenya. PLoS Clin Trials 1(6), e29.

Bejon, P., Ogada, E., Mwangi, T., Milligan, P., Lang, T., Fegan, G., Gilbert, S.C., Peshu, N., Marsh, K. and Hill, A.V. (2007) Extended follow-up following a phase 2b randomized trial of the candidate malaria vaccines FP9 ME-TRAP and MVA ME-TRAP among children in Kenya. PLoS ONE 2(1), e707.

Blanchard, T.J., Alcami, A., Andrea, P. and Smith, G.L. (1998) Modified vaccinia virus Ankara undergoes limited replication in human cells and lacks several immunomodulatory proteins: implications for use as a human vaccine. J Gen Virol 79 ( Pt 5), 1159-67.

Block, W., Upton, C. and McFadden, G. (1985) Tumorigenic poxviruses: genomic organization of malignant rabbit virus, a recombinant between Shope fibroma virus and myxoma virus. Virology 140(1), 113-24.

Boulanger, D., Brochier, B., Crouch, A., Bennett, M., Gaskell, R.M., Baxby, D. and Pastoret, P.P. (1995) Comparison of the susceptibility of the red fox (Vulpes vulpes) to a vaccinia-rabies recombinant virus and to cowpox virus. Vaccine 13(2), 215-9.

Carroll, M.W. and Moss, B. (1995) E. coli beta-glucuronidase (GUS) as a marker for recombinant vaccinia viruses. Biotechniques 19(3), 352-4, 356.

Carroll, M.W. and Moss, B. (1997) Host range and cytopathogenicity of the highly attenuated MVA strain of vaccinia virus: propagation and generation of recombinant viruses in a

Casey, C.G., Iskander, J.K., Roper, M.H., Mast, E.E., Wen, X.J., Torok, T.J., Chapman, L.E., Swerdlow, D.L., Morgan, J., Heffelfinger, J.D., Vitek, C., Reef, S.E., Hasbrouck, L.M., Damon, I., Neff, L., Vellozzi, C., McCauley, M., Strikas, R.A. and Mootrey, G. (2005) Adverse events associated with smallpox vaccination in the United States, January-October 2003. Jama 294(21), 2734-43.

Chernos, V.I., Antonova, T.P. and Senkevich, T.G. (1985) Recombinants between vaccinia and ectromelia viruses bearing the specific pathogenicity markers of both parents. J Gen Virol 66 ( Pt 3), 621-6.

Coupar, B.E., Oke, P.G. and Andrew, M.E. (2000) Insertion sites for recombinant vaccinia virus construction: effects on expression of a foreign protein. J Gen Virol 81(Pt 2), 431-9.

Dorrell, L., Williams, P., Suttill, A., Brown, D., Roberts, J., Conlon, C., Hanke, T. and McMichael, A. (2007) Safety and tolerability of recombinant modified vaccinia virus Ankara expressing an HIV-1 gag/multiepitope immunogen (MVA.HIVA) in HIV-1-infected persons receiving combination antiretroviral therapy. Vaccine 25(17), 3277-83.

Drexler, I., Heller, K., Wahren, B., Erfle, V. and Sutter, G. (1998) Highly attenuated modified vaccinia virus Ankara replicates in baby

hamster kidney cells, a potential host for virus propagation, but not in various human transformed and primary cells. J Gen Virol 79 ( Pt 2), 347-52.

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

Esposito, J.J. and Fenner, F. (2001) Poxviruses, 2885-2921 pp. Fields Virology, 4th ed., edited by B. Roizman, Howley, P., Straus, S., Martin, M., DE, G., Lamb, R., Knipe, D. Lippincott Williams and Wilkins, Philadelphia.

Fenner, F., Henderson, D.A., Arita, I., Jezek, Z., and and Ladnyi, I.D. (1988) Smallpox and its eradication. World Health Organization, Geneva.

Gallego-Gomez, J.C., Risco, C., Rodriguez, D., Cabezas, P., Guerra, S., Carrascosa, J.L. and Esteban, M. (2003) Differences in virus-induced cell morphology and in virus maturation between MVA and other strains (WR, Ankara, and NYCBH) of vaccinia virus in infected human cells. J Virol 77(19), 10606-22.

Gherardi, M.M. and Esteban, M. (2005) Recombinant poxviruses as mucosal vaccine vectors. J Gen Virol 86(Pt 11), 2925-36.

Guo, Z.S. and Bartlett, D.L. (2004) Vaccinia as a vector for gene delivery. Expert Opin Biol Ther 4(6), 901-17.

Hanke, T., McMichael, A.J., Dennis, M.J., Sharpe, S.A., Powell, L.A., McLoughlin, L. and Crome, S.J. (2005) Biodistribution and persistence of an MVA-vectored candidate HIV vaccine in SIV-infected rhesus macaques and SCID mice. Vaccine 23(12), 1507-14.

Hanke, T., McMichael, A.J. and Dorrell, L. (2007) Clinical experience with plasmid DNA- and modified vaccinia virus Ankara-vectored human immunodeficiency virus type 1 clade A vaccine focusing on T-cell induction. J Gen Virol 88(Pt 1), 1-12.

Hansen, H., Okeke, M.I., Nilssen, Ø., and Traavik, T. 2004. 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 the loss of the transgene. *Vaccine* 23, 499-506.

Kieny, M.P., Lathe, R., Drillien, R., Spehner, D., Skory, S., Schmitt, D., Wiktor, T., Koprowski, H. and Lecocq, J.P. (1984) Expression of rabies virus glycoprotein from a recombinant vaccinia virus. Nature 312(5990), 163-6.

Li, Y., Ropp, S.L., Zhao, H., Damon, I.K. and Esposito, J.J. (2007) Orthopoxvirus pan-genomic DNA assay. J Virol Methods 141(2), 154-65.

Mempel, M., Isa, G., Klugbauer, N., Meyer, H., Wildi, G., Ring, J., Hofmann, F. and Hofmann, H. (2003) Laboratory acquired infection with recombinant vaccinia virus containing an immunomodulating construct. J Invest Dermatol 120(3), 356-8.

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

Moroziewicz, D. and Kaufman, H.L. (2005) Gene therapy with poxvirus vectors. Curr Opin Mol Ther 7(4), 317-25.

Moss, B. (1996) Genetically engineered poxviruses for recombinant gene expression, vaccination, and safety. Proc Natl Acad Sci U S A 93(21), 11341-8.

Moss, B. (2001) Poxviridae: The Viruses and Their Replication, 2849-2883 pp. Fields Virology, 4th ed., edited by B. Roizman, Howley, P., Straus, S., Martin, M., DE, G., Lamb, R. and Knipe, D. Lippincott Williams and Wilkins, Philadelphia.

Mwau, M., Cebere, I., Sutton, J., Chikoti, P., Winstone, N., Wee, E.G., Beattie, T., Chen, Y.H., Dorrell, L., McShane, H., Schmidt, C., Brooks, M., Patel, S., Roberts, J., Conlon, C., Rowland-Jones, S.L., Bwayo, J.J., McMichael, A.J. and Hanke, T. (2004) A human immunodeficiency virus 1 (HIV-1) clade A vaccine in clinical trials: stimulation of HIV-specific T-cell responses by DNA and recombinant modified vaccinia virus Ankara (MVA) vaccines in humans. J Gen Virol 85(Pt 4), 911-9.

Okeke, M.I., Nilssen, Ø., and Traavik, T. 2006. 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* 87, 21-27.

Okeke, M.I. 2007. Recombination *in Vitro* between transgenic and wild type orthopoxviruses; characterization of parental and progeny hybrid viruses. PhD thesis, University of Tromsø, ISBN 978-82-7589-188-2

Okeke, M.I., Nilssen, Ø., and Traavik, T. 2008. Biological properties of recombinant viruses obtained from co-infection in vitro with Modified vaccinia virus Ankara vectored influenza vaccine and wild type cowpox virus. *Submitted*.

Parrino, J. and Graham, B.S. (2006) Smallpox vaccines: Past, present, and future. J Allergy Clin Immunol 118(6), 1320-6.

Pastoret, P.P., Boulanger, D. and Brochier, B. (1995) Field trials of a recombinant rabies vaccine. Parasitology 110 Suppl, S37-42.

Pastoret, P.P. and Vanderplasschen, A. (2003) Poxviruses as vaccine vectors. Comp Immunol Microbiol Infect Dis 26(5-6), 343-55.

Perkus, M.E., Tartaglia, J. and Paoletti, E. (1995) Poxvirus-based vaccine candidates for cancer, AIDS, and other infectious diseases. J Leukoc Biol 58(1), 1-13.

Ramirez, J.C., Gherardi, M.M. and Esteban, M. (2000) 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 74(2), 923-33.

Rupprecht, C.E., Blass, L., Smith, K., Orciari, L.A., Niezgoda, M., Whitfield, S.G., Gibbons, R.V., Guerra, M. and Hanlon, C.A. (2001) Human infection due to recombinant vaccinia-rabies glycoprotein virus. N Engl J Med 345(8), 582-6.

Sandvik, T., Tryland, M., Hansen, H., Mehl, R., Moens, U., Olsvik, O. and Traavik, T. (1998) Naturally occurring orthopoxviruses: potential for recombination with vaccine vectors. J Clin Microbiol 36(9), 2542-7.

Schneider, J., Gilbert, S.C., Blanchard, T.J., Hanke, T., Robson, K.J., Hannan, C.M., Becker, M., Sinden, R., Smith, G.L. and Hill, A.V. (1998) Enhanced immunogenicity for CD8+ T cell induction and complete protective efficacy of malaria DNA vaccination by boosting with modified vaccinia virus Ankara. Nat Med 4(4), 397-402.

Schneider, J., Langermans, J.A., Gilbert, S.C., Blanchard, T.J., Twigg, S., Naitza, S., Hannan, C.M., Aidoo, M., Crisanti, A., Robson, K.J., Smith, G.L., Hill, A.V. and Thomas, A.W. (2001) A prime-boost immunisation regimen using DNA followed by recombinant modified vaccinia virus Ankara induces strong cellular immune responses against the Plasmodium falciparum TRAP antigen in chimpanzees. Vaccine 19(32), 4595-602.

Shen, Y. and Nemunaitis, J. (2005) Fighting cancer with vaccinia virus: teaching new tricks to an old dog. Mol Ther 11(2), 180-95.

Smith, C.L., Mirza, F., Pasquetto, V., Tscharke, D.C., Palmowski, M.J., Dunbar, P.R., Sette, A., Harris, A.L. and Cerundolo, V. (2005) Immunodominance of poxviral-specific CTL in a human trial of recombinant-modified vaccinia Ankara. J Immunol 175(12), 8431-7.

Smith, G.L. and Moss, B. (1983) Infectious poxvirus vectors have capacity for at least 25 000 base pairs of foreign DNA. Gene 25(1), 21-8.

Staib, C., Drexler, I. and Sutter, G. (2004) Construction and isolation of recombinant MVA. Methods Mol Biol 269, 77-100.

Traavik, T. (2002) Enviromental risks of genetically engineered vaccines, 331-355 pp. Genetically engineered Organisms; Assessing Enviromental and Human Health Effects, edited by D.K. Letourneau and B.E. Burrows. CRC Press USA.

Weidinger, G., Ohlmann, M., Schlereth, B., Sutter, G. and Niewiesk, S. (2001) Vaccination with recombinant modified vaccinia virus Ankara protects against measles virus infection in the mouse and cotton rat model. Vaccine 19(20-22), 2764-8.

Weingartl, H., Czub, M., Czub, S., Neufeld, J., Marszal, P., Gren, J., Smith, G., Jones, S., Proulx, R., Deschambault, Y., Grudeski, E., Andonov, A., He, R., Li, Y., Copps, J., Grolla, A., Dick, D., Berry, J., Ganske, S., Manning, L. and Cao, J. (2004) Immunization with modified vaccinia virus Ankara-based recombinant vaccine against severe acute respiratory syndrome is associated with enhanced hepatitis in ferrets. J Virol 78(22), 12672-6.

Zhang, X., Cassis-Ghavami, F., Eller, M., Currier, J., Slike, B.M., Chen, X., Tartaglia, J., Marovich, M. and Spearman, P. (2007) Direct comparison of antigen production and induction of apoptosis by canarypox virus- and modified vaccinia virus ankara-human immunodeficiency virus vaccine vectors. J Virol 81(13), 7022-33.