Environmental Risk Assessment

Deliberate release of the GMO MVA-NSmut in a proposed clinical trial

Conducted according to the principles described in:
S.I. No. 500 of 2003 Genetically Modified Organisms (Deliberate Release) Regulations, 2003, Second Schedule
Guideline on Scientific Requirements for the Environmental Risk Assessment of Gene Therapy Medicinal Products
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Background

MVA-NSmut is a recombinant virus vaccine derived from the attenuated virus, Modified Vaccinia Ankara. It has a genetic modification leading to the expression of NSmut – which encodes a 1,985 amino acid sequence encompassing NS3 to NS5b of the non-structural region of HCV genotype 1b – the commonest subtype of Hepatitis C virus in Europe.

Modified Vaccinia virus Ankara (MVA) is a highly attenuated vector that is unable to replicate efficiently in human and most mammalian cells. Recombinant vaccines lead to protein expression that may have the native conformation, glycosylation and post-translational modifications that may occur during natural infection. They may lead to antibody responses as well as cytotoxic responses.

These are non-replicating, non-integrating viral vectors. They are not expected to be able to persist in the body or the environment. This means that the vector will only be transiently present in the human body. The mechanism of action of both vectors is the expression of the HCV immunogen NSmut encoded by the viral vectors and stimulation of a humoral and cellular immune response to the expressed protein.

Step 1 – Identification of wild type and GMO characteristics which may cause adverse effects

MVA-NSmut is the GMO we propose to use. It has incorporated vectors that encode of which encode a 1,985 amino acid sequence encompassing NS3 to NS5b of the non-structural region of HCV (NSmut). NSmut is 1985 amino acids long, with a genetically inactivated NS5B polymerase. The genetically inactivated polymerase mutation was performed for safety reasons to limit any potential replication capacity for the vaccine. The NS region is know to be dense in CD8+ and CD4+ T cell epitopes and is more conserved than the region encoding viral envelope proteins.

Modified Vaccinia Ankara is a desirable choice of virus vaccine for safety and immunogenicity reasons. Live unattenated vaccinia virus was used to eradicate smallpox with minimal fears for safety. A small but definite risk of developing illness as a result of unattenuated vaccinia was identified, and therefore steps were undertaken to develop an attenuated version. The Modified Vaccinia

Ankara is one such attenuated version, which, through multiple passages in chicken embryo fibroblasts, has lost its pathogenicity and its ability to replicate in mammalian cells, including human cells. MVA has been safe in human and animal studies. 150,000 humans were vaccinated with MVA at the end of the smallpox eradication program without any significant side effects reported [1]. In previous vaccine trials for malaria and other diseases MVA following genetic modification (to incorporate genes from pathogenic organisms) has been used with no safety/toxicity issues. [2-5].

MVA was originally derived from the vaccinia strain Ankara by over 500 serial passages in chicken embryo fibroblasts (CEF cells). MVA has 6 major genomic deletions compared to the parental Ankara genome and is severely compromised in its ability to replicate in mammalian cells. No replication has been documented in non-transformed mammalian cells. The viral genome has been proven to be stable through a large series of passages in CEFs. MVA also showed no cytopathic effect or plaque formation in cells of human origin. In irradiated mice, MVA did not elicit any morbidity or lethality even when administered at high doses intra-cerebrally, indicating its safety even in immune-compromised organisms.

The potential hazards of use of MVA-NSmut are as follows:

Phlebotomy and vaccination risks
Human toxicity
Human immunoreactivity
Recombination of MVA-NSmut with other agents leading to reversion to replication competence

There is no reason to expect that exposure to NSmut as a result of this vaccination would lead to any deleterious effect of any future treatment for Hepatitis C in an individual.

Step 2 – Evaluation of the Potential Consequences of Each Adverse Effect

Phlebotomy: The maximum volume of blood drawn (690mLs over the study period of 8 months) should not compromise these otherwise healthy HIV-1 positive patients. There may be minor bruising, local tenderness or pre-syncopal symptoms associated with venepuncture. Full blood counts will be monitored at regular intervals to ensure anaemia does not develop in participants.

Patients inoculated with the MVA-NSmut vaccine would be most at risk of unintended adverse effects, although there is a theoretical risk that leakage of the vaccine from the site of inoculation could lead to exposure of the GMO to others including staff members in the research site and members of the public in the community. Steps to minimize this risk will be in place in the study protocol.

Local potential adverse effects: This vaccine is due to be administered intramuscularly. Many vaccines are associated with minor possible immunogenic reactions at the site of inoculation, which manifest as erythema and discomfort

at the site. If incorrectly administered it is conceivable that the inoculating needle could damage underlying structures, such as nerves or blood vessels.

If MVA-NSmut re-acquired the ability to replicate reliably the consequences would likely be minimal, with local antibody responses likely adequate to suppress any infection. This follows on from the use of un-attenuated vaccinia being used safely in the campaign to eradicate smallpox from humanity.

If the vaccine came in contact with the cornea, there is a theoretical risk of cellular infection and ulceration. This will be unlikely given the use of eye protection and the use of standard operating procedures for drawing up and administering the vaccine, as well as safe disposal of waste.

As with any other vaccine, Guillan-Barre syndrome (GBS) or immune mediated reactions that can lead to organ damage can occur.

As with any other vaccine, serious allergic reactions including anaphylaxis can occur. Volunteers will be vaccinated in a clinical area where Advanced Life Support drugs and equipment are immediately available for the management of serious adverse reactions.

Indirect effects: There is no theoretical basis for the possibility that exposure to NSmut as a result of this vaccination would lead to any deleterious effect on any future treatment for Hepatitis C in an individual.

The genetic modification of MVA leading to expression of NSmut is unlikely to lead to any deleterious events in the event of this gene being transferred to other viruses. NSmut has a mutation inactivating the enzymatic activity of the encoded polymerase gene elimination any potential replicative capacity of the insert.

Step 3 – Evaluation of the likelihood of adverse effects

The MVA-NSmut candidate vaccine has previously been administered to humans.

MVA-NSmut was first evaluated in humans in the HCV003 trial in the UK. This was followed by the HCV004 phase I trial in Italy and the phase I/II trial, code 10-0069 in the USA. Data from HCV003 and HCV004 confirm the excellent safety profile of MVA-NSmut, consistent with other trials of recombinant MVA vaccines that have been conducted at Oxford University.

In HCV003, healthy volunteers received MVA-NSmut at a dose of 2×10^8 pfu. Local reactions occurred in 100% of healthy volunteers, local pain being the most frequent event followed by warmth of the vaccination site and erythema. Most local adverse events were mild (74%) and self-limited (median duration 3 days). Systemic reactions occurred in 89% of healthy volunteers, of which 10.4% were severe. They were, however, generally transient (< 2 days).

These data add to the existing safety profile of MVA as a vector for a range of antigens, including the malaria antigens ME-TRAP (study codes VAC033 and

Mal034), MSP1 (study code VAC037), CS (study code VAC038), AMA-1 (Study code VAC039), and L3SEPT; antigen 85A from Mycobacterium tuberculosis; and influenza antigens NP+M1. To date there have been no vaccine-related serious adverse events in these trials. There are also extensive safety data from trials of MVA HIV vaccines in healthy HIV-uninfected and HIV-positive ART-treated subjects [3, 5-7].

Preclinical data is also available for NSmut. The safety of MVA-NSmut as part of a prime-boost vaccination regimen has been demonstrated in pre-clinical good laboratory practice (GLP) toxicology studies in mice and macaques (documented in the Investigator's Brochure). Prime-boost vaccinations with AdCh3-NSmut followed by MVA-NSmut were performed at the Research Toxicology Centre (RTC), Pomezia, Italy, with analyses for the immunogenicity and bio-distribution being performed at the Okairos laboratories. Repeated dose toxicity studies with a heterologous prime/boost vaccination schedule (Ad6 and AdCh3NSmut or AdCh3NSmut1 followed by MVA NSmut) given via the intramuscular route were performed in Balb/c mice (RTC study 78130 for AdCh3NSmut and study 82930 for AdCh3NSmut1). No toxic effects were observed in any organ.

Immunogenicity was also assessed in this study and in macaques. All vaccinated animals developed strong T cell responses to multiple epitopes in NSmut. Although formal biodistribution studies were not performed with MVA-NSmut, the biodistribution and persistence of another MVA-vectored HIV vaccine, MVA.HIVA, was assessed in a GLP study of SIV-infected macaques and severe combined immunodeficient (SCID) mice. In SIV-infected macaques, MVA.HIVA was undetectable by nested PCR 6 weeks after dosing. In SCID mice, the MVA.HIVA vaccine was only detected by PCR at the site of injection 49 days after dosing in 4/6 mice, and these sites were negative by day 81 post-injection [8].These data supported regulatory applications for clinical evaluation of MVA vaccines in HIV-positive human subjects

From 1972 until 1980 (the end of compulsory smallpox vaccination) MVA was licensed in Germany and was included in the official immunization schedule. In a large field carried out in Germany in the late 1970s, over 120,000 previous unvaccinated individuals were vaccinated with MVA (0.2mL) administered either intra-dermally or subcutaneously. The study population included high-risk groups such as people suffering from allergies, elderly people and alcoholics. Given intra-dermally, a red nodule of up to 4mm was observed at the injection site at day 4 or 5. Only a small proportion showed any systemic side effects such as a fever >38.5C [1]. MVA proved to be non contagious and avirulent. Viral replication is blocked late during infection of cells but importantly viral and recombinant protein synthesis in unimpaired even during this abortive infection. Replication deficient recombinant MVA has been viewed as an exceptionally safe viral vector. When tested in animal model studies, recombinant MVAs have been shown to be a avirulent, yet protectively immunogenic as vaccines against viral disease and cancer. Recent studies in macaques severely immunocompromised by SIV infection have further supported the view that MVA should be safe in immunocompormised humans. MVA is currently in development as a vector for

multiple diseases including HIV [9, 10], Tuberculosis [11], Hepatitis C, Influenza [12] and melanoma.

As with any other vaccine, Guillan-Barre syndrome (GBS) or immune mediated reactions that can lead to organ damage may occur. However, such problems are very rare events with any vaccine. As with any vaccine, serious allergic reactions including anaphylaxis can occur. Volunteers should be vaccinated in a clinical are where Advanced Life Support drugs and equipment are immediately available for the management of serious adverse reactions.

Release to wider environment
Given the lack of replicative capacity in mammalian cells it is unlikely that a significant release to the wider environment could happen. Theoretically, there could be minor leakage of the vaccine from the inoculation site and this could be a mode of contaminating the local environment. The dose would be small, in comparison with the inoculation dose and the risk of transmission to other individuals extremely low. This risk would be further reduced as a result of the standard safety procedures for disposal of waste products and covering of inoculation sites with a dressing after vaccination, to absorb any virus that may have leaked through the needle track. The dressing will be removed from the injection site after 30 minutes (+/- 5 minutes) and will be disposed of as GMO waste by autoclaving.

MVA vector shedding has been assessed in a clinical trial evaluating MVA expressing human MUC1 as an antigen-specific anti-tumor immunotherapy [13] Urine samples collected after 4 hours and 8 days were negative for MVA, confirming that the vaccine vector is not excreted due to the lack of systemic distribution.

Likelihood of release to environment and other humans – low

Recombination with other viruses in the environment – very unlikely due to the lack of replicative ability

Step 4 – Risk assessment overall

A risk assessment matrix is used to estimate the risk to human health or the environment. Where a range of risks is available, the highest risk is used, so as not to underestimate risks.

	Magnitude	Likelihood	Risk Estimation
Safety / toxicity	Moderate	Low	Low
Immune hyper-reactivity	High	Low	Low
Reversion to replicative virus	Low	Low	Low
Effect on treatment	High	Low	Low
Release to other humans	Low	Low	Low
Release to environment			

Step 5 – Risk Management Strategies

Patients do not require hospitalization or isolation due to the low risks involved. Outpatient surveillance and clinical assessment at study visits is appropriate. Blood analysis and clinical assessment for toxicities is appropriate.

Patients will be supplied with patient information leaflets and have a discussion with the enrolling personnel, both of which will go through the potential risks to the patient. Protocol information is available to study staff. Standard operating procedures (SOPs) are to be learned by study staff and applied, minimizing the likelihood of unintended exposures or release of the GMO into the environment.

The study is for healthy HIV-1 infected volunteers. The inclusion and exclusion criteria are outlined below:

Inclusion Criteria

HIV-1 seropositive adults must satisfy all the following inclusion criteria to be eligible for the study:

- Healthy adults aged 18 to 60 years (inclusive)
- Resident in or near the trial sites for the duration of the vaccination study
- Able and willing (in the Investigator's opinion) to comply with all study requirements
    - Treatment with an effective ART regimen for at least 9 months prior to inclusion
    - HIV Viral Load <50 copies/mL at the last routine HIV follow-up visit within the last 9 months prior to inclusion
    - Willingness to remain on ART for the study duration
    - CD4 cell count above 350 cells/uL
    - Negative HCV serology and negative HCV RNA PCR testing
- For women of child bearing potential, willingness to practise continuous effective contraception during the study and a negative pregnancy test on the day(s) of vaccination. Effective contraception is defined as a contraceptive method with failure rate of less than 1% per year when used consistently and correctly and, when applicable, in accordance with the product label. For example:
    - Injectable progestogen
    - Male partner sterilisation prior to the female subject's entry into the study, and this male is the sole partner for that subject
    - Male condom combined with a vaginal spermicide (foam, gel, film, cream or suppository)
    - Intrauterine device or intrauterine system
    - Adequate contraception does not apply to subjects of child bearing potential with same sex partners, when this is their preferred and usual lifestyle
- Men, including those with pregnant partners, should use condoms until 3 months after the last vaccination
- Written informed consent

Exclusion Criteria

HIV-1 seropositive adults may not enter the study if any of the following exclusion criteria apply:

- Participation in another research study involving an investigational product in the 30 days preceding enrolment, or planned use during the study period
- Prior receipt of a recombinant simian or human adenoviral vaccine
- Clinical, biochemical (abnormal liver synthetic dysfunction defined by an elevated blood prothrombin time or a low blood albumin level), ultrasonographic, Fibroscan™, or liver biopsy (histology) evidence of cirrhosis or portal hypertension
- Ongoing or recent (<12 months) AIDS defining illness (US CDC definition)
- History of allergic disease or reactions likely to be exacerbated by any component of the vaccine
- History of clinically significant contact dermatitis
- Any history of anaphylaxis in reaction to vaccination
- Pregnancy, lactation or willingness/intention to become pregnant during the study
- Known active malignant disease (except basal cell carcinoma of the skin and cervical carcinoma in situ)
- Current suspected or known injecting drug abuse (except individuals participating in a heroin substitution program without known or suspected concomitant drug abuse). Participants will be counselled regarding the risk of HCV acquisition during the trial.
- Seropositive for hepatitis B surface antigen (HBsAg)
- Positive test for Hepatitis C antibody and/or PCR
- Severe neutropenia (Absolute neutrophil count of <500 cells/ uL)
- Severe thrombocytopenia (Platelet count <50,000 cells/uL)
- Anaemia (Haemoglobin <10g/dL)
- Uncontrolled autoimmune disease
- History of organ transplantation
- Severe uncontrolled psychiatric disease
- Significant coagulopathy or anticoagulant therapy at time of vaccination
- Any other significant disease, disorder or finding, which, in the opinion of the Investigator, may either put the patient at risk because of participation in the study, or may influence the result of the study, or the patient's ability to participate in the study

The following adverse events associated with vaccine immunisation constitute absolute contraindications to further administration of vaccine. If any of these events occur during the study, the subjects must be withdrawn and followed until resolution of the event, as with any adverse event.

- Anaphylactic reaction following administration of vaccine
- Pregnancy
- Elevation in ALT greater that 10 times the upper limit of normal at any time during the study

The following adverse events constitute contraindications to administration of vaccine at that point in time; if any one of these adverse events occurs at the time scheduled for vaccination, the subject may be vaccinated at a later date, or withdrawn at the discretion of the investigator. The subject must be followed until resolution of the event as with any adverse event.

- Acute disease at the time of vaccination. Acute disease is defined as the presence of a moderate or severe illness with or without fever. All vaccines can be administered to persons with a minor illness such as diarrhoea, mild upper respiratory infection with or without low-grade febrile illness, *i.e.*, temperature of <37.5°C/99.5°F).
- Temperature of ≥37.5°C (99.5°F) at the time of vaccination.

Following vaccination there will be clinical reviews at Days +1, +7, +14, +28, +56, +57, +63, +84 , +98 and a 9 month follow up visit. Blood draws for safety assessment (haematology and clinical chemistry) will be obtained at each of these visits except for day +1 and +57.

For every adverse event (AE), an assessment of the relationship of the event to the administration of the vaccine will be undertaken. The Investigator / Sponsor-Investigator and the internal PEACHI safety review board both determine causality and it is expected that communication and consultation may occur in the assessment of the causality of AEs. An intervention-related AE refers to an AE for which there is a possible, probable or definite relationship to administration of a vaccine. Interventions are considered unrelated if they fall into the category of no relationship or unlikely relationship. An interpretation of the causal relationship of the intervention to the AE in question will be made, based on the type of event, the relationship of the event to the time of vaccine administration, and the known biology of the vaccine therapy.

A study independent Safety Committee (SC) will be appointed to provide safety oversight. The SC will review Serious Adverse Events (SAEs) if deemed possibly, probably or definitely related to vaccination. The SC will be notified within 1 working day of the investigators' being aware of their occurrence. The SC has the power to terminate the study if deemed necessary following a vaccine-related SAE

Storage of the product will be in approved facilities with monitored temperature stable conditions. Vaccine preparation and handling will be in accordance with institutional policies.

Institutional procedures and guidelines will be used for the conduct of the study, including vaccination administration and waste management.

'General surveillance' will be done as per protocol and as outlined above. 'Monitoring as appropriate' will be done when needed. Patients will be told to contact the study team if any unexpected effects occur.

Step 6 - Determining overall risk

Given the analysis outlined above, the proven history of MVA-vaccines, and the benign nature of the protein product of the inserted gene the overall risk associated with this GMO is low. All safety monitoring and ethical procedures will be followed if this protocol is approved.

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