Environmental Risk Assessment

Deliberate release of the GMO AdCh3NSmut1 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
EMEA/CHMP/GTWP/125491/2006, 30 May 2008

Background

AdCh3NSmut1 is a modified recombinant virus derived from an attenuated replication-incompetent adenovirus with a genetic modification to promote the expression of NSmut. The extensive genetic variability of HCV is a major challenge for vaccine development. We have based our HCV vaccine on non structural (NS) amino acid sequences from the genotype 1, subtype b, BK strain isolate. The BK strain amino acid sequence is relatively conserved across several genomes (≤ 80% homology) covering 14 different subtypes belonging to the 6 major genotypes. The NS region encompasses approximately two thirds of the HCV genome and encodes five different proteins (NS3, NS4a, NS4b, NS5a and NS5b). Proteolytic cleavage of the HCV polyprotein is mediated by the encoded NS3 protease. This process recapitulates the same cascade of events occurring in vivo upon viral infection and therefore provides a physiological way of presenting the viral antigens to the host immune system.

Chimpanzee adenoviruses have been developed as viral vectors following concerns that pre-exisiting immunity to human adenoviral serotypes could limit future widespread use of these viruses as vaccine candidates. Simian adenoviruses are not known to cause pathological illness in humans and prevalence of antibodies to adenoviruses of chimpanzee origin is less that 5% in humans residing in the USA. AdCh3 is rendered replication incompetent by deletion of the E1 region that contains genes essential for the initiation of viral replication.

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 we intent to use is the expression of the HCV immunogen NSmut encoded by the viral vectors and stimulation of a cellular immune response to the expressed protein. 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.

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

AdCh3NSmut1 is the GMO that we propose to use and is derived from AdCh3, a recombinant attenuated chimpanzee adenovirus. Human adenoviruses are often asymptomatic infections but can produce respiratory tract infections, gastrointestinal disturbances and ocular infections. They are most common in children and their incubation period is between 1 and 10 days. Most members of the population are seropositive and can produce neutralizing antibodies against adenoviruses. Transmission of adenovirus can occur via aerosol droplet, the faecal-oral route and by contact with contaminated fomites.

Most adenoviral infections are minor and self-limited. Adenoviruses are usually not integrative and generally do not persist long-term in lymphoid tissue, although such cases have been reported rarely. This strain AdCh3NSmut1, as well as being a non-human virus, is replication incompetent making it safer for use.

The genetic insert NSmut is 1995 amino acids long, with a genetically inactivated NS5B polymerase. 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.

The potential hazards of use of AdCh3-NSmut are as follows:

Phlebotomy and vaccination risks
Human toxicity
Human immunoreactivity
Recombination of AdCh3-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 over the study period (Maximum of 690mLs in total over a period of 9 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.

Patients inoculated with the AdCh3NSmut1 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 and outlined in the Standard Operating Procedures.

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 may manifest as erythema and discomfort at the site. If incorrectly administered, it is conceivable that

underlying structures, such as nerves or blood vessels, could be damaged by the inoculating needle.

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. However, such problems are very rare events with any vaccine and have never occurred with AdCh3 vector vaccines to date.

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 AdCh3 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 eliminating any potential replicative capacity of the insert.

If AdCh3NSmut1 re-acquired the ability to replicate reliably the consequences would likely be minimal, with local antibody responses likely adequate to suppress any infection. Adenoviruses in humans rarely cause serious illness, although they commonly are associated with upper respiratory tract infections and less commonly gastrointestinal, ophthalmic, genitourinary and neurological disease

Step 3 – Evaluation of the likelihood of adverse effects

Ongoing and completed Phase I trials in Oxford include those using the AdCh3 and Ad6 vectors encoding the NS antigen (AdCh3NSmut and Ad6NSmut) in healthy volunteers (study HCV001), in HCV infected patients (study HCV002TV), and study HCV003 which is also assessing the safety and immunogenicity of AdCh3NSmut1 – identical to the AdCh3NS to be used in this study – and MVA-NSmut in healthy volunteers.

The phase I clinical trial HCV001 assessed the safety and immunogenicity of AdCh3NSmut in healthy volunteers. This was an open label study where 38 volunteers received AdCh3NSmut at least once during the trial. Priming vaccinations were administered to 22 volunteers at doses ranging from 5×10^8 vp to 7.5×10^{10} vp. A further 16 individuals received a single boosting

vaccination of AdCh3NSmut at 2.5 x 10^{10} vp following priming vaccinations with Ad6NSmut at varying doses [1].

In the on-going HCV003 trial the dose of AdCh3NSmut is 2.5x10^{10} vp, which was determined by complete safety analysis of the HCV001 trial data. This trial also introduces the use of the new improved batch of AdCh3NSmut (AdCh3NSmut1), at the dose of 2.5x10^{10} vp. This new batch AdCh3NSmut1 is being used in the staged phase I/II trial 10-0069 in progress in the USA (FDA approval, http://clinicaltrials.gov/ct2/show/NCT01436357). In HCV003 up to 24 healthy volunteers will be included.

The results of the HCV001 trial demonstrate that AdCh3NSmut is well tolerated and there were no reported Suspected Unexpected Serious Adverse Events (SUSARs). When AdCh3NSmut was administered as the priming vaccination, 93% of the observed local reactions were mild in severity, with 98% being resolved within 1 week. Of the observed systemic reactions, 96% were mild in severity (the remaining 4% were moderate), and 93% had resolved by 1 week. In the HCV003 trial (to date, 29 subjects have been vaccinated with either AdCh3NSmut or AdCh3NSmut1) local reactions occurred in 94% (of which 91% were mild and 2.2% classified as severe), local pain being the most common adverse events followed by warmth at the vaccination site. The majority lasted 1-3 days. Systemic reactions occurred in 81%, headache being the most common followed by fatigue; 74% were classified as mild.

Adenoviral vector shedding was explored in urine and throat swabs in study HCV001: there was no detectable virus in any clinical sample after intramuscular AdCh or AdHu immunisation. These finding are consistent with the pre-clinical studies demonstrating no vector dissemination beyond regional lymph nodes and no vector persistence.

Vaccine trials evaluating another chimpanzee adenovirus vector, AdCh63, in healthy volunteers have also been conducted at Oxford University. Vaccines for malaria incorporated ME-TRAP (a multi-epitope string and the thrombospondin related adhesion protein) inserts in trials VAC033 and MAL034 [2, 3], and an MSP1 (Merozoite surface protein-1) insert in trial VAC037 [4].

Local and systemic adverse events are comparable in incidence and severity to the manifestations observed in HCV001 and HCV003. An HIV vaccine incorporating a conserved region immunogen, HIVconsv, was evaluated in the phase I trial HIV-CORE 002 [5]. In all these trials there were no vaccine-related serious adverse events or SUSARs.

Pre-clinical data is also available for AdCh3NSmut. Pre-clinical studies with AdCh3NSmut / AdCh3NSmut1 were performed at the Research Toxicology Centre (RTC), Pomezia, Italy, with analyses for the immunogenicity and bio-distribution being performed at the Okairos laboratories. Each study involved intra-muscular injection of 6.0 x 10^{9} vp/dose of AdCh3NSmut.

In RTC study 57710, tissue distribution after AdCh3NSmut immunisation in mice was assessed in two ways: (i) inoculation of human embryonic kidney (HEK) 293 cells with mouse organ homogenates followed by culture and real-time PCR to detect infectious virus; (ii) quantification of adenoviral genomes in DNA extracted from organ homogenates by real-time PCR. This study showed that one week after intramuscular injection, AdCh3NSmut was barely detected only in the regional lymph nodes while muscles were negative in all mice. Thus in both compartments there is evidence of a loss of infectious virus. Similar results were obtained by qPCR of adenovirus DNA. These data indicate that AdCh3NSmut was not able to replicate within the mouse tissue.

Lack of replicative capacity of AdCh3NSmut1 was also confirmed by a replication competence assay [0 RCA per $3x10^{10}$ vp (specifications: <1 per $3x10^{10}$ vp)]. Because human adenoviral infection is transient and because the vaccination vector AdCh3 is replication-defective, detectable only at the site of injection (skeletal muscle is the route for vaccine, while wild type adenoviruses may be found at mucosal sites) and does not disseminate or persist, the theoretical risk of recombination of the vector with human adenoviruses in vivo is very low. Several repeated dose toxicity studies were performed using AdCh3NSmut in mice (RTC studies 57710, 70450 and 78130 for AdCh3NSmut, and 82930 for AdCh3NSmut1): these were designed to investigate potential toxicity arising from repeated administration of the vaccine in heterologous prime/boost regimens similar to those proposed for the clinical trials. No toxic effects were noted in mice receiving Ad6NSmut, AdCh3NSmut, AdCh3NSmut1 and MVA-NSmut when given by the intramuscular route.

Immunogenicity was also assessed during the studies outlined above (RTC 57710, 70450 and 78130 for AdCh3NSmut, and study 82930 for AdCh3NSmut1). It was found that all vaccinated mice developed very strong T cell-mediated immune responses directed against multiple epitopes. Similarly, in preclinical studies performed at Okairos laboratories using Rhesus macaques, potent responses targeting different NS antigens were induced in all immunised macaques.

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 (SOPs) 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 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.

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	Low	Low	Low

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 will be 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 infected male or female patients aged 18 – 60 years
- Resident in or near the trial site for the duration of the vaccination study

- Able and willing to comply with all the study requirements
- Continuous treatment with an effective cART regimen (HIV-1 viral load <40 copies/mL) for at least 9 months prior to inclusion
- Willingness to remain on antiretroviral therapy (ART) for the study duration
- Negative HCV serology and negative HCV RNA PCR
- Willingness to conduct harm-reduction sexual behavior
- Women only: Must practice continuous effective contraception for the duration of the study
- Written informed consent

Exclusion Criteria
- Participation in another research study involving an investigational product in the 30 days preceding enrolment, or planned use during the study period
- Ongoing or recent (<6 months) AIDS defining illness according to CDC definitions
- Previous receipt of a recombinant simian or human adenoviral vaccines
- 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 heroine 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 constitute contraindications to administration of vaccine at that point in time; if any one of these adverse events occurs at the time

scheduled for either of the two vaccinations, 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 illness at the time of vaccination, i.e. 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 <37.7°C).
- Temperature of 37.7°C at the time of vaccination.

The following adverse events associated with vaccination 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

Following vaccination at week 0 and week 8 subjects will have follow up visits at day 1, week 1, 2 and 4, day 57, week 9, 12, 24 and 38. Bloods for safety assessment will be done on each of these days except for day 1 and day 57.

For every adverse event (AE), an assessment of the relationship of the event to the administration of the vaccine will be undertaken. A vaccine-related AE refers to an AE for which there is a possible, probable or definite relationship to administration of a vaccine. Adverse events 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

All AEs occurring during the study that are observed by the Investigator or reported by the patient, whether or not attributed to study medication, will be reported in the case report form (CRF).

All serious adverse events (SAEs) must be reported to the Sponsor (and the Chief Investigator, if the event is being reported by another Investigator), within 24 hours of the Site Study Team becoming aware of the event being defined as serious. The Data Safety Monitoring Committee (DSMC) should be informed of SAEs within 7 days of awareness. The DSMC 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 temperatures and approved conditions. Vaccine preparation and handling will be in accordance with institutional policies.

General surveillance will be done as per protocol and as outlined above. Monitoring as appropriate will be done as 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 Adenoviral 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.

1. Barnes, E., et al., *Novel adenovirus-based vaccines induce broad and sustained T cell responses to HCV in man.* Sci Transl Med, 2012. **4**(115): p. 115ra1.
2. O'Hara, G.A., et al., *Clinical assessment of a recombinant simian adenovirus AdCh63: a potent new vaccine vector.* J Infect Dis, 2012. **205**(5): p. 772-81.
3. Ogwang, C., et al., *Safety and immunogenicity of heterologous prime-boost immunisation with Plasmodium falciparum malaria candidate vaccines, AdCh63 ME-TRAP and MVA ME-TRAP, in healthy Gambian and Kenyan adults.* PLoS One, 2013. **8**(3): p. e57726.
4. Sheehy, S.H., et al., *Phase Ia clinical evaluation of the safety and immunogenicity of the Plasmodium falciparum blood-stage antigen AMA1 in AdCh63 and MVA vaccine vectors.* PLoS One, 2012. **7**(2): p. e31208.
5. Borthwick, N., et al., *Vaccine-elicited Human T Cells Recognizing Conserved Protein Regions Inhibit HIV-1.* Mol Ther, 2014. **22**(2): p. 464-75.