TH HBV VV-001 Clinical study

MVA-HBV

Directive 2001/18/EC – Annex II

PRINCIPLES FOR THE ENVIRONMENTAL RISK ASSESSMENT (ERA)

Notifier: Glaxo SmithKline Biologicals

EudraCT number: 2017-001452-55

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Abbreviations

AE	Adverse Event
BHK	Baby Hamster Kidney
BSL1	Biosafety Level 1
CEF	Chick Embryo Fibroblasts
ChAd155-hIi-HBV	Investigational Recombinant Chimpanzee Adenovirus HBV Vaccine
DNA	Deoxyribonucleic Acid
DS	Drug Substance
FMDV	Foot-And-Mouth Disease Virus
FTIH	First-Time-In Human
GLP	Good Laboratory Practices
GMO	Genetically Modified Organism
GMP	Good Manufacturing Practices
GSK	GlaxoSmithKline
HBc	Hepatitis B Core Protein
HBs	Hepatitis B Surface Protein
HBV	Hepatitis B Virus
HCV	Hepatitis C
HHD	Hla.A2/Dr1
hIi	Human Invariant Chain
IDT	Impfstoffwerk Dessau-Tornau
IM	Intramuscular
iSRC	Internal Safety Review Committee
MHC	Major Histocompatibility Complex
MVA	Modified Vaccinia Virus Ankara
MVA-HBV	Modified Vaccinia Ankara-Hepatitis B Virus
MVA-NSmut	MVA Vectored Vaccine
MVS	Master Virus Seed
NA	Nucleo(S)Tide Analogue
OPV	Orthopox Virus
PCR	Polymerase Chain Reaction
pfu	Plaque-Forming Unit
RNA	Ribonucleic Acid
SAE	Serious Adverse Event
SRC	Safety Review Committee
vp	Viral Particles
VV	Vaccinia Virus

I. Introduction

1. Context of the release

As per the requirements of Directive 2001/18/EC, this document presents an assessment of the possible risks to the environment following deliberate release of a genetically modified organism.

The proposed release will occur during a clinical trial entitled:

'A first-time-in human (FTIH), Phase I, randomized, multi-centric, single-blind, controlled dose-escalation study to evaluate the reactogenicity, safety, immunogenicity and efficacy of GSK Biologicals' HBV viral vectored vaccines given in a prime-boost schedule with sequential or co-administration of adjuvanted proteins therapeutic vaccine (GSK3528869A) in chronic Hepatitis B patients (18-65 years old) well controlled under nucleo(s)tides analogues (NA) therapy'.

The EudraCT number of the study is: 2017-001452-55 and the Applicant's study code number is: 204852 / abbreviated title is: TH HBV VV-001.

Duration of the vaccination phase of the clinical trial Th HBV VV-001 will be approximately 2.5 years starting from Q3-2018 to the date of last dose of the study in Q1-2021. With safety follow-up the total duration of the study is 4.5 years with study completion in Q1-2023.

2. Characteristics of the GMO

a. Description of the GMO and doses

The GMO is a modified vaccinia virus Ankara vector (MVA) encoding a fusion of sequences derived from two hepatitis B virus (HBV) protein antigens. The two HBV proteins are the truncated core nucleocapsid protein antigen (HBc) and the full-length small surface antigen (HBs), separated by the self-cleaving 2A region of the foot-and-mouth disease virus. The 2A region allows processing of the HBc-2A-HBs transcript into the expression of two separate HBc and HBs protein antigens.

MVA is a highly attenuated vaccinia virus strain that was developed by repeated passaging (> 570 passages) of the chorioallantois vaccinia virus Ankara (CVA) in primary cell culture of chicken embryo fibroblasts (Mayr et al., 1978). The resulting MVA strain was used during the smallpox eradication campaign to vaccinate over 120,000 people considered at high risk of adverse events for the vaccinia vaccine (Stickl et al., 1974). While the vaccinia virus exhibits a wide host range, is able to efficiently replicate in human cells, and has caused laboratory-acquired vaccinia vius infections (Isaacs, 2012). In contrast, MVA exhibits a narrow host range and is not able to replicate in human cells. For the reasons, the vaccinia virus is classified as a risk group 2 biological agent, whereas the MVA strain is risk group 1 (Stellberger 2016).

The investigational MVA-HBV vaccine is presented as a sterile liquid suspension filled in stoppered 2 mL glass vials filled at a nominal volume of 0.7 mL per vial and are stored at <-60°C. The MVA-HBV vaccine is formulated in Tris-NaCl buffer without addition of a preservative. The vaccine is presented as a single-dose (monodose) at a concentration of 4.10e8 pfu/mL for intramuscular (IM) administration.

Two concentrations will be evaluated in the Phase 1 clinical study: a higher potency dose of 2 x 10e8 plaque forming unit (pfu) per dose and a lower potency dose of 2 x10e7 pfu per dose.

b. Detection and identification of the GMO

The following techniques are used to detect and identify the GMO:

- The identity of the recombinant MVA-HBV vector is confirmed at the MVS level by DNA sequencing of the transgene.
- Identity of the MVA-HBV viral vector is also confirmed by PCR at the level of purified bulk and filled vaccine, where PCR primers are designed to target sites on the the HBV transgene.
- In addition, Western blots are performed at the level of purified bulk and filled vaccine, that are relevant to detect and identify the GMO insert since they use specific antibodies targeting the HBc and the HBs proteins.

3. Characteristics of the release

a. Previous release of the GMO

The GMO MVA-HBV is to be assessed for safety, reactogenicity and immunogenicity in a Phase 1 trial (EudraCT: 2017-001452-55) entitled:

A first-time-in human (FTIH), Phase I, randomized, multi-centric, single-blind, -controlled dose-escalation study to evaluate the reactogenicity, safety immunogenicity and efficacy of GSK Biologicals' HBV viral vectored vaccines given in a prime-boost schedule with sequential of co-administration of adjuvanted proteins therapeutic vaccine (GSK3528869A) in chronic Hepatitis B patients (18-65 years old) well controlled under nucleo(s)tides analogues therapy. This study is multi-centric and will be held in several European countries.

As this will be the first in human trial of the MVA-HBV GMO, no data is available about the previous release of the proposed MVA-HBV GMO.

Clinical studies conducted with similar recombinant MVA GMO's containing other transgenes (e.g. malaria, Ebola, HCV, HIV, RSV) have not raised safety concerns. No significant adverse effects have been reported and the GMO appears to be generally safe and well tolerated (Verheust et al. 2012, Goossens et al. 2013).

b. Characteristics of the planned release

The planned released will occur during a clinical study to be held at several investigational sites under the responsibilities of local principal investigators.

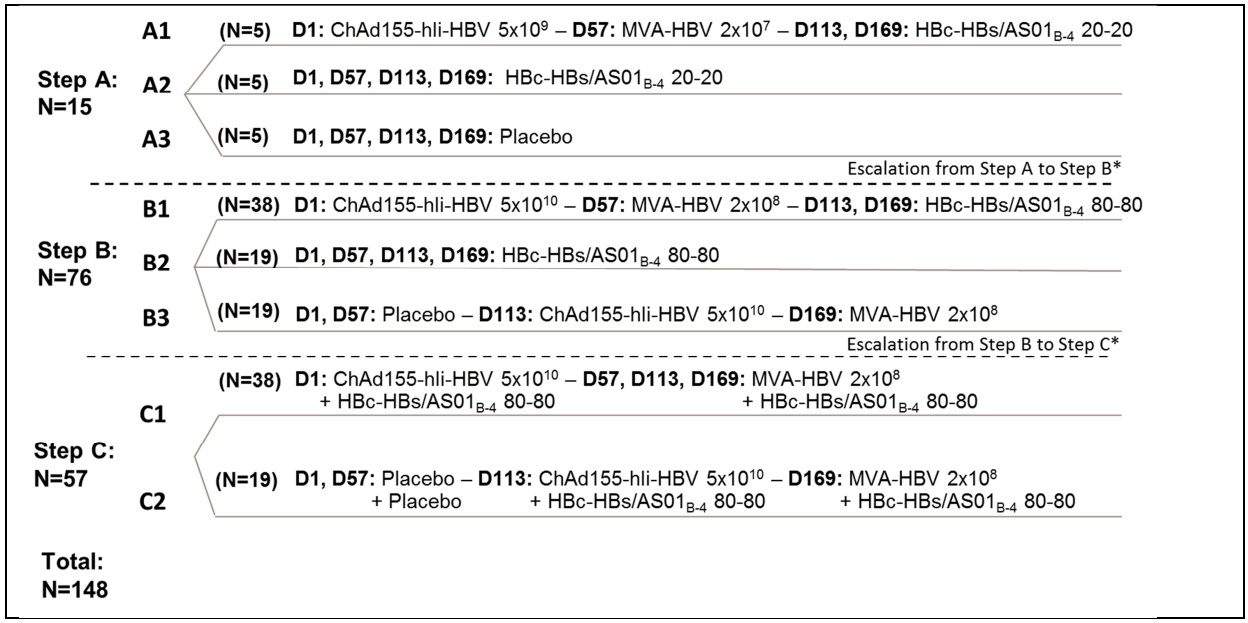
The study cohort is planned to consist of 148 enroled patients. The GMO will be administered in steps according to the schedule provided in Figure 1. For the GMO MVA-HBV vaccine the administrations are as follows:

- Step A: Three randomized groups. Group A1 (N=5) will receive one dose of MVA-HBV vaccine at 2x10e7 pfu per dose at Day 57.

- Step B: Three randomized groups. Group B1 (N=38) will receive one dose of MVA-HBV vaccine at 2x10e8 pfu/dose at Day 57. Group B3 (N=19) will receive one dose of MVA-HBV vaccine at 2x10e8 pfu/dose at Day 169.
- Step C: Two randomized groups. Group C1 (N=38) will receive three (3) doses of MVA-HBV vaccine at 2x10e8 pfu/dose at Days 57, 113 and 169. Group C2 (N=19) will receive one dose of MVA-HBV vaccine at 2x10e8 pfu/dose at Days 57, 113 and 169.

Based on the dose levels of 2x10e7 pfu/dose and 2 x 10e8 pfu/dose, the planned number of patients per group, and the number of doses to be administered (5 low dose and 190 high dose), it is estimated that a total of 3.81x10e10 pfu will be administered (released) during the proposed study. This value represents a pool of all doses administered at all investigational sites in all countries.

Figure 1 Study steps and study groups



II. Identification of GMO characteristics which may cause adverse effects

1. Possible negative effects including allergenic and toxic effects in Humans

a. Allergenic effects

As with all injectable vaccines/medicinal products, immediate local and/or delayed systemic allergic reactions to vaccination can occur. However, there is no basis to indicate the MVA-HBV GMO will provoke adverse allergenic effects in the vaccinated study population beyond transient local or systemic reactions associated with vaccination.

b. Toxic effects

As with all injectable vaccines/medicinal products, immediate local and/or delayed systemic allergic reactions to vaccination can occur. The most likely reaction to occur following IM administration of the GMO is a transient and self-limiting local inflammatory reaction. This may typically include

redness, swelling and tenderness at the site of injection. Mild systemic reactions following vaccination may include: fatigue, fever, headache, stomach or intestinal troubles, muscle pain or feeling cold (chills).

c. Replication competent virus

The MVA GMO is replication-deficient. One of the possible risks associated to its release is the reversion to a virulent or a replication competent vector following recombination events with naturally occurring homologs. See section III.c. for an analysis of the potential for reversion.

d. Shedding/Survivability

The potential for shedding and survivability of the GMO is addressed in section III.d.

e. Reactivation of endogenous retroviruses

As up to 8% of the human genome is of retroviral origin (Hurst et al. 2017, Meyer et al. 2017). The evaluation of the potential reactivation of HERVs by the GMO is covered in sections III.1.e. and IV.1.e.

2. Possible negative effects including allergenic and toxic effects in animals and plants

The possibility of any interaction between the GMO and other species is minimal under the conditions of the proposed release. If the interaction were to occur there is no basis to indicate any negative toxic effects would result. The risk to plants is null since the GMO is not capable of transducing plant cells.

3. Effects on the dynamics of populations of species in the receiving environment and the genetic diversity of these populations

The possibility of any interaction between the GMO and other species, besides the target population in the clinical study, is minimal under the conditions of the proposed release. If interaction were to occur, given the severe host restriction, replication-deficiency and extrachromosomal non-integrative nature of MVA, there is no basis to indicate the GMO could have any effect on the dynamics or genetic diversity of the populations in the environment.

4. Altered susceptibility to pathogens facilitating the dissemination of infectious diseases and/or creating new reservoirs or vectors

MVA is a highly attenuated orthopoxvirus that has been rendered replication-deficient and is not pathogenic. MVA is not naturally found in the environment or natural ecosystems and has no known animal reservoirs. MVA exhibits severe host cell restriction, and although it replicates well in CEF and BHK cells, it replicates poorly in most mammalian cells and is unable to spread in normal human cells. The GMO is not capable of transducing plant cells and therefore this section is not applicable for plant protection treatments.

While a plasmid containing an ampicillin antibiotic resistance gene was used as a selection tool for the recombinant engineering manipulations leading to the construction of the GMO. It is important to note that no antibiotic resistance gene is found in the final MVA-HBV construct.

Given all the conditions that lend its non-propagative and non-integrative nature, there is negligible risk that the GMO could impact susceptibility to pathogens or facilitate pathogen dissemination.

5. Compromising prophylactic or therapeutic medical, veterinary, or plant protection treatments

Plasmids used for construction of the GMO bear antibiotic resistance genes. However, the GMO does not encompass any of these antibiotic resistance genes. Therefore, the probability of transfer of antibiotic resistance gene compromising future prophylactic or therapeutic treatments to the target host (human chronically infected with HBV) is negligible.

Identical rational applies for veterinary treatments since the GMO but is not applicable for plant since the GMO is not capable of transducing plant cells.

6. Effects on biogeochemistry

The GMO is an investigational medicinal product to be released in a highly controlled clinical setting and should have no impact on biogeochemical cyles.

III. Evaluation of the magnitude of the potential consequences and likelihood of occurrence of adverse effects

1. Possible negative effects including allergenic and toxic effects in Humans

a. Allergenic effects

There is no basis to indicate the MVA-HBV GMO will provoke allergenic effects in the vaccinated study population.

The immune response generated after infection with the *native species* Vaccinia Virus protects individuals against smallpox (i.e. as a smallpox vaccine); the Vaccinia Virus vaccine-induced infection is mild and usually asymptomatic in healthy individuals. However, during historical Vaccinia Virus small pox vaccination campaigns, complications and side effects occurred with a higher likelihood in immune compromised persons. Therefore, in order to reduce the likelihood adverse events occurring during vaccination, the attenuated MVA strain was developed. The attenuated MVA strain was used in the 1970's, during the end of global smallpox eradication efforts, to vaccinate some 120,000 people in Germany who were considered susceptible to the adverse events with the Vaccinia Virus vaccine. It was found that MVA was safe and well tolerated with the most frequent adverse reactions reported being local reactions, fever and flu-like symptoms.

As with all injectable vaccines/medicinal products, immediate local and/or delayed systemic allergic reactions to vaccination can occur. In order to treat immediate systemic allergic reaction to GMO vaccination, all study subjects will remain under observation (visual follow-up, no specific procedure) at the study site for at least 60 minutes after vaccination.

b. Toxicity/Pathogenicity

Clinical studies conducted with similar recombinant MVA GMO's containing other transgenes (e.g. malaria, Ebola, HCV, HIV, RSV) have not raised safety concerns. No significant adverse effects have been reported and the GMO appears to be generally safe and well tolerated (Pierantoni et al. 2015, Green et al. 2015, Verheust et al. 2012, Goossens et al. 2013).

Preclinical GMO toxicity data. Two GLP-compliant toxicity studies were performed using GMO batches comparable to the clinical trial material including: a single-dose local tolerance and a repeat-dose toxicity studies. The toxicology studies were performed using the same dose and volume, number of administrations- plus-one, and route of administration, as will be used in the clinical study.

In the repeat-dose rabbit study, 5 doses of each vaccine were administered i.e. 1 more dose than the clinical dosing regimen that anticipates 4 vaccinations. Three different schedules of vaccination (HBc-HBs/AS01B-4 alone; combination of ChAd155-hli-HBV, MVA-HBV and HBc-HBs/AS01B-4; co-administrations of HBc-HBs/AS01B-4 with ChAd155-hli-HBV or MVA-HBV) were used. The frequency of administration was compressed (every 2 weeks) vs. the clinical regimen (every 8 weeks).

Results of the single dose toxicity studies demonstrated that administration of the GMO (0.5 mL at 3.9x10e8 pfu/mL) simultaneously to adjuvanted recombinant proteins HBc-HBs/AS01B-4 (in the opposite leg, both via intramuscular route), did not induce safety concerns. No sign of systemic toxicity was reported.

In the repeated dose toxicity study, the animals received a priming dose with the ChAd155-hli-HBV vector (that encodes the same HBV antigens in its transgene), followed by 4 administrations of the MVA-HBV GMO, while they received on the opposite leg concurrently HBc-HBs/AS01B-4 as immune enhancer. Note, the transgene for the ChAd155-hli-HBV vector encodes the same HBc and HBs antigens as are encoded by the MVA-HBV transgene. The ChAd155-hli-HBV transgene differs from MVA-HBV in that the N-terminus of the gene encoding the HBc protein has been fused to the gene encoding the human Major Histocompatibility Complex (MHC) class II-associated invariant chain p35 isoform (hli). This vaccination schedule was clinically well tolerated, and all vaccinated animals had anti-HBc and anti-HBs antibodies at the end of the treatment and recovery periods. The in-life findings were all consistent with the inflammatory reaction and the immune response that may occur after administration of vaccines. Hematology findings mainly consisted in increased neutrophil counts, which were accompanied by increased fibrinogen and C-reactive protein levels, and by decreased albumin/globulin ratio at blood biochemistry. All these parameters were returned to normalcy within 7 days after dosing. 3 days after the last injection, the administration of HBV therapeutic candidate vaccines given alone or in combination/co-administration induced inflammatory reaction at the injection sites along with slight changes indicative of an immune stimulation in draining lymph nodes and spleen. Similar changes but of lower severity were seen 28 days after the last injection, suggesting that the recovery was ongoing. When compared to controls, the severity and/or incidence of the changes were more pronounced in animals injected sequentially with ChAd155-hli-HBV and MVA-HBV in the right site and HBc-HBs/AS01B-4 in the left site, than in animals treated sequentially with the 3 HBV candidate vaccines ChAd155-hli-HBV, MVA-HBV and HBc-HBs/AS01B-4 in the right site, and finally in animals given HBc-HBs/AS01B-4 alone in the right

site. Overall, the HBV therapeutic candidate vaccines were considered to be well tolerated since the microscopic findings indicative of an inflammatory reaction/immune response are those expected after an antigenic stimulation by the intramuscular route.

c. Replication competent virus

There is no known poxvirus able to complement MVA to generate a replication competent virus, and spontaneous reversion of MVA to replication competent vaccinia virus has not been documented (Goossens et al. 2013).

d. Shedding/Survivability

The GMO will be administered by intramuscular (IM) injection. With this route of administration, studies show there is limited virus shedding and limited spread to other tissues, as the virus vector remains localized to the site of injection. In addition, intramuscular injection, as compared to subcutaneous injection, reduces the probability of viral particles being present on the skin close to the injection site in so-called “skin pock lesion” and thereby reduces potential shedding via the needle track.

Due to the severely restricted host range of MVA, its lack of virulence in animals and humans, and its highly attenuated replication, we do not expect the vector to survive or spread in the environment.

The potential for shedding of infectious MVA-HBV particles into the environment is considered negligible especially when administered by the intramuscular injection route. Human clinical studies conducted with similar MVA constructs administered by this route have been mainly unable to detect vector shedding from study subjects in biological samples (sputum, saliva, urine, feces) (Goossens et al. 2013). There is no indication that the HBV transgene could influence the shedding behaviour of recombinant MVA vectors.

Preventive measures implemented during the conduct of the clinical trial will also minimize inadvertent dissemination from spills or accidents. Poxviruses are readily inactivated by a number of detergents. There is also minimal risk of persistence or survivability of the MVA vector in the environment due to its loss of viability and decay at ambient temperatures.

2. Possible disease including allergenic and toxic effects in animals and plants

Toxicology studies have ruled out the potential pathogenicity of the GMO in rodents. The GMO is therefore not expected to be allergenic or toxic in animals. In addition, the GMO is not capable of transducing plant cells.

3. Effects on the dynamics of populations of species in the receiving environment and the genetic diversity of these populations

There is no identified potential for competitive increase in the human population as study subjects who will receive the GMO will have no selective advantage over non-treated humans.

The MVA GMO has been modified from the parental vaccinia virus to be replication-deficient and there is no basis to consider that addition of the HBV transgene sequence to the GMO would

promote any post-release selection for increased invasiveness. Therefore, no competitive advantage was conferred to the GMO in relation to the parental organism.

4. Altered susceptibility to pathogens facilitating the dissemination of infectious diseases and/or creating new reservoirs or vectors

The administration of the GMO should not increase the dissemination of infectious disease. Neither should GMO administration into the target host alter susceptibility to pathogens in a negative sense. In fact, the role of the MVA-HBV is to induce an antigen-specific immune response designed to eliminate HBV-infected cells in a population already ill with chronic HBV infection.

The GMO is not expected to create new reservoirs since it is not integrative and will be cleared from the host thanks in particular to the host immune response.

5. Compromising prophylactic or therapeutic medical, veterinary, or plant protection treatments

There is no basis to indicate the GMO MVA backbone nor the expressed transgene products will have any negative impact on prophylactic or therapeutic medical or veterinary treatments. This section is not applicable for plant protection since the GMO is not capable of transducing plant cells.

6. Effects on biogeochemistry

Not applicable

IV. Estimation of the risk posed by each identified characteristic of the GMO.

1. Possible negative effects including allergenic and toxic effects in Humans

a. Allergenic effects

See section II.1.a. and II.1.b. There is no basis to indicate the GMO will provoke allergenic adverse effects in the vaccinated study population.

b. Toxicity/Pathogenicity

See section II.1.a. and II.1.b. Preclinical toxicology studies have demonstrated the absence of toxicity, and confirmed the safety of the GMO at equivalent dosage and number of administrations to be performed in the clinical study. The GMO is highly attenuated and rendered avirulent in animal and humans. MVA was originally developed as a safer alternative to vaccinia virus vaccination during the final phase of the small pox eradication effort. Recombinant MVA vectors have been extensively used in many clinical study settings and are considered safe for use in humans without risk from serious adverse events. The risk of toxicity and or pathogenicity to humans following GMO administration in the planned release is therefore expected to be low.

c. Replication competent virus

There is no known poxvirus able to complement MVA to generate a replication competent virus, and spontaneous reversion of MVA to replication competent vaccinia virus has not been documented. Therefore the risk of reversion to a replication competent vector is considered negligible.

d. Shedding/Survivability

The potential for shedding of infectious MVA-HBV particles into the environment is considered negligible especially when administered by the intramuscular injection route. Human clinical studies conducted with similar MVA constructs administered by this route have been mainly unable to detect vector shedding from study subjects in biological samples (sputum, saliva, urine, feces) (Goossens et al. 2013). There is no indication that the HBV transgene could influence the shedding behaviour of recombinant MVA vectors. Preventive measures implemented during the conduct of the clinical trial will also minimize inadvertent dissemination from spills or accidents. Poxviruses are readily inactivated by a number of detergents. There is also minimal risk of persistence or survivability of the MVA vector in the environment due to its loss of viability and decay at ambient temperatures.

e. Reactivation of endogenous retroviruses

The overall risk of reactivation of endogenous retroviruses is considered negligible because MVA remains epichromosomal.

2. Possible disease including allergenic and toxic effects in animals and plants

The potential negative effects to animals are similar to those identified for humans. The likelihood of occurrence is almost null since the proposed release will occur during a human clinical study, and no interaction of the GMO with the environment is expected. In addition, the GMO has a severely restricted host cell range. Therefore, the overall risk to animals is considered negligible. The risk to plants is null since the GMO is not capable of transducing plant cells.

3. Effects on the dynamics of populations of species in the receiving environment and the genetic diversity of these populations

The risk of modification of the population dynamics and of the genetic diversity is considered negligible.

4. Altered susceptibility to pathogens facilitating the dissemination of infectious diseases and/or creating new reservoirs or vectors

The risk of alteration of the susceptibility to pathogens is considered null. Neither facilitation of infectious diseases nor creation of new reservoirs is therefore expected.

5. Compromising prophylactic or therapeutic medical, veterinary, or plant protection treatments

The risk of compromising prophylactic or therapeutic medical protection treatments is considered negligible since no antibiotic resistance gene will be transferred to the host. The risk to animals is

also considered negligible since they are not targeted hosts and risk of accidental transmission is considered null. Finally, the risk to plants is null since the GMO is not capable of transducing plant cells.

6. Effects on biogeochemistry

The risk of effects on biogeochemistry is null.

V. Application of management strategies for risks from the deliberate release of GMO

1. Management strategies for vaccinated patients

The investigational MVA-HBV vaccine will be administered for the first time in a human population. Particular attention will be paid following GMO administration to monitor any immediate or delayed adverse event. Dosage and administration of study vaccines will be performed in steps as described in Figure 1.

Patients will receive a prime-boost administration of the GMO (i.e. ChAd155-hIi-HBV) and another GMO (MVA-HBV), both encoding the hepatitis B core (HBc) and surface (HBs) antigens, and a recombinant protein vaccine containing HBc and HBs antigens adjuvanted with the adjuvant system $AS01_{B-4}$ (HBc-HBs/$AS01_{B-4}$) administered either sequentially or concomitantly to the prime-boost. All vaccines will be administered intramuscularly.

Prior to administration of the next consecutive dose or to escalation to the subsequent step, the safety data will be reviewed by an internal Safety Review Committee (iSRC) independent from the study team. The iSRC will conduct unblinded reviews of all available safety data and will escalate any safety signal to the Applicant which may lead to the modification or suspension of the study.

The safety holding rules which will be assessed by the iSRC are displayed in Table 1:

- Holding rules 1, 2 and 3 will be assessed by the iSRC during the safety evaluation.
- Holding rules 1 and 3 will also be monitored by the investigator on a continuous basis irrespective of the number of patients enrolled. If an investigator detects one of the holding rules mentioned above, he/she will immediately put the enrolment or the vaccination on hold and will immediately inform the Sponsor and enter the data in the eCRF. It is the Sponsor's responsibility to put the enrolment or the vaccination on hold at all sites.

Table 1: Holding rules during the planned iSRC

Holding Rule	Event	Number of patients
1a	Death or any life-threatening SAE	≥ 1
1b	Any SAE that is considered as related to the vaccine in an investigational group	≥ 1
1c	Any withdrawal from the study (by investigator or patient request) following a Grade 3 AE that cannot reasonably be attributed to a cause other than vaccination	≥ 1
1d	Any local or general solicited AE leading to hospitalization, or fever > 40°C (104°F) that cannot reasonably be attributed to a cause other than vaccination, or necrosis at	≥ 1

Holding Rule	Event	Number of patients
	the injection site, within the 7-day (days 1-7) post-vaccination period	
2a	Any Grade 3 solicited local AE (lasting 48h or more) in an investigational group, within the 7-day (day 1-7) post-vaccination period	At least 25% AND ≥ 2 in a vaccine group
2b	Any Grade 3 solicited general AE (lasting 48h or more) in an investigational group, that cannot reasonably be attributed to a cause other than vaccination, within the 7-day (day 1-7) post-vaccination period	At least 25% AND ≥ 2 in a vaccine group
2c	Any Grade 3 unsolicited AE in an investigational group, that cannot reasonably be attributed to a cause other than vaccination, within the 7-day (day 1-7) post-vaccination period or Any Grade 3 abnormality in pre-specified hematological or biochemical laboratory parameters in an investigational group within the 7-day (day 1-7) post-vaccination period	At least 25% AND ≥ 2 in a vaccine group
3a	Any acute exacerbation or severe hepatitis flare (intermittent elevation of ALT to more than 10 times the ULN)*	≥ 1
3b	Any acute exacerbation or moderate hepatitis flare for more than 2 weeks (intermittent elevation of ALT to > 5 to < 10 X ULN)*	≥ 1
3c	Any ALT flare (ALT > 3XULN) with other substantial liver biochemical change defined as an increase in serum bilirubin to ≥2 x ULN and/or international normalized ratio (INR) >1.5*	≥ 1
3d	Any hepatic decompensation defined as the occurrence of 1 or more of the following events: ascites, spontaneous bacterial peritonitis, hepatorenal syndrome, variceal bleeding, or hepatic encephalopathy	≥ 1
3e	Any reactivation of chronic hepatitis B as characterized by HBV-DNA breakthrough accompanied with 1 or more of the following: ALT elevation to > 3 X ULN, substantial biochemical changes, or hepatic decompensation as defined above	≥ 1
3f	Any AE related to spontaneous local or general bleeding AND Thrombocytopenia < 50,000/mm^3	≥ 1

* The abnormal value should be confirmed by an additional testing preferably within 48-72 hours; if no additional value is available within one week, the initial value will be considered as confirmed. ULN for ALT = 48 U/L (Q^2 Solutions Laboratory); ULN for bilirubin = 22 µmol/L (Q^2 Solutions Laboratory). In case the test is repeated locally, the reference range of the local laboratory should be used and recorded.

2. Management strategies for workers protection

All study personnel who will handle, store, prepare or administer the GMO will be appropriately trained. All personnel handling the GMO will be required to wear appropriate personal protective equipment. Accidental exposure in the form of a needle-stick injury will be minimised by the completion and demonstration of competency in the trial specific requirements for every member of staff involved with the study. All relevant standard and study specific operating procedures must be followed in the event of such as accident or incident occurring. No accidental inoculation is expected of staff but if it was to occur it would be considered as being safe and followed up by existing local guidance.

3. Management strategies in case of accidental spillage

Accidental spillages will be reported according to local procedures. Key staff members of the clinical study team will be contacted immediately. A report of the spillage will be documented and the clean-

up procedure will be monitored according to local procedures. Records of staff training and competency will be documented.

VI. Determination of the overall risk of the GMO

The active ingredient of the MVA-HBV investigational vaccine is the highly attenuated replication-deficient MVA encoding a fusion of the HBc and HBs proteins of the HBV, separated by the 2A cleaving region of the FMDV. There are no risks associated with the expressed products of the transgene. The expression of the transgene in the vaccine reciepient or study subject will be transient since the GMO is a replication-deficient, non-propagative and a cytoplasm-localized non-integrative virus.

MVA is a genetically stable strain of vaccinia virus that does not integrate its viral DNA into the host cell genome as the virus remains localized in the cell cytoplasm. And in terms of genetic stability, MVA is a double-stranded DNA virus, and as all orthopoxviruses, encodes its own DNA polymerase that serves a proofreading role which results in typically low rates of mutation from one passage to the next.

Genetic stability of the MVA-HBV GMO has been been assessed and demonstrated by analytical testing performed throughout development starting from the primary virus seed (PVS), to the master virus seed (MVS), and at different stages during the manufacture of clinical material. All steps of the manufacture of the recombinant MVA-HBV vaccine are conducted using current Good Manufacturing Practices (cGMP) based on a seed lot system. Clinical lots of MVA-HBV vaccine are produced from a GMP-manufactured MVA-HBV MVS lot.

Genetic stability of the MVA-HBV GMO is verified at various steps through assessment of identity, purity, potency and extensive safety testing. Analytical measures include the determinaton of infectious titer in permissive primary cell culture, DNA sequencing of the transgene, restriction analysis, identity and purity testing by PCR amplification of specified target sequences, and transgene expression by Western blot analysis.

The long-term stability of the MVA-HBV MVS starting material and the GMO vaccine when stored frozen at temperatures <-60°C will be followed according to pre-defined stability plans up to 48 months and 60 months, respectively. Stability data is available following 24 months storage indicating no change in the stability of the MVS starting material. Long-term stability data for the GMO vaccine has been obtained for up to 18 months when stored at < -60°C, showing the material meets product stability specifications throughout this period of time.

In summary, testing performed at different stages of the production process provides phenotypic and genotypic verification of the genetic stability of the MVA-HBV GMO material as compared to reference standards.

The GMO is replication-deficient, is not integrative and is not expected to survive, multiply or disperse following its release during the proposed clinical study. Moreover, it will be administrated intramuscularly, a route which limits its potential shedding. The recipient is incapable of establishing a productive, transmissible infection in humans. It is therefore not considered to be virulent.

All available nonclinical data suggests that the MVA-HBV vaccine candidate has acceptable immunogenicity, tolerability and toxicity profiles for conducting the clinical trial and is not expected to pose risks during the proposed release.

MVA was originally developed as a safer alternative to vaccinia virus vaccination during the final phase of the small pox eradication effort. Recombinant MVA vectors have been extensively used in many clinical study settings are considered safe for use in humans without risk from serious adverse events.

VII. Conclusion on the potential environmental impact from the release of the GMO

A. Likelihood of the GMO to become persistent and invasive in natural habitats under the conditions of the release

We do not expect the vector to survive or spread in the environment. The possibility of gene transfer of the MVA-HBV vector to other species is negligible minimal under the conditions of the proposed release.

MVA viral vectors have been used extensively in clinical trials both as direct administration and cell therapy strategies. While no data exists on the environmental impact of the MVA-HBV vector, as the Phase I study proposed in this application, will be the first human trial with this particular MVA construct, there is no scientific basis to suspect that the presence of HBV transgene in the MVA viral vector will change its distribution characteristics, shedding, or replicative capacity compared to other inserts used in the same MVA vector backbone.

Therefore it is not expected that deliberate release of MVA-HBV in this clinical study will impair other humans, flora or fauna, near or far to the release area.

The likelihood of the GMO becoming persistent and invasive in the environment is low for the following reasons:

- The MVA-HBV vector to be used in the proposed clinical study is replication-deficient, is unable to produce vector particles, and therefore cannot establish propagative infections of host cells or non-targetted host cells.
- Due to its extensive attenuation, MVA exhibits a severely restricted host range and is incapable of producing disease in permissive animal hosts.
- MVA remains in the cytoplasm of infected cells, its genome remains epichromosomal thus avoiding the risk of integration of the GMO DNA into the host genome.
- No known poxvirus is able to complement MVA to generate a replication competent virus. Spontaneous reversion of MVA to replication competent vaccinia virus has not been documented.
- Shedding of the GMO into the environment is considered negligible especially when adminstred by the intramuscular injection route. Human studies with similar MVA constructs administered by this route have been mainly unable to detect vector shedding from study subjects in biological samples (sputum, saliva, urine, feces).

- The release of the GMO will occur during the conduct of a highly controlled clinical study setting via the intrasmuscular route of administration to study subjects, where procedures will minimize dissemination and inadvertent transmission.
- Any GMO waste generated during the conduct of the clinical study will be managed according to procedures specific to the GMO as approved by the local institutional policy and the clinical study protocol.
- The GMO is capable of transducing only animal cells and not plant cells.

B. Any selective advantage or disadvantage conferred to the GMO and the likelihood of this becoming realised under the conditions of the proposed release

No selective advantage or disadvantage will be conferred by the GMO, neither from the MVA backbone nor from the HBV transgene, to the target host.

C. Potential for gene transfer to other species under conditions of the proposed release of the GMO and any selective advantage or disadvantage conferred to those species

The GMO will be released during a clinical study conducted according to the Good Clinical Practices, at identified investigational sites and under the responsibility of principal investigators. Therefore and since no shedding is expected, there is no expected gene transfer to other species than the target host (i.e. patients enrolled in the clinical study). The only non-target organism that might accidently receive an administration of the GMO is clinical study personnel following a needle-stick injury. If an accidental administration, or transfer of the GMO to a non-target host, were to occur there is no advantage or disadvantage conferred by the GMO.

D. Potential immediate and/or delayed environmental impact of the direct and indirect interactions between the GMO and target organisms (if applicable)

The expected biological activity of the GMO following intramuscular injection is the induction of an immune response against the HBV proteins that constitute the transgene. This should translate into a clinical benefit to the study subjects who are chronically infected with HBV.

E. Potential immediate and/or delayed environmental impact of the direct and indirect interactions between the GMO with non-target organisms, including impact on population levels of competitors, prey, hosts, symbionts, predators, parasites and pathogens

The GMO will be released in a highly controlled clinical study setting with a neglible likelihood of the GMO coming into contact with the environment.

F. Possible immediate and/or delayed effects on human health resulting from potential direct and indirect interactions of the GMO and persons working with, coming into contact with or in the vicinity of the GMO release

The GMO will be released during a clinical study via the intramuscular route of administration. Human studies with similar MVA constructs administered by this route have been unable to detect vector shedding from study subjects. Since there is no basis to expect shedding of the GMO from the vaccinated cohort in the proposed study, no immediate and/or delayed effect resulting from

potential indirect interactions with persons working with or coming into contact with or in the vicinity of the GMO release is expected.

Furthermore the MVA vector cannot cause disease in animals or humans, and according to EEC regulations for the protection of workers (reference needed … might be NIH or CDC guidelines), persons working with MVA are not required to be immunized prophylactically.

G. Possible immediate and/or delayed effects on animal health and consequences for the feed/food chain resulting from consumption of the GMO and any product derived from it, if it is intended to be used as animal feed

Not applicable.

H. Possible immediate and/or delayed effects on biogeochemical processes resulting from potential direct and indirect interactions of the GMO and target and non-target organisms in the vicinity of the GMO release

No immediate or delayed effects on biogeochemical processes are expected.

I. Possible immediate and/or delayed, direct and indirect environmental impacts of the specific techniques used for the management of the GMO where these are different from those used for non-GMOs

The GMO will be released in a clinical study setting with a neglible likelihood of the GMO coming into contact with the environment. The only techniques foreseen to manage the GMO upon release (upon administration to study subjects), is a decontamination of the immediate area and to manage biohazard waste according to study protocol and local or institutional procedures specified for the GMO. These procedures will serve to further limit any contact or spread of the GMO to the environment. There are no expected immediate and/or delayed direct or indirect environmental impacts from these procedures.

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