TH HBV VV-001 Clinical study

Recombinant vaccine vector Modified Vaccinia Ankara-HBV

(MVA-HBV)

Annex IIIA according to Directive 2001/18/EC

Information Required in Notifications Concerning Releases of Genetically Modified Organisms Other than Higher Plants

Notifier: GlaxoSmithKline Biologicals

EudraCT number: 2017-001452-55

Version 1.0 – February-2018

Table of contents

List of Figures

List of Tables

Abbreviations

AE	Adverse Event
BSL1	Biosafety Level 1
CEF	Chick Embryo Fibroblast
CHB	Chronic Hepatitis B
CMI	Cell-Mediated Immunity
DNA	Deoxyribonucleic Acid
DP	Drug Product
DS	Drug Substance
EEC	European Economic Community
eGFP	Enhanced Green Fluorescent Protein
FMDV	Foot-And-Mouth Disease Virus
FTIH	First-Time-In Human
GLP	Good Laboratory Practices
GMO	Genetically Modified Organism
GMP	Good Manufacturing Practice
GSK	GlaxoSmithKline
HBc	Hepatitis B Core Nucleocapsid
HBs	Hepatitis B Surface Antigen
HBsAg	Hepatitis B Surface Antigen
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HHD	Hla.A2/Dr1
HIV	Human Immunodeficiency Virus
IM	Intramuscular
iSRC	Internal Safety Review Committee
IU	Infectious Units
MVA	Modified Vaccinia Ankara Virus
MVS	Master Virus Seed
NA	Nucleo(S)Tides Analogues
PCR	Polymerase Chain Reaction
Pfu	Plaque Forming Unit
SAE	Serious Adverse Event
sp	Synthetic Promoter
SPF	Specific Pathogens Free
VSS	Virus Seed Stock
VV	Vaccinia Virus

I. GENERAL INFORMATION

A. Name and address of the notifier (company or institute)

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B. Name, qualifications and experience of the responsible scientist(s)

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C. Title of the project

The release of the GMO will take place during a clinical study 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".

EudraCT number of the study is: 2017-001452-55 and the Company code is: TH HBV VV-001.

II. INFORMATION RELATING TO THE GMO

A. Characteristics of (a) the donor, (b) the recipient or (c) (where appropriate) parental organism(s):

Please find below the proposed nomenclature of terms used in this document:

- **The donor organism**: the organism(s) from which the sequences encoded by the GMO are derived
- **The recipient organism**: the engineered vector with an "empty" cassette (i.e. without the transgene)
- **The parental organism**: the organism from which the engineered vector is derived

1. scientific name;

(a) Donor

Donors are identified as:

- Gene sequences from the human hepatitis B virus (HBV) encoding the truncated core protein (HBc) and the full-length surface antigen (HBs).
- Gene sequence from the foot-and-mouth disease virus (FMDV) encoding the 2A cleavage sequence.

(b) Recipient

The recipient is the MVA Red vector system which is derived from the parental MVA organism.

(c) Parental

The parental organism is the modified vaccinia virus Ankara, known as MVA, from which the recipient MVA Red vector system is derived. 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). The vaccinia virus exhibits a wide host range, is able to efficiently replicate in human cells, and has caused laboratory-acquired vaccinia virus infections (Isaacs, 2012). In contrast, MVA exhibits a narrow host range and is not able to replicate in human cells. For those reasons, the vaccinia virus is classified as a risk group 2 biological agent, whereas the MVA strain is is classified as a risk group 1 agent (Stellberger 2016).

2. taxonomy;

(a) Donor

HBV: Family: Hepadnaviridae; Genus: Orthohepadnaviruses; Species: Human hepatitis B virus

FMDV: Family: Picornaviridae; Genus: Aphthoviruses; Species: foot-and-mouth disease virus

Human: Family: Hominidae; Genus: Homo, Species: sapiens

(b) Recipient

MVA vector system: Not applicable

(c) Parental

MVA: Order: Poxviridae/Chordopoxviridae; Genus: Orthopoxvirus; Species: Vaccinia Virus

3. other names (usual name, strain name, etc.);

(a) Donor

None

(b) Recipient

MVA Red vector system

(c) Parental

MVA

4. phenotypic and genetic markers;

(a) Donor

Hepatitis B virus (HBV) is a highly contagious DNA virus restricted to humans, transmitted via infected blood and semen, which affects the liver. Infection with HBV can cause both acute resolving infection and chronic hepatitis B (CHB) and accounts for about 780,000 related deaths per year. More than 240 million people worldwide are chronically infected with hepatitis B.

The GMO transgene is constituted by a sequence derived from two HBV proteins: the core nucleocapsid (HBc) and the small surface antigen (HBs), separated by the 2A region of the foot-and-mouth disease virus (FDMV), that allows processing of the HBc and HBs fusion into separate protein antigens. Foot and mouth disease virus (FMDV) infects cattle as the main host. Humans can also harbor FMDV in their respiratory tract.

The HBV transgene is under the transcriptional control of the control P7.5 MVA early promoter. The FMDV 2A region between the HBc protein and the HBs protein, mediates polyprotein processing by a translational effect known as ribosomal skip (Donnelly et al. 2001). After transfection into mammalian cells, cleavage occurs.

The region 2A-mediated protease cleavage occurs at the C-terminus of 2A just ahead of the last proline in the amino acid sequence. The proline remains at the N-terminus of the HBs protein, while the 23 amino acids preceding the proline cleavage site remain with the HBc-2A polypeptide. The 2A region (18 amino acids) has been supplemented with a spacer of 6 amino acids at its N-terminus; spacers of this nature have been reported to increase the efficiency of 2A mediated cleavage.

The expression of the transgene, following protease processing, thereby results in the production of two separate polypeptides: HBc-spacer-2A and HBs. For brevity the HBc-spacer-2A polypeptide will be referred to as the HBc protein throughout the dossier.

Each antigenic sequence was codon-optimised for expression in eukaryotic cells, chemically synthesised and assembled.

(b) Recipient

The recipient organism is a recombinant MVA-Red vector system that is derived from the MVA virus parent.

The MVA-Red vector system originates from a vial of MVA parent provided by the State of Bavaria. The attenuated parent MVA was generated by passaging vaccinia virus more than 570 serial passages in chick embryo fibroblast (CEF) cells. The MVA stock at passage 572 was frozen in 1978 (MVA SB) and was supplied to Okairos (later transferred to GSK) under agreement with the Ministry of Bavaria. The State of Bavaria identified the MVA strain as follows: "*MVA virus vectors in their original, unmodified form ("MVA SB") as manufactured at the former Bayerische Landesimpfanstalt by using the then approved virus seed batch 460 MG (571th passage) - growth substrate: HFE-cells) as described in Mayr, A., Hochstein-Mintzel, V. and Stickl, H. (1975), Infection 3, 6-14, which date back to earlier than December 31, 1978*."

Therefore, the MVA-Red vector system is derived from the original MVA virus seed batch 460 MG (from attenuation passage 571 (termed MVA-571)) that was described by Prof. Anton Mayr (Mayr, A. et al. Infection 1975) and manufactured by the Bayerisches Landesimpfanstalt. This is the same MVA strain that originated from Prof. Anton Mayr, as derived from MVA-572, that was used in Germany during a 1978 vaccination program in more than 120,000 human subjects (as part of a two-step vaccination protocol with a conventional vaccinia virus vaccine against smallpox).

Dr. Antonio Siccardi (University of Milan, Italy) has constructed the MVA-Red vector system by sub-cloning the coding sequence for red fluorescent protein (RFP) from the plasmid pHCRed1-1 (Clontech) into a transfer plasmid pΔIII-HR-sP, which harbors the MVA deletion III (ΔIII) genomic flanking regions. Transfection with that transfer plasmid into CEFs, already harboring the wild type MVA Mu77, allowed the red virus to recombine at the homologous ΔIII sites resident in both the virus and the recombinant plasmid, an event readily detectable by red fluorescence from infected recombinant cells. Absence of ΔIII parental virus sequence was confirmed by PCR

(c) Parental

The MVA viral vector is a highly attenuated strain of vaccinia virus originally developed as a smallpox vaccine. The MVA vector, derived from the replication-competent dermal vaccinia strain Chorioallantois vaccinia virus Ankara (CVA), has been attenuated by more than 570 serial passages in primary cultured chicken embryo fibroblasts (CEF). The passaging has resulted in many mutations in the parental virus genome, as well as six major deletions resulting in the loss of roughly 15% (30 kbp) of the original genetic information. Deletions include a number of genes that contribute to viral evasion from host immune responses and that determine virus host range (Meisinger-Henschel et al. 2010). These mutations have rendered the MVA virus highly attenuated, unable to productively infect most mammalian cell lines and replicates very poorly, if at all, in most mammalian cell types, including primary human cells and most transformed human cell lines. Following infection by MVA, non-infectious immature virions and abnormal particles are produced but no infectious particles.

MVA has subsequently been extensively used as a viral vector to induce antigen-specific immunity against transgenes, both in animal models and in humans. Numerous MVA-based vaccines have been tested in clinical trials (Goossens et al. 2013).

5. degree of relatedness between donor and recipient or between parental organisms;

The donors do not have any relatedness with the recipient or parental organisms.

The recipient is derived from the parental organism as described in section II.A.4.c above.

6. description of identification and detection techniques;

(a) Donor

Several assays are now commercially available for identification and detection of HBV. HBV is most commonly detected in serum or whole blood although the virus can also be detected in dried blood, hepatocytes and other tissues such as renal tissues.

Serological methods are the most widely used methods since they are rapid and cost effective to detect different markers such HBsAg, anti-HBcAg, HBeAg, and anti-HBeAg. Amongst these assays, ELISA and chemiluminescent enzyme immunoassay are dominant methods for HBV detection and quantification followed by molecular techniques such as RT PCR.

(b) Recipient

Identification of the MVA Red vector system can be performed using a PCR-based assay with primers specific to the MVA Red vector backbone.

(c) Parental

PCR-based assays have been developed allowing the differentiation between human pathogenic vaccinia viruses and attenuated MVA strains. To differentiate between MVA and other vaccinia strains, these assays take advantage of the fact that MVA has lost about 15% of its genome as compared to other vaccinia viruses with six described major deletion regions (Del-I, -II, -III, -IV, -V, and -VI).

7. sensitivity, reliability (in quantitative terms) and specificity of detection and identification techniques;

(a) Donor

See Section 6a. Please refer to each manufacturer notice for sensitivity and specificity of the assay.

(b) Recipient

The PCR assay is specific to the MVA-Red vector as primers are designed based on the MVA-Red vector sequence to be identified. The assay has not been validated yet.

(c) Parental

See Section II.6.(c) above.

8. description of the geographic distribution and of the natural habitat of the organism including information on natural predators, preys, parasites and competitors, symbionts and hosts;

(a) Donor

HBV is a highly contagious pathogen that infects mostly humans but can also infect related species such as primates (usually for R&D purposes).

FMDV is an animal (cloven-hoofed) pathogen which rarely causes infections in humans.

(b) Recipient

The recipient is a recombinant MVA vector system engineered by molecular techniques and maintained in laboratories; there is therefore no natural habitat.

(c) Parental

MVA is a highly attenuated virus that must be maintained frozen or in primary cell culture in laboratories. MVA does not exist in the natural habitat, and since the successful completion of the smallpox eradication vaccination campaign, neither does vaccinia virus.

9. organisms with which transfer of genetic material is known to occur under natural conditions;

(a) Donor

There is no transfer of genetic material from the donors to their natural hosts.

(b) Recipient

The recipient is a recombinant MVA vector system engineered by molecular techniques and maintained in laboratories; there is therefore no natural habitat.

(c) Parental

MVA and vaccinia virus do not exist in the natural habitat.

10. verification of the genetic stability of the organisms and factors affecting it;

(a) Donor and (b) Recipient

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 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 of development and production process through integrity analysis of the vector and transgene insert through assessment of identity, purity, potency and extensive safety testing. Analytical measures include the determination 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 pre-GMP research seed is tested to exclude the presence of non-recombinant MVA viruses, and the identity of the construct is confirmed by PCR, restriction mapping and sequencing of the HBV transgene. The master virus seed (MVS), produced under GMP conditions, is tested for identity by PCR and by sequencing of the HBV transgene. For each production lot, identity is demonstrated by PCR, while purity, biological potency and safety are also confirmed.

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.

(c) Parental

See Section II.A.10.(a) and (b) above.

11. pathological, ecological and physiological traits:

(a) classification of hazard according to existing Community rules concerning the protection of human health and/or the environment;

(a) Donors

HBV. Hepatitis B virus has been classified, as other Hepadnaviruses, as Class 3 under the Directive 2000/54/EC on the protection of workers from risks related to exposure to biological agents at work. With however a note stating that HBV presents a limited risk of infection for workers because it is not normally infectious by air-borne route.

FMDV. The Picornaviridae family has been classified as Class 2 under the Directive 2000/54/EC. FMDV has not been specifically classified under the Directive 2000/54/EC.

However, only a very small portion of DNA from the donor HBV and FMDV organisms is present in the transgene, not enough to create infectious HBV or FMDV particles.

(b) Recipient

The recombinant MVA vector is not classified by the EEC directive, but most competent authorities view recombinant MVA vectors as belonging to biohazard safety level 1 (BSL1), since it is a highly attenuated virus strain that is replication-deficient in human cells, exhibits a severely limited host range for infectivity, is non-virulent to animals, and is unable to cause human disease (Goossens, 2013).

No reports of MVA transmission to health-care personnel from vaccine recipients have been published. Furthermore, laboratory and other health-care personnel who work with highly attenuated strains of VV (including MVA) do not require routine vaccinia vaccination.

(c) Parental organism

See Section II.11.(b). Due it's highly attenuated replication-deficient state and inability to cause infection, the MVA virus parent is viewed by most competent authorities as belonging to hazard group BSL1.

(b) generation time in natural ecosystems, sexual and asexual reproductive cycle;

(a) Donors

For detailed information on the generation time of the hepatitis B virus please refer to (Lin et al. 2015). However, not relevant, as only a very small portion of DNA from the donor HBV and FMDV organisms is present in the transgene, not enough to create infectious HBV or FMDV particles.

(b) Recipient

Not applicable since the recipient MVA vector system is replication deficient.

(c) Parental organisms

Not applicable since the MVA parental strain is replication deficient.

(c) information on survival, including seasonability and the ability to form survival structures;

(a) Donors

The transgene gene segments, which are based on donor sequences from HBV (HBc and HBs antigens), and FMDV (2A region), are unable to reverse the replication-deficient genotype of vector. The recipient is incapable of completing a reproductive cycle and hence cannot survive in the natural ecosystem.

(b) Recipient

The MVA vector system cannot persist in the environment due to its loss of viability and decay at ambient temperatures. In the targeted human host cell, MVA cannot form complete viral particles, therefore cannot form the structures necessary to survive more than temporally within the targeted host nor in the environment.

(c) Parental organisms

Same as for recipient organism.

(d) pathogenicity: infectivity, toxigenicity, virulence, allergenicity, carrier (vector) of pathogen, possible vectors, host range including non-target organism. Possible activation of latent viruses (proviruses). Ability to colonise other organisms;

(a) Donors

While the wild type HBV and FMDV viruses are known to be pathogenic, the HBc, HBs and 2A region gene sequences extracted from them and compiled into the GMO transgene, are not known to alter the infectivity, toxigenicity, virulence or allergenicity of the GMO vector.

(b) Recipient

The MVA vector is replication-deficient and only capable of transducing a highly restricted range of host animal cells (mammalian ones). It is devoid of any pathogenic sequences. Toxicology studies performed using the GMO at the same dosage and numbers of intramuscular administrations as will be used in the clinical study, have ruled out potential pathogenicity of the final GMO (see section II.C.2.(i)).

(c)Parental organism

MVA has been demonstrated safe for use in human populations. During the small pox eradication campaign, vaccination with Vaccinia Virus resulted in complications and side effects that 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 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 in being local reactions, fever and flu-like symptoms.

(e) antibiotic resistance, and potential use of these antibiotics in humans and domestic organisms for prophylaxis and therapy;

(a) Donors and (c) Parental

Not applicable. Neither HBV nor FDMV nor MVA encode antibiotic resistance genes.

(b) Recipient

Although plasmids used for construction of the recipient MVA vector system bear antibiotic resistance genes, the GMO does not encompass any of these resistance genes. Therefore, the probability of transfer of any antibiotic sequence to the target host (Human) is very unlikely.

(f) involvement in environmental processes: primary production, nutrient turnover, decomposition of organic matter, respiration, etc.

Not applicable

12. nature of indigenous vectors:

This section is not applicable since there are no indigenous sequences that might enhance the transfer of the genetic material.

(a) sequence;

(b) frequency of mobilisation;

(c) specificity;

(d) presence of genes which confer resistance.

13. history of previous genetic modifications.

This will be a First-Time-In-Human study with the proposed GMO MVA-HBV.

The recombinant MVA vector, with a variety of different transgenes, has been used extensively in clinical studies for vaccination and gene therapy applications, generating a human safety database without raising major safety concerns. No significant adverse effects have been reported and the GMO appears to be generally safe and well tolerated with the main adverse events reported as minor injection site reactions (Verheust et al., 2012; Goossens et al., 2013).

The attenuated MVA strain was used during the end of smallpox eradication campaign in Germany to vaccinate some 120,000 people considered at high risk for complications with the vaccinia virus vaccine. In this high risk population, the vaccine did not produce serious adverse events, but instead was associated with only minor local reactions, fever and flu-like symptoms. Today MVA exists as a licensed product as a vaccine used to protect against smallpox in adults.

B. Characteristics of the vector:

1. nature and source of the vector;

The recipient is a recombinant MVA vector system derived from the MVA virus parent. The recombinant MVA-HBV GMO is produced by homologous recombination between the MVA Red vector system and the shuttle plasmid (p94-HBV-A) containing the HBV transgene.

2. sequence of transposons, vectors and other non-coding genetic segments used to construct the GMO and to make the introduced vector and insert function in the GMO;

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3. frequency of mobilisation of inserted vector and/or genetic transfer capabilities and methods of determination;

MVA is highly attenuated, replication deficient and has negligible likelihood of mobilization. Its release is restricted to the delivery via the intramuscular route of administration to human study subjects in a highly controlled clinical trial setting.

A potential hazard for any gene transfer viral vector is a recombination event between the GMO and its naturally occurring homolog that results in either the transfer of the transgene to the wild type virus, or the reverse, the transfer of essential genomic elements from the WT homolog to the vector resulting in a return to virulence of the vector.

However, the probability of MVA reversion is negligible for a few reasons. Firstly, for homologous recombination to occur it requires co-localization, in this case neither MVA nor its vaccinia virus homologs exist in nature. Vaccinia virus has been eradicated, and MVA can only be maintained frozen or in primary cell culture in laboratories.

Secondly, the extensive attenuation process (> 500 successive cell culture passages) has resulted in the loss of roughly 15% of the parental genome, and there is no known poxvirus able to complement MVA to generate a replication competent virus. Indeed, spontaneous reversion of MVA to replication competent vaccinia virus has never been documented.

Additionally, MVA is a non-integrative virus, following infection of the target human host cell, it remains exclusively in the cytoplasm, its DNA remains outside the cell nucleus thus eliminating any risk of integration of the viral DNA into the host genome. Also because of the severely restricted host range of MVA, its lack of virulence in animals, and its highly attenuated replication, we do not expect the vector to survive or spread in the environment.

The identification and detection assays detailed in Section II.A.6. above could be used to determine genetic transfer in case of suspicion.

4. information on the degree to which the vector is limited to the DNA required to perform the intended function.

Recombinant MVA-HBV is composed only of the MVA backbone and the inserted transgene. In terms of the MVA backbone, sequences rendering the vaccinia virus pathogenic have been removed by extensive serial passage. What remains of the MVA backbone functions as an efficient viral vector for the selective transfer of genetic material into the targeted host cell.

In terms of the transgene, the expected biological activity of the vaccine candidate is the induction of an antigen-specific immune response against both HBV proteins (HBc an HBs antigens) that constitute the HBV transgene.

C. Characteristics of the modified organism:

1. information relating to the genetic modification:

(a) methods used for the modification;

Genetic modifications to generate the final GMO involve insertion and deletion of genetic material using molecular biology techniques (detailed below).

(b) methods used to construct and introduce the insert(s) into the recipient or to delete a sequence;

The production of the recombinant MVA-HBV viral vector was performed according to Di Lullo et al. (2010)

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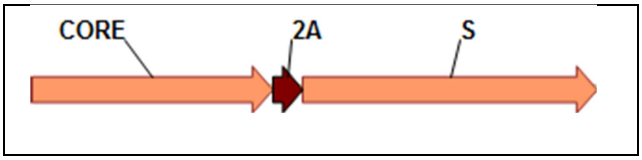
(c) description of the insert and/or vector construction;

The MVA-HBV transgene encodes the truncated core nucleocapsid protein (HBc) from adw2 strain genotype A and the full-length small surface antigen (HBs) from adw2 strain of the hepatitis B virus (HBV). The HBc-HBs sequence is separated by the self-cleaving 2A region of the foot-and-mouth disease virus that allows processing of the fusion protein into separate HBc and HBs antigens. A schematic representation of the transgene is provided in **Error! Reference source not found.**.

Figure 1: Schematic representation of the MVA-HBV transgene



(d) purity of the insert from any unknown sequence and information on the degree to which the inserted sequence is limited to the DNA required to perform the intended function;

The insert has been described above. Its identity and purity are assessed at several steps of the manufacturing process of the GMO:

- Identity and purity by PCR are performed on the master viral seed and drug product,
- Transgene sequencing is performed on the master virus seed in its final container,
- Transgene expression and identity is assessed by Western blot using two antibodies (one against the HBc, the second one against the HBs) on the master virus seed in its final container and on the drug product .

(e) methods and criteria used for selection;

Rationale for choosing the HBc and HBs proteins encoded by the transgene is detailed below:

i. Core protein

Several published studies compared the HBV antigen-specific T-cells in different segments of patients affected by HBV (post-acute infection, patients recovering from a chronic infection, active chronic infection and inactive carriers). The outcome of these studies highlighted that a strong, multi-specific T-cell response, particularly to the HBcAg, is essential for the clearance of HBV. When comparing T cells from patients with a chronic HBV resolving infection versus patients with unresolved chronic HBV infection, higher $CD4^+$ T-cells and $CD8^+$ T-cells specific to the core protein was evidenced in patients with resolving infection (Li et al. 2011, Liang et al. 2011, Boni et al. 2012).

Further evidence of the role of T-cells targeting the HBc antigen in resolving HBV infection comes from data in bone marrow transplants. Bone marrow recipients with chronic hepatitis B effectively cleared their infection after they received bone marrow from donors naturally immune against HBV infection. The clearance of infection was associated with the transfer of core-specific $CD4^+$ T-cells from the donor to the recipient and an increase in $CD4^+$ T-cells and $CD8^+$ T-cells predominantly specific to the HBc antigen (Lau et al 2002).

In addition, the core protein is highly conserved across HBV genotypes and subtypes.

ii. Surface antigen

HBs as the principle surface antigen of HBV, contains the key antigenic determinants (defining the genotype) as well as some of the key cross-genotype-preserved B-cell epitopes responsible for induction of broad neutralizing responses (Bhatnagar et al. 1982, Ryu et al. 1997). Although the HBs sequence is variable across genotypes, the Applicant's licensed prophylactic hepatitis B vaccine, Engerix-B, using the same HBs sequence, is protective against HBV across genotypes.

(f) sequence, functional identity and location of the altered/inserted/deleted nucleic acid segment(s) in question with particular reference to any known harmful sequence.

The transgene sequence (see **Error! Reference source not found.**) does not encode any known harmful, pathological or allergenic products. Toxicology studies performed with the GMO have demonstrated its safety at the same dosages to be administered in the clinical study.

2. information on the final GMO:

(a) description of genetic trait(s) or phenotypic characteristics and in particular any new traits and characteristics which may be expressed or no longer expressed;

The MVA-HBV GMO consists of a recombinant replication-defective MVA engineered to express the HBc and HBs proteins of HBV.

(b) structure and amount of any vector and/or donor nucleic acid remaining in the final construction of the modified organism;

The structure of the transgene in the GMO vaccine candidate construct has been confirmed by DNA sequencing to conform to the desired transgene encoding HBc-2A region-HBs, and is identical to the transgene carried by the shuttle plasmid. Testing is performed to ensure no selection marker genes are left in the GMO.

(c) stability of the organism in terms of genetic traits;

Genetic stability is assessed at several steps of the manufacturing process to ensure that there is no genetic modification during GMO manufacturing:

Genetic stability is verified by following assays:

- Identity and purity by PCR is performed at the harvest step of the master virus seed and drug product.
- Genetic characterization by DNA sequencing of the transgene is performed on the master viral seed
- Transgene identity is assessed by monitoring its expression via Western blot on the master viral seed and drug product.

(d) rate and level of expression of the new genetic material. Method and sensitivity of measurement;

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(e) activity of the expressed protein(s);

The expected biological effect of the expressed HBc and HBs proteins is to trigger an antigen-specific immune response against the HBV proteins as the GMO is intended for HBV treatment.

In nonclinical development, the subsequent administration of MVA-HBV following ChAd155-hIi-HBV in HLA.A2/DR1 (HHD) transgenic mice further increased the $CD8^+$ T-cell response to both antigens. Following the MVA boost, a higher frequency of HBc-specific $CD8^+$ T-cells was observed in mice primed with ChAd155-hIi-HBV versus mice primed with ChAd155-HBV, while HBs-specific $CD8^+$ T-cell responses were not further enhanced. When administered to HHD transgenic mice, the full vaccination regimen (i.e. sequential or concomitant administration of viral vectors and adjuvanted proteins) leads to robust $CD4^+$ T-cell, $CD8^+$ T-cell and antibody responses to both vaccine antigens. Moreover vaccine-induced HBs- and HBc-specific $CD4^+$ and $CD8^+$ T-cells were detected in the liver of animals vaccinated with both vaccine regimens.

(f) description of identification and detection techniques including techniques for the identification and detection of the inserted sequence and vector;

A test for identity by polymerase chain reaction (PCR) has been developed using specific primers for the transgene. The resulting amplicons from this assay provide complete coverage of the transgene. The pattern is then visualized by electrophoresis on agarose gel. This assay is performed on the MVS and each lot of drug product.

Genetic characterization by DNA sequencing of the transgene is performed on the MVS in its final container.

Western blot immunoassays are used to confirm the identity of the GMO construct by evaluating transgene expression of the HBc and HBs proteins.

(g) sensitivity, reliability (in quantitative terms) and specificity of detection and identification techniques;

Identity testing of MVA-HBV construct as confirmed by DNA sequencing of the transgene is considered sensitive, reliable and specific since the obtained nucleotide sequence must conform to the predicted sequence of the transgene as carried by the shuttle plasmid.

Identity testing of the GMO vaccine is performed at several stages during manufacture of the product using various methods including: identity testing by PCR and identity testing of transgene expression by Western blot immunoassay . The identity test by PCR is considered sensitive, specific and fit for purpose as the multiple sets of primers used in the assay have been designed specifically to target nucleotide sequences located on the transgene. The Western blot immunoassay is also considered sensitive, reliable and specific since it is performed using primary antibodies targeted specifically to the expression products of the transgene.

(h) history of previous releases or uses of the GMO;

The recombinant MVA vector, with a variety of different transgenes, has been used extensively in clinical studies for vaccination and gene therapy applications, generating a large human safety database without raising major safety concerns. No significant adverse effects have been reported and the GMO appears to be generally safe and well tolerated with the main adverse events reported as minor injection site reactions (Verheust et al. 2012, Goossens et al. 2013). This will be a First-Time-In-Human study with the proposed GMO, MVA-HBV.

Vaccination using a priming dose of recombinant chimpanzee-derived adenoviral (ChAd) vector followed by a booster dose of recombinant MVA vector have shown that heterologous prime-boost regimens are a safe and synergistic immunogenic delivery platform. Clinical studies have been conducted in the context of different vaccine development programs including: malaria, HIV, HCV and Ebolavirus.

The MVA given as a boost after ChAd priming induced antigen-specific T-cell responses with mixed CD4+/CD8+ phenotypes, as well as substantial specific functional IgG responses, with prominent antigen-specific CD8+ T-cells responses (Hodgson et al. 2015; Ogwang et al. 2013; Reyes-Sandoval et al. 2010; Sheehy et al. 2011; Sheehy et al. 2012; Swadling et al. 2014).

More advanced studies recently supported that such a prime-boost strategy using the same antigenic insert generated sustained memory and unprecedented effector T-cell responses including a high proportion of cytokine-secreting CD8+ T-cells (Ewer et al. 2013; Swadling et al. 2014).

(i) considerations for human health and animal health, as well as plant health:

(i) toxic or allergenic effects of the GMOs and/or their metabolic products;

The GMO is not expected to have any toxic nor allergenic effects.

- Potential allergenic effects

As with all injectable vaccines, immediate systemic allergic reactions to vaccination can occur. These are however very rare and are estimated to occur once per 450,000 to once per 1,000,000 vaccinations for vaccines which do not contain allergens such as gelatin or egg protein (Zent et al. 2002).

In order to be able to treat patients with an immediate systemic allergic reaction to vaccination in the proposed trial, patients will remain under observation (visual follow-up) at the study site for at least 60 minutes after vaccination.

- Potential toxic effects

Vaccination in general may lead to local reactions at injection site, such as pain, redness and swelling. Systemic reactions may also occur, such as fever, malaise, fatigue, gastro-intestinal symptoms, or chills. Such reactions are usually transient.

Two toxicity studies were performed in New Zealand White rabbits in a GLP compliant environment using GMO batches that are comparable to the clinical trial materials and using the same administration route: a single-dose local tolerance and a repeat-dose toxicity studies. The dose that will be used in clinic and identical volume were administered. 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 (adjuvanted recombinant proteins 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 study demonstrated that administration of the GMO (0.5mL at 3.9x108 pfu/mL) simultaneously to 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 repeat toxicity study, the animals received a prime with the ChAd155-hli-HBV vector (the transgene also contains the HBc-2A-HBs sequence) followed by 4 administrations of the GMO while they received on the opposite leg concurrently HBc-HBs/AS01B-4 as immune enhancer. 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 CRP 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, then 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 intra muscular route.

- Conclusions

All available nonclinical data suggest that the GMO has demonstrated safety and an acceptable tolerability/toxicity profile for conducting the FTIH clinical trial.

(ii) comparison of the modified organism to the donor, recipient or (where appropriate) parental organism regarding pathogenicity;

The parental organism is MVA which does not cause disease in animals or humans. The GMO transgene is derived from the HBc and HBs sequences from the HBV. There is no basis to indicate that the presence of the transgene in the GMO MVA-HBV will alter its expected non-virulence.

(iii) capacity for colonisation;

The GMO is not expected to display capacity for colonization since it is replication-deficient.

(iv) if the organism is pathogenic to humans who are immunocompetent:

MVA was originally developed expressly for use in immune compromised or other groups thought to be at risk for small pox vaccination using the vaccinia virus. Likewise, the recombinant MVA-HBV GMO is not expected to be pathogenic in this population, and there is no indication that the transgene will change these characteristics since the encoded transgene is not pathogenic or toxic.

(v) other product hazards

None known

III. INFORMATION RELATING TO THE CONDITIONS OF RELEASE AND THE RECEIVING ENVIRONMENT

A. Information on the release

1. description of the proposed deliberate release, including the purpose(s) and foreseen products,

The GMO will be released during clinical trial Th HBV VV-001 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 therapy".

The GMO MVA-HBV has been developed as a therapeutic vaccine candidate for treatment of chronic hepatitis B virus disease. The GMO is intended to be used in a prime-boost vaccine regimen to induce robust antibodies and/or T-cells against different HBV antigens to restore immune control of HBV infection and ultimately to achieve hepatitis B immunological cure, defined by HBsAg loss and sustained HBV DNA suppression in chronic hepatitis B patients (Terrault et al. 2015). The proposed release is aimed at assessing the safety, reactogenicity and immunogenicity of the GMO in chronic HBV patients 18-65 years old well controlled under nucleo(s)tides analogues. Treatment with the GMO aims to clear HBsAg (i.e. HBsAg below detectable level defined as HBsAg loss) or reduce HBsAg level to minimal values in

order to allow at least 15% more patients to safely discontinue NA therapy without virological or clinical relapse.

The proposed vaccination regimen includes a heterologous prime-boost schedule with two viral vectored vaccines coding for the hepatitis B core (HBc) and the hepatitis B surface (HBs) antigens in order to induce a strong CD8+ T-cell response, together with sequential or concomitant administration of AS01B-4-adjuvanted HBc-HBs proteins in order to induce strong antigen-specific CD4+ T-cell and antibody responses.

The GMO is a recombinant replication-deficient MVA vector engineered to express two HBV antigens, the truncated HBc core protein and the full-length HBs surface antigen. It is manufactured in a GMP compliant environment. The MVA-HBV vaccine is formulated in buffer composed of Tris pH 7.7 and sodium chloride. The vaccine is presented as a single-dose (monodose) for intramuscular (IM) administration. The volume per nominal dose is 0.5 ml at 4 x 10e8 plaque forming units (pfu)/mL. The vaccine is stored at <-60°C.

Two separate doses of the MVA-HBV investigational vaccine will be evaluated in the Th HBV VV-001 clinical study: a higher potency dose of 2 x 10e8 pfu per dose and a lower potency dose of 2 x 10e7 pfu per dose.

Table 1: GMO doses in the Th HBV VV-001

Vaccine candidate	Dose
MVA-HBV (low dose)	2 x 10e7 pfu/dose
MVA-HBV (high dose)	2 x 10e8 pfu/dose

2. foreseen dates of the release and time planning of the experiment including frequency and duration of releases,

The study is expected to be initiated in Q3-2018 with a target enrolment of approximately 148 eligible patients. The duration of the study is 120 weeks per patient with a 6 month vaccination followed by a 22 month safety follow-up phase.

3. preparation of the site previous to the release,

The GMO release will occur during a clinical study via intramuscular injection in subjects enrolled in the trial. The GMO release will be performed in designated rooms within a hospital or clinical setting. There is therefore no specific preparation of the site previous to the GMO release other than gathering study materials required to prepare and administer the vaccine, and disinfectants to clean surfaces post-release.

4. size of the site,

The GMO will be administered in clinical rooms.

5. method(s) to be used for the release,

The GMO will be administered to study subjects by intramuscular injection. The vaccine will be prepared and administered by trained clinical study staff following procedures outlined in the clinical study protocol. After each vaccination, the injection site will be covered with a dressing in order to absorb any virus that may leak out through the needle track. The dressing will be removed after 30 minutes and will be disposed as GMO waste by autoclaving or in accordance with the applicable guidelines/standard operating procedures at the investigator's site.

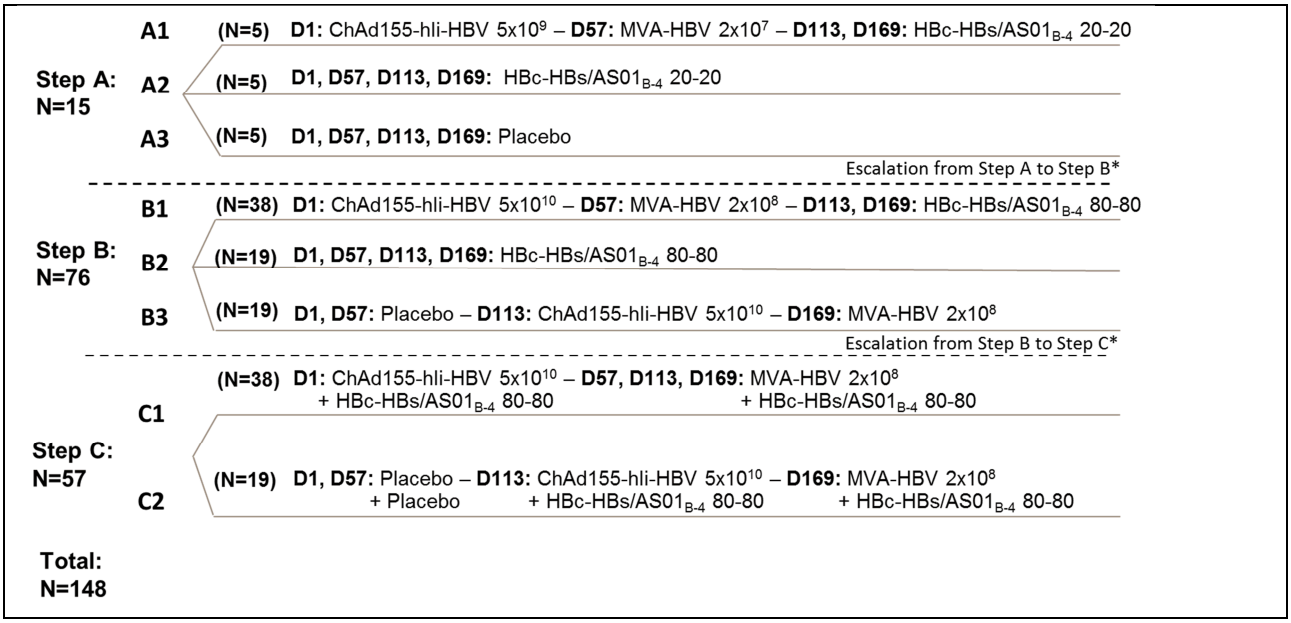
6. quantities of GMOs to be released,

The Th HBV VV-001 is a three step dose-escalating study, randomised within each step to evaluate the safety, immunogenicity and efficacy of different therapeutic HBV vaccination regimens. It will include 8 study groups totaling approximately 148 patients as shown in Figure 2.

The first step of the study (Step A) plans to evaluate a lower dose of ChAd155-hIi-HBV and MVA-HBV followed by two lower doses of the adjuvanted recombinant protein vaccine. Higher doses of the ChAd155-hIi-HBV and MVA-HBV vaccines will be subsequently assessed in Steps B and C. Step B will evaluate either a regimen with both the viral vectored vaccines and the adjuvanted proteins given sequentially, or with the adjuvanted proteins only, or with the viral vectored vaccines only. Step C will evaluate vaccination schedules where viral vectored vaccines and the adjuvanted proteins are co-administered.

Each subject will be followed for safety, immunogenicity and efficacy up to 22 months post last vaccine dose; those patients in groups reaching study predefined success criteria will be followed up to two years after the last vaccine dose (Week 120).

Figure 2: Study steps and study groups



The amount of GMO MVA-HBV to be released during Th HBV VV-001 is calculated as follows:

Table 2: Calculation of released GMO on basis of administered quantities

Step	Dose group	Number of patients	Number of vaccinations	Total quantities administered (pfu)
A	2 x 10e7 pfu	5	5 total doses 1 dose (N=5 at D57)	Total number of low dose administrations =5 1.0 x 10e8 pfu
B	2 x 10e8 pfu	57	57 total doses 1 dose (N=38 at D57) + 1 dose (N=19 at D169)	Total number of high dose administrations 57+133=190 3.80 x 10e10 pfu
C		57	133 total doses 3 doses (N=38 at D57, D113, D169) + 1 dose (N=19 at D169)	
Total quantity of GMO released				3.80 x 10e10

When enrolled at the same study site, these patients should be vaccinated sequentially, and at least 60 minutes apart as acute adverse reactions, like anaphylactic shock, typically occur within 1 hour after vaccination.

7. disturbance on the site (type and method of cultivation, mining, irrigation, or other activities),

Not applicable

8. worker protection measures taken during the release,

Clinical study staff involved in the storage, preparation and administration of the GMO will be appropriately trained. To minimize exposure, all personnel handling the GMO will be required to wear appropriate personal protective equipment, according to institutional procedures established for handling GMO's classified as BSL1 organisms. Most authorities view the use MVA in clinical studies as a BSL1 organism given the fact that MVA does not cause disease and has an extensive human safety record.

9. post-release treatment of the site,

After each vaccination, the injection site will be covered with a dressing in order to absorb any virus that may leak out through the needle track. The dressing will be removed after 30 minutes and will be disposed as biohazard waste in accordance with institutional procedures. A subsequent dressing applied to cover the site of injection may be disposed of by the study subject in household trash without special precautions.

For post-release treatment of the clinical rooms where preparation and administration of the GMO takes place see section V.B.

10. techniques foreseen for elimination or inactivation of the GMOs at the end of the experiment,

All empty vaccine vials, needles and syringes are to be discarded in biohazard waste containers after vaccine preparation/administration is completed for each subject. Keep the secondary containers for vaccine reconciliation by the monitor

Upon reconciliation and accountability, used study materials and unused study vaccine will either be destroyed following institutional procedures for the disposal of biohazard material, or will be returned to the sponsor for destruction.

11. information on, and results of, previous releases of the GMOs, especially at different scales and in different ecosystems.

The GMO has been released at the same dosage and route of administration during the conduct of two toxicology studies performed in rabbits. Otherwise, study TH HBV-001 will be the first release of the GMO in the targeted human host.

B. Information on the environment (both on the site and in the wider environment):

1.geographical location and grid reference of the site(s) (in case of notifications under part C the site(s) of release will be the foreseen areas of use of the product),

The MVA-HBV GMO will be administered during the proposed clinical trial at the following sites:

Clinical Study Site Address	Principal Investigator
Belgium	
Hôpital Erasme; Route de Lennik 808, Brussels	Christophe Moreno
UZ Gent; De Pintelaan 185, Gent	Hans Van Vlierberghe
UZ Antwerpen, Wilrijkstraat 10; Edegem	Thomas Vanwolleghem
SGS Life Science Services, Lange Beeldekensstraat 267, Antwerpen	Stefan Bourgeois
UZ Leuven, Herestraat 49, Leuven	Frederik Nevens
Cliniques Universitaires Saint-Luc, Avenue Hippocrate 10, Brussels	Yves Horsmans
Germany	
Klinikum der J. W. Goethe-Universitaet-Med. K. I, Theodor-Stern-Kai 7, Frankfurt	Stefan Zeuzem
Universitaetsklinikum Aachen-Med. Klinik III, Pauwelsstr. 30, Aachen	Christian Trautwein
Medizinische Hochschule Hannover-Gastroenterologie, Carl-Neuberg-Str. 1, Hannover	Markus Cornberg
Universitaetsklinikum Tuebingen-Innere Medizin I, Otfried-Mueller-Str. 10,Tuebingen	Christoph Berg
Universitaetsklinikum Essen-Gastroenterologie, Hufelandstr. 55, Essen	Guido Gerken
Universitaetsklinikum Bonn-Med. Klinik I, Sigmund-Freud-Str. 25, Bonn	Ulrich Spengler
Johannes-Gutenberg-Universitaet Main, Langenbeckstr. 1, , Mainz	Martin Sprinzl
Universitaetsklinikum Eppendorf-Ambulanzzentrum, Martinistr. 52, Hamburg	Julian Schulze zur
United Kingdom	
Royal London Hospital NHS Foundation, Whitechapel road, London	Patrick Kennedy
John Radcliffe Hospital; Headley Way; Oxford	Paul Klenerman
Southampton General Hospital, Tremona Road, Southampton	Salim Khakoo
Kings College Hospital 1; Denmark Hill, London	Kaushik Agarwal
Queens Medical Centre, Derby Road, Nottingham	Stephen Ryder

2. physical or biological proximity to humans and other significant biota,

The GMO will be released during a clinical study. Except the study subject and the required clinical study staff, no other person is authorized to be present during GMO preparation and administration; thus limiting the proximity to humans.

3. proximity to significant biotopes, protected areas, or drinking water supplies,

Not applicable since the GMO will be administered in a clinical setting.

4. climatic characteristics of the region(s) likely to be affected,

Not applicable since the GMO will be administered during a clinical study.

5. geographical, geological and pedological characteristics,

Not applicable since the GMO will be administered during a clinical study.

6. flora and fauna, including crops, livestock and migratory species,

Not applicable since the GMO will be administered during a clinical study.

7. description of target and non-target ecosystems likely to be affected,

Not applicable since the GMO will be administered during a clinical study.

8. a comparison of the natural habitat of the recipient organism with the proposed site(s) of release,

The recipient organism is an engineered MVA vector maintained in laboratories; there is therefore no natural habitat.

9. any known planned developments or changes in land use in the region which could influence the environmental impact of the release.

Not applicable since the GMO will be administered during a clinical study.

IV. INFORMATION RELATING TO THE INTERACTIONS BETWEEN THE GMOs AND THE ENVIRONMENT

A. Characteristics affecting survival, multiplication and dissemination

1. biological features which affect survival, multiplication and dispersal,

The GMO MVA-HBV is not expected to survive, multiply or disperse following its release during the proposed clinical study.

MVA was developed by extensive attenuation that has resulted in the loss of approximately 15% of its parental genome. This has resulted in a virus that is replication-deficient, has a severely restricted host range, and lacks virulence in animals and humans. 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).

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.

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 behavior 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.known or predicted environmental conditions which may affect survival, multiplication and dissemination (wind, water, soil, temperature, pH, etc.),

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.

3. sensitivity to specific agents.

MVA is susceptible to different chemical agents, commonly used as disinfectants, and has shown sensitivity to heat inactivation. A completely effective elimination is achieved by autoclaving at 121 ° C for 15 minutes.

B. Interactions with the environment

1. predicted habitat of the GMOs,

Following its intramuscular administration, the GMO is expected to remain mainly at the site of injection with some vectors migrating to peripheral draining lymph nodes. As a non-integrative virus, in the infected host cell MVA is localized to the cytoplasm, does not enter the nucleus, and does not integrate its genome into that of the targeted host cell. MVA is an epichromosomal vector.

2. studies of the behaviour and characteristics of the GMOs and their ecological impact carried out in simulated natural environments, such as microcosms, growth rooms, greenhouses,

Since no interaction or survival in the environment can be expected, no specific studies on the potential ecological impact of the GMO have been performed.

3. genetic transfer capability

(a) post release transfer of genetic material from GMOs into organisms in affected ecosystems;

While the GMO viral vector is an effective vehicle to enable the transfer of the transgene into the targeted host cell for the transient expression of the transgene, as delivered by the intramuscular route of administration in a clinical trial setting. Because it is an epichromosomal virus, the GMO is not capable of gene transfer to the host cell genome.

Additionally, the GMO is a replication-deficient non-propagative vector exhibiting a highly restricted host range which limits its ability to interact with organisms other than the targeted host.

The possibility of gene transfer to other species is minimal under the conditions of the proposed clinical release of the GMO. The GMO will be administered to subjects in a clinical setting and is unlikely to come in contact with other animal species.

(b) post release transfer of genetic material from indigenous organisms to the GMOs;

No such post release transfer from indigenous organisms to the GMO is expected since the GMO will be provided in a sealed vial and the release will occur during a clinical trial fulfilling the GCP with traceability of the GMO during the whole study, and therefore the GMO will not come into contact with indigenous organisms in the environment.

4. likelihood of post release selection leading to the expression of unexpected and/or undesirable traits in the modified organism,

The likelihood of post release selection leading to the expression of unexpected and/or undesirable traits in the modified organism is negligible, because the GMO organism will not survive in the environment.

5. measures employed to ensure and to verify genetic stability. Description of genetic traits which may prevent or minimise dispersal of genetic material. Methods to verify genetic stability,

Techniques to detect and identify the GMO and its genetic stability have been described in section II.2.(f). Genetic stability is assessed throughout the GMO manufacturing process. In addition, expression and identity of the transgene are assessed using Western blots with specific antibodies. The GMO is replication deficient which minimizes further the genetic instability and probability of dispersal of the genetic material.

6. routes of biological dispersal, known or potential modes of interaction with the disseminating agent, including inhalation, ingestion, surface contact, burrowing, etc.,

The GMO MVA is a laboratory-developed viral vector that being replication-deficient, and incapable of producing virus particles in the targeted human host, has lost its biological modes of transmission.

Accidental exposure in the form of a needle-stick injury will be minimized 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 an accident or incident occurring.

7. description of ecosystems to which the GMOs could be disseminated,

Not applicable since the GMO will be released in the context of a clinical study and administered by appropriately trained personnel.

8. potential for excessive population increase in the environment,

Not applicable since the GMO will be released in the context of a clinical study

9. competitive advantage of the GMOs in relation to the unmodified recipient or parental organism(s),

There is no basis to consider that addition of the HBV transgene in the MVA vector will promote any post-release selection for any phenotypic traits. Therefore, no competitive advantage is conferred to the GMO in relation to the recipient and parental organism.

10. identification and description of the target organisms if applicable,

The release will be performed in the context of a clinical trial that will enroll patients with the following main eligibility criteria:

- Male or female between, 18-65 years old (at the time of the first study vaccination)
- Chronically Hepatitis B infected subjects* adherent to entecavir or tenofovir treatment given as per approved label/dosage as a first course of HBV oral therapy for least 30 months
  Specific criteria with regards to serology have been established as well as the criteria to document the medical history
- Stabilized liver disease for at least 24 months (specific criteria implemented).

11. anticipated mechanism and result of interaction between the released GMOs and the target organism(s) if applicable,

The expected biological activity of the GMO following intramuscular injection is the induction of an immune response against HBV. More specifically, a strong CD8+ T-cell responses as well as strong antigen-specific CD4+ T-cell and antibody responses are expected following the proposed heterologous prime-boost regimen.

12. identification and description of non-target organisms which may be adversely affected by the release of the GMO, and the anticipated mechanisms of any identified adverse interaction,

The only non-target organism that may potentially receive the GMO is clinical study personnel in the unlikely event a needle-stick injury occurs. Even if a needle-stick injury were to occur, the risk remains the same as for enrolled study subjects receiving the GMO in the proposed clinical study, there is no identified safety risk.

13. likelihood of post release shifts in biological interactions or in host range,

Not applicable since the GMO is replication-deficient and remains extrachromasomal post administration.

14. known or predicted interactions with non-target organisms in the environment, including competitors, preys, hosts, symbionts, predators, parasites and pathogens,

Not applicable since the GMO is an investigational medicinal product

15. known or predicted involvement in biogeochemical processes,

Not applicable since the GMO is an investigational medicinal product

16. other potential interactions with the environment.

None identified

V. INFORMATION ON MONITORING, CONTROL, WASTE TREATMENT AND EMERGENCY RESPONSE PLANS

A. Monitoring techniques

1. methods for tracing the GMOs, and for monitoring their effects,

The GMO will be released in a clinical study. As reported from clinical studies where recombinant MVA containing different transgenes were administered by intramuscular injection, the potential for shedding of MVA-HBV particles is limited. No environmental monitoring of the clinical sites for the released GMO is planned during the conduct of the study.

Monitoring of the functional effects resulting from GMO vaccination will be performed to assess the antigen-specific cell-mediated and humoral immunity from blood samples collected at several time points following vaccination. The table below lists the cell-mediated immunity and humoral immunity assays, respectively.

Table 3: Cell-mediated immunogenicity

System	Component	Challenge	Method	Unit
PBMC	HBs-specific (CD4/CD8) T-cells	HBs peptide pool	ICS	Events per million T-cells
	HBc-specific (CD4/CD8) T-cells	HBc peptide pool	ICS	Events per million T-cells

ICS: intracellular staining

Table 4: Humoral immunogenicity

System	Component	Method	Kit / Manufacturer	Unit	Cut-off
Serum	Anti-HBs IgG	CLIA	ADVIA Centaur anti-HBs2 (Siemens Healthcare)	mIU/ml	6.2 mIU/ml
	Anti-HBc IgG	TBD	TBD	TBD	TBD

2. specificity (to identify the GMOs, and to distinguish them from the donor, recipient or, where appropriate, the parental organisms), sensitivity and reliability of the monitoring techniques,

See Sections II.A.6. and II.A.7.

3. techniques for detecting transfer of the donated genetic material to other organisms,

In case of a suspicion of accidental transfer from the target host (patient enrolled in the clinical study) to a non-targeted organism, antigen-specific humoral immunity testing could be performed on blood samples.

4. duration and frequency of the monitoring.

The study duration per patient is 120 weeks (48 weeks of vaccination phase plus 72 weeks of follow-up).

Safety and immunogenicity will be monitored during 26 monitoring visits which have been scheduled as follows: at Day 1 (pre-vaccination); Day3; Day 8; Day 15; Day 31; Day 57; Day 64; Day 71; Day 87; Day 113; Day 120; Day 127; Day 143; Day 169; Day 176; Day 183; Day 199; Day 225; Day 253; Day 281; ; Day 309 Day 337; Day 421; Day 505; Day 673; Day 841. Hence, they are more frequent just after each GMO administration than outside these “windows”.

B. Control of the release

1. methods and procedures to avoid and/or minimise the spread of the GMOs beyond the site of release or the designated area for use,

The GMO will be stored in a secured and dedicated storage area with restricted access to authorized personnel. Personal protective equipment appropriate to the containment level will be worn at all times during handling and vaccine preparation/administration. All clinical study staff will receive GMO-specific training in all study-specific and GMO-associated procedures.

2. methods and procedures to protect the site from intrusion by unauthorised individuals,

The GMO will be released during the conduct of a highly controlled multi-centre international clinical study. Access to areas within each study site where GMO material is stored, prepared and administered will be accessible only by trained clinical study staff.

3. methods and procedures to prevent other organisms from entering the site.

The GMO will be released during the conduct of a highly controlled multi-centre international clinical study. Access to areas within each study site where GMO material is stored, prepared and administered will be accessible only by trained clinical study staff.

C. Waste treatment

1. type of waste generated,

Waste generated following the IM administration of the GMO vaccination to each study subject will be minimal and consists mostly of GMO vials, cotton swabs, and material used to perform the IM administration (needle + syringe).

2. expected amount of waste,

One (1) vial of GMO vaccine (plus syringe and needle) will be used per study subject per injection. In addition, disposable personnel protective equipment used by clinical study staff will be treated as biohazardous waste.

3. description of treatment envisaged.

All empty vaccine vials, needles and syringes are to be discarded in biohazard waste containers after vaccine preparation/administration is completed for each subject. Keep the secondary containers for vaccine reconciliation by the monitor

Upon reconciliation and accountability, used study materials and unused study vaccine will either be destroyed following institutional procedures for the disposal of biohazard material, or will be returned to the sponsor for destruction.

D. Emergency response plans

1. methods and procedures for controlling the GMOs in case of unexpected spread,

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

2. methods for decontamination of the areas affected, for example eradication of the GMOs,

The GMO vector is susceptible to most common disinfectants. All surfaces will be disinfected using appropriate means. Practical spill training sessions will be provided to all staff prior to working on the study. Record of staff training and competency will be documented.

3. methods for disposal or sanitation of plants, animals, soils, etc., that were exposed during or after the spread,

This is not applicable since the release will occur during a clinical study held at hospitals sites. Therefore, no contact with plants, animals or soils with the GMO is foreseen.

4. methods for the isolation of the area affected by the spread,

In case of accidental spread, an absorbent tissue will be immediately placed to absorb the spilled GMO, and then the contaminated surface will be decontaminated with a standard disinfectant according to appropriate measures in place.

5 plans for protecting human health and the environment in case of the occurrence of an undesirable effect.

The proposed study is a FTIH for the proposed MVA-HBV candidate vaccine. The target recruitment is approximately 148 patients who will receive different dose regimens.

In order to be able to treat patients with an immediate systemic allergic reaction to vaccination, all patients will need to remain under observation (visibly followed, no specific procedure) at the study site for at least 60 minutes after vaccination. First aid kit for anaphylactic reaction will be available at sites.

In addition to the planned iSRC evaluations, ad hoc safety evaluations can take place if a safety concern is identified by an investigator.

The safety holding rules which will be assessed by the iSRC are defined in Table 6.

- 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 5: 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 the injection site, within the 7-day (days 1-7) post-vaccination period	≥ 1
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/mm3	≥ 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.

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