ChAd155-hIi-HBV
and
MVA-HBV

PART 1 (COUNCIL DECISION 2002/813/EC)

SUMMARY NOTIFICATION INFORMATION FORMAT FOR THE RELEASE OF GENETICALLY MODIFIED ORGANISMS OTHER THAN HIGHER PLANTS IN ACCORDANCE WITH ARTICLE 11 OF DIRECTIVE 2001/18/EC

Notifier: GlaxoSmithKline Biologicals

EudraCT number: 2017-001452-55

Version 1.0 – February-2018

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List of Abbreviations

Ad5	Adenovirus 5
AE	Adverse Event
BGHpA	Bovine Growth Hormone Polyadenylation Signal
BSL1	Biosafety Level 1
ChAd155-hIi-HBV	Recombinant Chimpanzee Adenovirus type 155 HBV Vaccine
CEF	Chick Embryo Fibroblast
CHB	Chronic Hepatitis B
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
HBsAg	Hepatitis B Surface Antigen
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HEK-293	Human Embryonic Kidney 293
hIi	Human MHC Class II-associated Invariant Chain p35 Isoform
huCMV	Human Cytomegalovirus
IM	Intramuscular
MHC	Major Histocompatibility Complex
mRNA	Messenger Ribonucleic Acid
MVA	Modified Vaccinia Ankara
MVA-HBV	Modified Vaccinia Ankara-Hepatitis B Virus Vaccine
MVS	Master Virus Seed
NA	Nucleos(T)Ide Analogue
NHP	Non-Human Primates
ORF	Open-Reading Frame
pfu	Plaque-Forming Unit
PVS	Primary Virus Seed
Q-PCR	Quantitative Polymerase Chain Reaction
RSV	Respiratory Syncytial Virus
SAE	Serious Adverse Event
SRC	Safety Review Committee
vp	Viral Particles

VV	Vaccinia Virus

A. General information

1. Details of notification
   (a) Member State of notification Belgium
   (b) Notification number B/../../….
   (c) Date of acknowledgement of notification ../../….
   (d) 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 Applicant's study code is: 204852 (abbreviated title: TH HBV VV-001).

(e) Proposed period of release

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. Notifier:
Name of institution or company:
GlaxoSmithKline Biologicals SA
Rue de l'Institut, 89
1330 Rixensart, Belgium

3. GMO characterisation

Two viral vector GMOs will be evaluated in the clinical trial. The GMOs are named: ChAd155-hIi-HBV and MVA-HBV.

Please find below the proposed nomenclature for terms used in this document to describe the ChAd155-hIi-HBV construct:

- The donor organism: the organism(s) from which sequences encoded by the GMO are derived.
- The recipient organism: the "empty" (i.e. without the transgene) replication-defective ChAd155 simian-derived adenovirus vector backbone.
- The parental organism: the replication-competent simian-derived ChAd155 adenovirus isolate from which the engineered vector backbone is derived.

Please find below the proposed nomenclature for terms used in this document to describe the MVA-HBV construct:

- The donor organism: the organism(s) from which sequences encoded by the GMO are derived.

- The recipient organism: the engineered vector "empty" (ie without the transgene)
- The parental organism: the organism from which the engineered vector is derived.

(a) Indicate whether the GMO is a:

ChAd155-hIi-HBV:

viroid	(.)
RNA virus	(.)
DNA virus	(X)
bacterium	(.)
fungus	(.)
animal	
- mammals	(.)
- insect	(.)
- fish	(.)
- other animal	(.)
specify phylum, class	…

MVA-HBV:

viroid	(.)
RNA virus	(.)
DNA virus	(X)
bacterium	(.)
fungus	(.)
animal	
- mammals	(.)
- insect	(.)
- fish	(.)
- other animal	(.)
specify phylum, class	…

(b) Identity of the GMO (genus and species)

ChAd155-hIi-HBV:

(i) order:	Adenoviridae
(ii) genus:	Mastadenovirus
(iii) species:	Simian adenovirus
(iv) subspecies:	Subgroup C
(v) strain:	Serotype 155
(vi) pathovar:	…

(vii) common name: ChAd155

The GMO ChAd155-hIi-HBV is a viral suspension of a recombinant replication-defective simian (chimpanzee-derived) group C adenovirus serotype 155 (ChAd155) viral vector 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, that allows processing of the HBc-HBs fusion into separate protein antigens. In addition, the N-terminal part 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 (hIi).

The ChAd155 viral vector backbone (recipient organism) is derived from a simian adenovirus serotype 155 (parental organism) that was isolated from a healthy young chimpanzee housed at the New Iberia Research Center facility (The University of Louisiana at Lafayette, Louisiana, USA). The viral genome of the parental isolate was then cloned in a plasmid vector and subsequently modified to carry the deletion of E1 and E4 regions and the insertion of E4orf6 derived from human adenovirus type 5 (Ad5).

MVA-HBV:

Identity of the GMO:	Modified Vaccinia Virus Ankara (MVA)
Genus:	Orthopoxvirus
Species:	Vaccinia Virus

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 et al. 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 et al. 2016).

(c) Genetic stability – according to Annex IIIa, II, A(10)

ChAd155-hIi-HBV:

Parental adenoviruses are stable in nature. The GMO (ChAd155-hIi-HBV) is a simian adenovirus that upon administration to the target organism localizes in the nucleus of the host cell however does not integrate its DNA into the host cell genome. Integration of adenovirus DNA into the host cell genome has been observed as an extremely rare event in some human primary cell line cultures.

The genetic structure of the GMO vaccine is verified at different steps of the process of production to demonstrate the integrity of the vector and identity of the insert (such as restriction pattern and DNA sequencing of the full genome. All genetic characterization analyses on tested products showed conformity to predicted sequences.

One of the factors that may affect genetic stability is the occurrence of replication competent adenoviruses (RCA) during the manufacturing process. Formation of RCA from homologous recombination between the ChAd155 viral vector and the huAd5 E1 region of the production cell could arise. Although the risk of occurrence of this event is considered very low, the ChAd155-hIi-HBV material (MVS and DS) is tested for the presence of RCA using a RCA assay (specification: < 1 RCA / 3x10e10 vp). The confirmation of absence of RCA has been demonstrated in all batches manufactured to date.

Other factors that may affect genetic stability are temperature, UV light and cleaning procedures as adenoviruses are commonly susceptible to those. Adenoviruses can survive for long periods on environmental surfaces. They are resistant to lipid disinfectants (because they are non-enveloped), but are inactivated by heat (>56°C) and other disinfectants including: 1% sodium hypochlorite, ethyl alcohol, 2% glutaraldehyde, 0.25% sodium dodecyl sulphate.

The long-term stability of the ChAd155-hIi-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 72 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 GMO material as compared to reference standards.

MVA-HBV:
An issue of particular importance is the genetic stability of the viral preparation and potential for recombination and reconversion.

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 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 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.

4. Is the same GMO release planned elsewhere in the Community (in conformity with Article 6(1)), by the same notifier?

Yes (X) No (.)

If yes, insert the country code(s) UK, DE, BE…

5. Has the same GMO been notified for release elsewhere in the Community by the same notifier?

Yes (.) No (X)

If yes:

- Member State of notification …
- Notification number B/../../…

Please use the following country codes:

Austria AT; Belgium BE; Germany DE; Denmark DK; Spain ES; Finland FI; France FR; United Kingdom GB; Greece GR; Ireland IE; Iceland IS; Italy IT; Luxembourg LU; Netherlands NL; Norway NO; Portugal PT; Sweden SE

6. Has the same GMO been notified for release or placing on the market outside the Community by the same or other notifier?

Yes (.) No (X)

If yes:

- Member State of notification …
- Notification number B/../../…

7. Summary of the potential environmental impact of the release of the GMOs.

ChAd155-hIi-HBV:

While there is no available data on the environmental impact of the release of the ChAd155-hIi-HBV, as the proposed study is a FTIH study, it is not expected that deliberate release of the GMO in this clinical study will impair other humans, flora or fauna, near or far to the release area.

The low likelihood for the ChAd155-hIi-HBV vector to become persistent and invasive in the environment, as a result of its deliberate release during this clinical study is based on the following:

- *High genetic stability*: Lack of prolonged transgene expression has made replication incompetent adenoviruses attractive viral vectors for vaccine development. They possess a stable virion, allowing inserts of foreign genes to remain intact and they can infect many different cell types. The ChAd155 vector to be used in the proposed clinical study is replication-defective and only capable of transducing animal cells. In addition, the adenovector genome remains epichromosomal thus avoiding the risk of integration of the viral DNA into the host genome after infection of host cells (Feuerbach et al. 1996).
- *Absence of replication competent adenovirus (RCA)*: The risk of occurrence of the formation of RCA from homologous recombination between the ChAd155 viral vector and the human Ad5 E1 region of the host cell is considered very low, due to the lack of sequence homology between the human E1 flanking regions of human Ad5 and chimpanzee adenovirus E1. It has been shown that recombination and production of RCA does not occur when they are propagated in HEK-293 cells (Colloca et al. 2013), thus eliminating the problem of RCA generation during the adenovector manufacture. In

addition, the ChAd155-hIi-HBV vaccine is tested for the presence of RCA during different steps of the manufacture's process (see section A.3.(c)).

- *Absence of replication*: The ChAd155-hIi-HBV is incapable of surviving outside a host (animal) cell since the vector is replication-defective.
- *Controlled manufacturing processes*: To minimize release of the recombinant vectored vaccine virus into the environment, each vaccine is produced under good manufacturing practice (GMP) conditions with the handling of live material in appropriate laboratory facilities. This is to ensure that any release of modified organism is contained, inactivated and incinerated, using single use equipment as much as possible; to avoid release of modified genetic material into the environment.
- *Limited biodistribution:* A GLP-compliant biodistribution study with the ChAd155-hli-HBV vaccine was assessed in Sprague-Dawley rats. Following a single intramuscular (IM) administration at a constant dose-volume of 100 µL and at a concentration of 1x10e11 vp/mL (1/5th of the maximal intended human dose), ChAd155-hli-HBV was found in the injection site (muscle) and in the draining iliac lymph node from day 2 with viral particle numbers decreasing over time 7, 28 and 48 days after treatment. Systemic exposure to ChAd155-hli-HBV was demonstrated at 24 hours, 7 days and/or 48 days after treatment in inguinal lymph node and spleen, and at 24 hours after treatment in blood and popliteal lymph node where test item DNA became not detectable or not quantifiable) on day 8 and day 29. ChAd155-hli-HBV was not detected in brain, heart, kidney, liver, lung, ovary and testis samples.
- *Clinical experience indicating low risk of viral shedding:* Defective recombinant adenoviruses have been used extensively in clinical trials, either through direct administration or cell therapy strategies (contained in the cells). The majority of the studies have not detected viral release in biological samples (sputum, saliva, urine, feces) and whenever detected by PCR assay in urine or saliva, it disappeared a few days after administration. Following administration of a similar E1/E4-deleted simian adenovirus (ChAd3), but expressing a hepatitis C virus transgene, no viral vector shedding (in urine and throat swabs) was observed after intramuscular chimpanzee or human adenovirus immunization (clinical study HCV-001, EudraCT Number: 2007-004259-12).
- *Clinical study site management:* The release of the GMO will take place during the conduct of clinical study where the administration of the vaccine will occur in a hospital or clinic setting. Clinical study staff will be trained in the preparation, administration and waste clean-up of the GMO to minimize dissemination and inadvertent transmission. Materials used during the administration of the GMO will be treated as biohazard waste and eliminated in accordance with institutional guidelines.
- *Route of administration in the clinical study:* Finally, as the release is planned during a clinical trial, and will be administered intramuscularly to the patients, it is highly unlikely that the GMO will come into contact with the environment.
- *Lack of toxicity:* A GLP single-dose local tolerance toxicity study was conducted in New Zealand white rabbits with the ChAd155-hli-HBV vaccine when co-administrated with another HBV therapeutic candidate vaccine (MVA-HBV) with formulation intended to be used in the clinical study, and ruled out any systemic toxicity. A GLP repeat-dose toxicity study with administration of ChAd155-hli-HBV vaccine given alone or in combination/co-administration of other HBV therapeutic candidate vaccines showed that all vaccination schedules were clinically well tolerated, and all vaccinated animals had anti-HBc and anti-HBs antibodies at the end of the treatment and recovery periods.

As a conclusion, the potential for a detrimental environmental impact due to the release of the GMO is negligible.

MVA-HBV:
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 for the MVA-HBV vector to become persistent and invasive in the environment, as a result of its deliberate release during this clinical study is negligible, hence limiting any potential environmental impact. This reasoning is supported as follows:

The possibility of gene transfer of the MVA-HBV vector to other species is minimal under the conditions of the proposed release. A potential hazard for any GMO arises from recombination events between the GMO and its naturally occurring homologs, in this case orthopox virus (OPV), that could lead to either transfer of the transgene (the insert) to replication competent viruses or result in a return to virulence of the GMO vector. However, in the case of MVA, since the wild type vaccinia virus and the parental MVA are not naturally found in the environment, the probability of co-localization is negligible precluding any possibility of recombination events. Even if co-localization were to occur with another orthopoxvirus, the MVA vector has lost roughly 15% of its parental genome as a result of its attenuation process that encompassed > 500 passages in culture. There is no known poxvirus able to complement MVA to generate a replication competent virus, and finally, spontaneous reversion of MVA to replication competent vaccinia virus has not been documented. MVA is a non-intregrative virus as it is found exclusively in the cytoplasm of infected cells thus eliminating any risk of DNA integration into the host genome. MVA is unable to produce vector particles in human cells and so cannot establish a propagative infection. Furthermore biodistribution studies with similar MVA vectors containing different transgenes show they are rarely found outside the site of injection, which demonstrates the non-spreading character of the MVA vector (Verheust et al. 2012). The extensive attenuation process has also resulted in the severely restricted host range of MVA, its lack of virulence in animals, and its highly attenuated replication,

Furthermore, the potential for shedding of infectious MVA-HBV particles into the environment is limited due to the lack of viral shedding observed from subjects vaccinated with MVA vectors (Verheust et al. 2012).

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

During the nonclinical development of the GMO, GLP single dose and repeat doses toxicity studies performed in male and female rabbits ruled out potential systemic toxic effects directly related to the GMO, administrated via the clinical route (intramuscularly).

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.

In summary, under the conditions of this release we do not expect the MVA vector to spread to, survive in, or have any detrimental impact on the environment.

B. Information relating to the recipient or parental organism from which the GMO is derived

Please note that in the following section, information relating to both the recipient organism (the engineered vector "empty" (i.e. without the transgene)) and the parental organism (the organism from which the engineered vector is derived) are provided when appropriate.

1. Recipient or parental organism characterisation:

Applies to ChAd155-hIi-HBV and MVA-HBV:

(a) Indicate whether the recipient or parental organism is a:

viroid (.)
RNA virus (.)
DNA virus (X)
bacterium (.)
fungus (.)
animal
- mammals (.)
- insect (.)
- fish (.)
- other animal (.)
(specify phylum, class) …

other, specify …

2. Name

ChAd155-hIi-HBV:

(i) order: Adenoviridae
(ii) genus: Mastadenovirus
(iii) species: Simian adenovirus
(iv) subspecies: Subgroup C
(v) strain: Serotype 155
(vi) pathovar (biotype, ecotype, race, etc.): …
(vii) common name: ChAd155

Parental organism is chimpanzee adenovirus. Recipient organism contains deletions in the E1 and E4 regions and substitution of the native E4 by the E4ORF6 from human adenovirus 5 (Ad5).

MVA-HBV:

(i) order: Poxviridae/Chordopoxviridae
(ii) genus: Orthopoxvirus
(iii) species: Vaccinia Virus
(iv) subspecies …
(v) strain: Modified Vaccinia Virus Ankara
(vi) pathovar (biotype, ecotype, race, etc.): …
(vii) common name: MVA

3. Geographical distribution of the organism

(a) Indigenous to, or otherwise established in, the country where the notification is made:
Yes (.) No (X) Not known (.)

(b) Indigenous to, or otherwise established in, other EC countries:
(i) Yes (.)
If yes, indicate the type of ecosystem in which it is found:
Atlantic ..
Mediteranean ..
Boreal ..
Alpine ..
Continental ..
Macaronesian ..

(ii) No (X)
ChAd155-hIi-HBV:
The recipient organism is engineered in the laboratory and not found in the environment.

MVA-HBV:
The parental organism (MVA) is not naturally found in the environment.

(iii) Not known (.)

(c) Is it frequently used in the country where the notification is made?
Yes (.) No (X)

(d) Is it frequently kept in the country where the notification is made?
Yes (.) No (X)

4. Natural habitat of the organism
(a) If the organism is a microorganism
water (.)
soil, free-living (.)
soil in association with plant-root systems (.)
in association with plant leaf/stem systems (.)
other, specify
ChAd155-hIi-HBV:
The natural host of ChAd155 adenovirus is the chimpanzee. The parental ChAd155 adenovirus is not found in natural ecosystem outside of its natural host.

The recipient is a replication-defective chimpanzee adenovirus modified in the laboratory. It is not found in natural ecosystems. It has be grown in E1-complementing human embryonic kidney (HEK-293) based cell line dedicated to the propagation of viruses with deletions of key genes for replication, such as the E1 and E4.

MVA-HBV:
The parental organism MVA is a highly attenuated replication deficient recombinant vaccinia virus. MVA is known to replicate well in chicken embryo fibroblasts and

baby hamster cells, but poorly in most mammalian cells and is unable to spread in normal human cells. The parental MVA is found only in laboratory settings and not in natural ecosystems.

(b) If the organism is an animal: natural habitat or usual agroecosystem:
Not applicable

5. (a) Detection techniques
ChAd155-hIi-HBV:
Detection of the GMO is performed using a polymerase chain reaction (PCR) developed using specific sets of primers.

MVA-HBV:
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).

(b) Identification techniques
ChAd155-hIi-HBV:
The PCR mentioned in B.5.(a) above also allows the identification of the GMO.
Additional identification assays include:
- Full genome DNA sequencing.
- Restriction fragment analysis.
- Transgene expression by Western blot (using anti-HBc and anti-HBs antibodies) is assessed to ensure identity of the GMO at the final manufacturing stages.

MVA-HBV:
As described in B.5.a. above

6. Is the recipient organism classified under existing Community rules relating to the protection of human health and/or the environment?
Yes (X) No (.)
If yes, specify
ChAd155-hIi-HBV:
In terms of classification of hazard, human adenovirus is considered as a group 2 biological agent as per the European Economic Community classification for the protection of workers with biological agents (Directive 2000/54/EC). The group 2 designation applies to agents that can cause human disease and might be a hazard to workers, that are unlikely to spread to the community and for which there is usually effective prophylaxis or treatment available.

However, neither the parental nor the recipient organisms (simian adenovirus deleted of the E1 gene and rendered therefore replication-defective) are specifically classified by the EEC directive. Based on the inability of simian adenovirus to cause human disease and results of toxicity studies that demonstrated safety and tolerability, the GMO is not considered to pose a risk to human health. Similar recombinant simian adenovirus vectors, with different transgenes, have previously been used in human clinical studies where competent regulatory authorities have viewed them as BSL1 agents for the conduct of the clinical study.

MVA-HBV:
The human vaccinia virus (VV) is classified as a group 2 biological agent (BSL2) according to the EEC classification for the protection of workers with biological agents (Directive 2000/54/EC). BSL2 designation applies to agents that can cause human disease and might be a hazard to workers, that are unlikely to spread to the community, and for which there is usually effective prophylaxis or treatment available.

The recombinant MVA strain is not classified by the EEC directive: however, most competent authorities view MVA as belonging to hazard group BSL1, since it is a highly attenuated strain of VV 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 (Goosens et al. 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.

7. Is the recipient organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?

Yes (.) No (X) Not known (.)

If yes:

(a) to which of the following organisms:

humans (.)
animals (.)
plants (.)
other (.)

(b) give the relevant information specified under Annex III A, point II. (A)(11)(d) of Directive 2001/18/EC

ChAd155-hIi-HBV:
Adenoviruses are classified as Class 2 under Directive 2000/54/EC due their limited pathogenicity. Human adenoviruses commonly cause asymptomatic infection in humans, although they can also cause respiratory tract infections, gastrointestinal discomfort or eye infections with severity being variable. They are more common in children and in immunocompromised population. The incubation period ranges from 1 to 10 days. The majority of the population is seropositive for more than one subspecies of adenovirus and can quickly produce neutralizing antibodies. The principal host is human, and the minimal infectious dose is >150 plaque-forming units intra-nasally. Normally, the virus enters the respiratory tract or the eyes through aerosols produced by infected individuals. Most infections are minor in nature and self-limiting. Adenoviruses are usually not integrated into the host cells genome and do not persist in lymphoid tissues. Adenovirus may be transmitted between individuals via fecal-oral route, respiratory droplets, hand-to-eye and venereal transfer.

Adenovirus infections in non-human primates (NHP) are also predominantly subclinical, except for some cases of pneumonia in immunosuppressed simian immunodeficiency virus (SIV)-infected animals. Defective recombinant adenoviruses have been widely used in clinical trials, both as direct administration or cell therapy

strategies (contained in the interior of cells). The non-replicative adenoviruses lacking the E1 were classified as biosafety level class 1 for R&D purposes.

The recipient organism, the ChAd155 backbone, is replication-defective and a chimpanzee-derived species and therefore considered not to be pathogenic to humans or other non-target organisms. Moreover, adenoviruses are not integrated in the host genome and do not present a risk to activate latent provirus.

Finally, toxicity studies (single dose and repeat doses) performed in a GLP compliant environment showed that vaccination schedules using ChAd155-hli-HBV were clinically well tolerated and failed to demonstrate any systemic toxic effect.

MVA-HBV:
MVA is characterized by severe host cell restriction with efficient viral replication observed only in CEF and baby hamster kidney cells, with incomplete replication in human and most other mammalian cells tested. In non-permissive cells, there is no production of virions which could propagate and infect other cells. MVA presents no risk of integration into the host genome or activation of latent provirus, since the vector has a fully cytoplasmic cycle of propagation. Biodistribution studies performed with recombinant MVA have shown limited spread of infectious particles outside the injection site, even in immune-compromised animal models (Hanke et al. 2005). MVA is not an animal pathogen as it has been administered in several animal species and found not to be virulent.

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 of 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 (Verheust et al. 2012, Goossens et al. 2013).

8. Information concerning reproduction

(a) Generation time in natural ecosystems:

ChAd155-hIi-HBV:
Not applicable. The replication-defective recipient organism does not generate in natural ecosystems.

MVA-HBV:
Not applicable. MVA is not found in the environment or natural ecosystems and has no known animal reservoirs. MVA exhibits severe host cell restriction, and replicates well only in primary CEF cell culture and in the continuous BHK cell line. Therefore MVA exists only in a laboratory setting.

(b) Generation time in the ecosystem where the release will take place:
Not applicable. The replication-defective recipient organisms will not generate in the ecosystem where the release will take place.

(c) Way of reproduction: Sexual .. Asexual ..
Not applicable.

(c) Factors affecting reproduction:
Not applicable.

9. Survivability

(a) ability to form structures enhancing survival or dormancy:

(i)	endospores	(.)
(ii)	cysts	(.)
(iii)	sclerotia	(.)
(iv)	asexual spores (fungi)	(.)
(v)	sexual spores (funghi)	(.)
(vi)	eggs	(.)
(vii)	pupae	(.)
(viii)	larvae	(.)
(ix)	other, specify	

Not applicable. The GMOs are replication-defective.

(b) relevant factors affecting survivability:
ChAd155-hIi-HBV:
While adenoviruses can survive for up to 8 weeks on environmental surfaces at ambient temperatures, the GMO is replication-defective and is not expected to survive, multiply or disperse following its release during the proposed clinical study. Furthermore, the presence of replication competent adenovirus (RCA) is assessed on the drug substance as a quality control release test. 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 mainly localized to the site of injection (Sheets et al. 2008). In the unlikely event of shedding or accidental spills, while adenoviruses are resistant to lipid disinfectants because they are non-enveloped, adenoviruses are inactivated by common chemical agents (e.g. sodium hypochlorite as a 1-10% dilution of fresh bleach or ethyl alcohol). The virus is also susceptible to inactivation by heat and autoclaving at 121°C for 15 minutes. Identical factors are expected to apply to the GMO.

MVA-HBV:
The MVA-HBV GMO will be administered by intramuscular 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 (Hanke et al. 2005, Goosens et al. 2013, Schenk et al. 2007)

In the unlikely event of shedding or accidental spills, bioactivity of MVA at room temperature decays logarithmically. It is also susceptible to various chemical agents (e.g. 1% sodium hypochlorite, ethyl alcohol, and 2% glutaraldehyde) commonly used as disinfectants. The virus also shows sensitivity to heat inactivation with complete

elimination achieved by autoclaving at 121°C for 15 minutes. Based on these conditions as applied to the release of the GMO during the clinical study, we do not expect any survival of the MVA in the environment.

10. (a) Ways of dissemination

ChAd155-hIi-HBV:

Adenoviruses are transmitted effectively by direct contact via contaminated aerosols and water droplets, and indirectly via contact with objects contaminated with respiratory secretions from an infected person. The minimal infectious dose of adenovirus is 150 plaque forming units when administered intra-nasally. Adenovirus may also be spread via the faecal-oral route.

As the release will take place during a clinical study held under Good Clinical Practices (GCP), precautions will be taken to minimize the production of aerosols during the handling, preparation and administration of the GMO. Also any contaminated surfaces or objects will be disinfected immediately with appropriate adenoviral-active disinfectants and standard institutional procedures for decontamination of biohazard waste.

Biodistribution studies demonstrated that following intramuscular injection, the GMO was undetectable in brain, heart, kidney, lung, ovary or testis samples (from day 2) and in blood samples, liver and spleen (from day 8). The GMO remain mainly localized at the injection site and the draining lymph nodes, including the iliac, inguinal, and to some extent, the popliteal lymph node.

Of note, no shedding using GMOs derived from other simian adenovirus strains was ever observed during clinical studies (Wold et al. 2013).

MVA-HBV:

MVA-vectored GMOs remain localized in the cell cytoplasm until the destruction of the cell. In the proposed clinical study, the GMO will be administered intramuscularly, hence reducing the probability of viral particles presence on the skin close to the injection site and potential shedding via this route.

Human clinical studies conducted with similar MVA constructs, administered by the intramuscular 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 alter or influence the shedding behavior of recombinant MVA vectors.

(b) Factors affecting dissemination

ChAd155-hIi-HBV:

Factors affecting the dissemination of adenoviruses include the administered dose, formation of aerosols, and proximity of susceptible uninfected hosts to immunized subjects.

Strict adherence to the safety measures to be taken during the handling, preparation and administration of the GMO during the clinical trial as specified in the clinical protocol will adequately control any potential spread of the GMO.

MVA-HBV:
The release will take place during a clinical study performed under Good clinical practices. Hence, strict adherence to the safety measures to be taken during the handling, preparation and administration of the GMO during the clinical trial as specified in the clinical protocol will adequately control any potential spread of the GMO.

11. Previous genetic modifications of the recipient or parental organism already notified for release in the country where the notification is made (give notification numbers)
…, B/../../…
ChAd155-hIi-HBV:
This will be a First-Time-In-Human study with the proposed GMO, the ChAd155-hIi-HBV.

Another GMO vaccine candidate with the same ChAd155 backbone but encoding an RSV antigen (ChAd155-RSV) has been assessed for safety, reactogenicity and immunogenicity in a Phase 1 trial in healthy adults aged 18 to 45 years (EudraCT: 2014-005333-31) entitled: "A Phase I, randomised, observer-blind, controlled study to evaluate the safety, reactogenicity and immunogenicity of a GlaxoSmithKline Biologicals' respiratory syncytial virus (RSV) investigational vaccine based on viral proteins F, N and M2-1 encoded by chimpanzee-derived adenovector (ChAd155-RSV) (GSK3389245A), when administered intramuscularly according to a 0, 1 month schedule in healthy adults aged 18 to 45 years."

The ChAd155-RSV candidate is also currently assessed in a Phase1/2 clinical study in paediatric population (EudraCT: 2016-0001117-76) in Spain and Italy under the protocol entitled "A Phase 1/2, randomized, observer-blind, controlled, multi-centre, dose-escalation study to evaluate safety, reactogenicity and immunogenicity of GSK Biologicals' respiratory syncytial virus (RSV) investigational vaccine based on the RSV viral proteins F, N and M2-1 encoded by chimpanzee-derived adenovector (ChAd155-RSV) (GSK3389245A), when administered intramuscularly according to a 0, 1-month schedule to RSV-seropositive infants aged 12 to 17 months".

Several other adenoviral vectors derived from subgroup C adenovirus have been produced using a similar manufacturing process and assessed for safety and efficacy in clinical trials. Three different simian-derived recombinant adenoviruses have been evaluated in clinical trials including: ChAd63 adenovirus (Biswas et al. 2011) belongs to serotype E (Colloca et al. 2012) and has been mainly used in malaria trials (Sheehy et al. 2011, O'Hara et al. 2012, de Barra et al. 2014, Hodgson et al. 2015) where more than 1,000 healthy volunteers have been vaccinated, including two-month old babies. The ChAd3 (Peruzzi et al. 2009) and PanAd3 (Vitelli et al. 2013) recombinant adenoviruses belonging to the serotype C group (Colloca et al. 2012) have been evaluated in hepatitis C virus (HCV) and Ebolavirus trials with more than 1,500 vaccinees, and in a Phase I clinical RSV trial enrolling 42 volunteers, respectively.

All simian adenovectors tested so far in the clinic showed an acceptable safety profile in the study populations with no reported vaccine related serious adverse advents (Sheehy et al. 2011, Barnes et al. 2012, O'Hara et al. 2012, Capone et al. 2013, de Barra et al. 2014, Hodgson et al. 2015, Ledgerwood et al. 2015).

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

MVA and recombinant MVA vectors have been used extensively in clinical studies and have consistently demonstrated a good safety record in both healthy and immunocompromised study populations (Goosens et al 2013).

C. Information relating to the genetic modification

1. Type of the genetic modification

ChAd155-hIi-HBV:

(i)	insertion of genetic material	(X)
(ii)	deletion of genetic material	(X)
(iii)	base substitution	(.)
(iv)	cell fusion	(.)
(v)	others, specify …	

MVA-HBV:

(i)	insertion of genetic material	(X)
(ii)	deletion of genetic material	(.)
(iii)	base substitution	(.)
(iv)	cell fusion	(.)
(v)	others, specify …	

2. Intended outcome of the genetic modification

ChAd155-hIi-HBV:

The intended outcome of the genetic modifications described below is to develop a replication-defective recombinant simian adenoviral vector capable of expressing two hepatitis B virus (HBV) protein antigens in infected cells and to activate a HB antigen-specific immune response in the host. The two HBV proteins include: 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 that allows processing of the HBc-HBs fusion into separate protein antigens. In addition, the N-terminal part 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 (hIi) that is acting as a genetic adjuvant to the associated antigen and will help inducing a more robust HB antigen-specific immune response in the host.

The vector ChAd155 system was obtained by introducing deletions into the E1 and E4 regions of the viral genome, and substituting the native E4 region with human adenovirus type 5 E4 region open-reading frame (ORF) 6, rendering the ChAd155 backbone replication-defective.

The DNA of hIi-HBV transgene was cloned into a shuttle vector under the control of human cytomegalovirus (hCMV) promoter and bovine growth hormone polyadenylation signal (BGHpA), with its expression controllable through the bacterial tetracycline-sensitive repressor (Stanton et al. 2008). The transgene expression cassette was inserted into the ChAd155 backbone by homologous recombination in *E. coli* SW102. The resulting ChAd155-hIi-HBV virus was rescued in a HEK-293 derivative cell line by transfection and was then further amplified by serial passaging.

The intended outcome of the GMO vector is to serve as a therapeutic approach for chronic HBV patients that will induce a robust immune response in terms of antibodies, CD4+ and CD8+ T-cells to the HBc and HBs antigens.

MVA-HBV:
The aim of the genetic modification described below is to develop a replication-deficient recombinant MVA vector capable of expressing two key HBV protein antigens in patients chronically infected with HBV to activate an antigen-specific immune response. The two HBV proteins include: 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 that allows processing of the HBc-HBs fusion into separate protein antigens.

The intended outcome of the GMO is to serve as a therapeutic approach for chronic HBV patients that will induce a robust antigen-specific immune response to reduce levels of HBsAg allowing the cessation of NA therapy.

3. (a) Has a vector been used in the process of modification?
Yes (X) No (.)
If no, go straight to question 5.

(b) If yes, is the vector wholly or partially present in the modified organism?
Yes (X) No (.)
If no, go straight to question 5.

4. If the answer to 3(b) is yes, supply the following information
(a) Type of vector

ChAd155-hIi-HBV:
Two vectors are considered here:

plasmid	(X) (plasmid carrying the hIi-HBV transgene)
bacteriophage	(.)
virus	(.)
cosmid	(.)
transposable element	(.)
other, specify:	Bacterial Artificial Chromosome (BAC) vector (recipient organism)

MVA-HBV:

plasmid	(X)
bacteriophage	(.)
virus	(.)
cosmid	(.)
transposable element	(.)
other, specify	…

(b) Identity of the vector
ChAd155-hIi-HBV:
The DNA of hIi-HBV transgene was synthesized and cloned into a shuttle vector (plasmid) under the control of hCMV promoter with the TetO inserted downstream

the hCMV promoter TATA box and bovine growth hormone polyadenylation signal (BGHpA).

Standard DNA manipulation techniques in *E. coli* (direct cloning and homologous recombination) were used to clone the ChAd155 viral genome into a Bacterial Artificial Chromosome (BAC) vector and to modify the plasmid vector in order to introduce the deletion of native E1 and E4 regions as well as the introduction of Ad5E4orf6 region. HIi-HBV transgene was then inserted in the plasmid BAC/ChAd155 (ΔE1, ΔE4_Ad5E4orf6) by different recombination steps in *E. coli*.

MVA-HBV:
The transfer plasmid encoding the HBV transgene.

(c) Host range of the vector
ChAd155-hIi-HBV:
The vectors will replicate in laboratory strain of *E.coli*. The final adenovirus vector can then only replicate in cells which express E1 (such as E1-complemented HEK 293 cells).

MVA-HBV:
Escherichia coli

(d) Presence in the vector of sequences giving a selectable or identifiable phenotype

	Yes	No
antibiotic resistance	(X)	
other, specify:		(.)

Indication of which antibiotic resistance gene is inserted
ChAd155-hIi-HBV:
A kanamycin resistance gene is inserted in the plasmid expressing transgene cassette. However, after homologous recombination with the vector containing the modified ChAd155 viral genome, the kanamycin resistance gene is not present in the final recombined adenovirus vector.

A selection cassette including the suicide gene SacB, ampicillin–resistance gene and lacZ (Amp-LacZ-SacB selection cassette) is inserted in the BAC vector during the process. However, this Amp-LacZ-SacB selection cassette is substituted by the HBV transgene cassette and is therefore not present in the final GMO.

MVA-HBV:
The plasmid transfer vector contains the ampicillin resistance gene. However, this ampicillin resistance sequence is not present in the GMO.

(e) Constituent fragments of the vector
ChAd155-hIi-HBV:
The DNA fragment inserted as the transgene in the chimpanzee adenovirus vector (ChAd155) encodes sequences derived from 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 that allows processing of the HBc-HBs fusion into separate protein antigens. In addition, the N-terminal part 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 (hIi). The plasmid expressing hIi-HBV transgene also contains the hCMV promoter with the TetO inserted downstream the hCMV promoter TATA box to provide transcriptional control of the transgene while in the packaging cell line, and bovine growth hormone poly-adenylation signal (BGHpA). The ChAd155 viral genome was cloned into a BAC shuttle vector by homologous recombination in *E. coli*. Subsequent genetic modifications were performed to effect the deletion of the E1 and E4 genes and replace the native E4 by E4ORF6 of the human adenovirus type 5 (Ad5).

MVA-HBV:
The plasmid vector contains DNA sequences encoding the HBV transgene antigens (HBc and HBs proteins separated by the 2A region). The transgene cassette is flanked by two MVA genomic regions that allow insertion of the transgene in the recipient MVA by homologous recombination.

(f) Method for introducing the vector into the recipient organism

ChAd155-hIi-HBV:

(i)	transformation	(.)
(ii)	electroporation	(.)
(iii)	macroinjection	(.)
(iv)	microinjection	(.)
(v)	infection	(.)
(vi)	other, specify …	(X)

Homologous recombination in *E.coli*

MVA-HBV:

(i)	transformation	(.)
(ii)	electroporation	(.)
(iii)	macroinjection	(.)
(iv)	microinjection	(.)
(v)	infection	(.)
(vi)	other, specify …	(X)

Homologous recombination between the plasmid transfer vector and the MVA vector system was accomplished in primary CEF culture.

5. If the answer to question B.3(a) and (b) is no, what was the method used in the process of modification?

(i)	transformation	(.)
(ii)	microinjection	(.)
(iii)	microencapsulation	(.)
(iv)	macroinjection	(.)
(v)	other, specify …	

6. Composition of the insert

(a) Composition of the transgene insert

ChAd155-hIi-HBV:
The DNA fragment inserted as the hIi-HBV transgene in the chimpanzee adenovirus vector (ChAd155) encodes sequences derived from two HBV proteins antigens: 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, that allows processing of the HBc-HBs fusion into separate protein antigens. In addition, the N-terminal part 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 (hIi).

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 hIi-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: hIi-HBc-spacer-2A and HBs. For brevity the hIi-HBc-spacer-2A polypeptide will be referred to as the hIi-HBc protein throughout the dossier.

MVA-HBV:
The insert contains the HBV gene (encoding the HBc and HBs antigens separated by the 2A region under the control of the P7.5 vaccinia virus promoter to drive transgene expression. The FMDV 2A region allows processing of the fusion protein into separate HBc and HBs antigens.

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.

(b) Source of each constituent part of the insert
ChAd155-hIi-HBV:
The HBc and HBs antigens are derived from the hepatitis B virus. At least nine genotypes (A through I) of HBV have been identified, differing in their genome by more than 8%. Within a given HBV genotype, multiple geno-subtypes have been identified, differing by 4-8%. The sequences encoding the HBc and HBs antigens are derived from genotype/subtype A2. The 2A region is derived from the foot and mouth disease virus. The hIi sequence is derived from the human gene coding for CD74 also called the human invariant chain (hIi) that acts as a genetic adjuvant to optimize the CD8+ T-cell immune response to the HBc antigen.

MVA-HBV:

The HBc and HBs antigens are derived from the hepatitis B virus. The P7.5 promoter is derived from vaccinia virus. The 2A region is derived from the foot and mouth disease virus.
At least nine genotypes (A through I) of HBV have been identified, differing in their genome by more than 8%. Within a given HBV genotype, multiple geno-subtypes have been identified, differing by 4-8%. The sequences encoding the HBc and HBs antigens are derived from genotype subtype A2.

(c) Intended function of each constituent part of the insert in the GMO
The intended function of the gene insert in ChAd155-hIi-HBV expressing the hIi-HBc and HBs antigens, and likewise for gene insert in MVA-HBV expressing the HBc and HBs antigens, is to elicit an antigen-specific immune response (in particular CD8+ T-cells and to a lesser extent CD4+ T-cells) with the goal of clearing or reducing HBsAg to undetectable levels to allow patients to discontinue NA therapy without virological or clinical relapse.

Rationale for antigens selected for the vaccine:
Efficient control of HBV infection as observed after resolution of acute HBV infection is associated with the induction and persistence of helper and cytotoxic T-cells targeting different HBV proteins and production of anti-HBV envelope antibodies (Bertoletti et al. 2013).

The ChAd155-hIi-HBV and MVA-HBV vectors include a transgene coding for both the HBc and the HBs proteins. At least nine genotypes (A through I) of HBV have been identified, differing in their genome by more than 8%. Within a given HBV genotype, multiple geno-subtypes have been identified, differing by 4-8%. In the ChAd155-hIi-HBV and MVA-HBV vectors, the sequences of the HBc and of the HBs are those from genotype/subtype A2.

Hepatitis B core protein (HBc): The HBc plays a key role when forming nucleocapsides packaging the HBV genome in the cytoplasm of infected cells during viral replication. HBc sequence is highly conserved across HBV genotypes and geno-subtypes. Immune system cell response specific to HBV core antigen (HBcAg) and, to a less extent, to other HBV antigens play a major role in control and resolution of HBV. Induction of CD8+ T-cells targeting HBc antigen (HBcAg) and although to a less extent, hepatitis B surface (HBs) and hepatitis B polymerase (HBpol) antigens, had been demonstrated to correlate with clearance of acute and chronic infections (Li et al. 2011; Liang et al, 2011, Boni et al. 2012, Block et al. 2017).

Hepatitis B surface protein (HBs): HBs is the principle surface antigen and 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). Clearance of HBsAg along with appearance of anti-HBs antibodies is a marker of resolution of hepatitis B infection. The efficacy of antibodies to HBsAg (anti-HBs) in preventing HBV infection has been largely established. Although the HBs sequence is variable across genotypes, HBs is already included in the well-studied family of GSK commercialised hepatitis-B prophylactic vaccines (Engerix B™ (adult and paediatric), Fendrix™, Twinrix™ (adult and paediatric)/Ambirix™ and Infanrix

hexa™), has been demonstrated to be protective against hepatitis B, regardless of genotypes.

The ChAd155-hIi-HBV vector contains the human invariant chain (hIi). The human invariant chain (hIi), also known as CD74 when expressed on the plasma membrane, is an evolutionarily conserved type II membrane protein which has several roles within the cell and throughout the immune system (Borhese et al 2011). HIi associates with MHC class II α and γ chains and directs the transport of the αβIi complexes to endosomes and lysosomes, exerting its main function in antigen presentation. Ii has been shown to increase MHC class I presentation of antigenic peptides when genetically linked to an antigen, leading to enhanced T cells, most likely by targeting the transport of this antigen to the endosomal/lysosomal compartments.

Enhanced and sustained CD8+ T-cells responses were demonstrated in mice and non-human primates using an adenoviral vector-based vaccine encoding a fusion protein of target antigen with hIi (Capone et al 2014). Hence, in order to increase further the induction of antigen-specific CD8+T-cell responses from the candidate ChAd155-HBV vaccine, the DNA fragment coding for the hIi has been N-terminally fused to the DNA coding for the HBcAg of the HBV transgene, thus aiming to act as a genetic adjuvant to the HBcAg. The ChAd155-hIi-HBV priming was shown to induce a more robust core antigen-specific CD8+ T-cells than a construct without hIi when tested in the MHC-class I/II human transgenic mice.

(d) Location of the insert in the host organism

ChAd155-hIi-HBV:

- on a free plasmid (.)
- integrated in the chromosome (.)
- other, specify integrated in the genome of the adenovirus

MVA-HBV:

- on a free plasmid (.)
- integrated in the chromosome (.)
- other, specify integrated in the MVA genome
  Following co-transfection with the MVA vector system and the plasmid shuttle vector containing the transgene, the transgene is integrated in a site-directed manner into the MVA genome by homologous recombination.

(e) Does the insert contain parts whose product or function are not known?

Yes (.) No (X)

If yes, specify …

D. Information on the organism(s) from which the insert is derived

1. Indicate whether it is a:

ChAd155-hIi-HBV:

viroid	(.)
RNA virus	(X) foot-and-mouth disease virus (FMDV)
DNA virus	(X) human hepatitis B virus (HBV)
bacterium	(.)

fungus (.)
animal
- mammals (X) human: fragment coding for the hIi
- insect (.)
- fish (.)
- other animal (.)
(specify phylum, class) …
- other, specify

MVA-HBV:
viroid (.)
RNA virus (X) foot-and-mouth disease virus (FMDV)
DNA virus (X) human hepatitis B virus (HBV)
bacterium (.)
fungus (.)
animal
- mammals (.)
- insect (.)
- fish (.)
- other animal (.)
(specify phylum, class) …
- other, specify

2. Complete name

Applies to ChAd155-hIi-HBV and MVA-HBV.
For human hepatitis B virus (HBV):

(i)	order and/or higher taxon (for animals)	…
(ii)	family name	Hepadnaviridae
(iii)	genus	Orthohepadnavirus
(iv)	species	Human hepatitis B virus (HBV)
(v)	subspecies	…
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	HBV

Applies to ChAd155-hIi-HBV and MVA-HBV.
For foot-and-mouth disease virus (FMDV):

(i)	order and/or higher taxon (for animals)	Picornavirales
(ii)	family name	Picornaviridae
(iii)	genus	Aphthovirus
(iv)	species	
(v)	subspecies	…
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	FMDV

Applies to ChAd155-hIi-HBV only:
For human: fragment coding for the hIi

(j)	order and/or higher taxon (for animals)	Primates
(ii)	family name	Hominidae
(iii)	genus	Homo
(iv)	species	sapiens
(v)	subspecies	…
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	human

3. Is the organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?
Yes (X) No (.) Not known (.)
If yes, specify the following:
(a) to which of the following organisms:

humans (X)
animals (X)
plants (.)
other ..

(b) are the donated sequences involved in any way to the pathogenic or harmful properties of the organism
Yes (.) No (X) Not known (.)
If yes, give the relevant information under Annex III A, point II(A)(11)(d):

4. Is the donor organism classified under existing Community rules relating to the protection of human health and the environment, such as Directive 90/679/EEC on the protection of workers from risks to exposure to biological agents at work?
Yes (X) No (.)
If yes, specify
HBV has been classified as other hepadnaviruses as Class 3 under the Directive 2000/54/EC. with a note stating that HBV presents a limited risk of infection for workers because it is not normally infectious by air-borne route.

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.

5. Do the donor and recipient organism exchange genetic material naturally?
Yes (.) No (X) Not known (.)

E. Information relating to the genetically modified organism

1. Genetic traits and phenotypic characteristics of the recipient or parental organism which have been changed as a result of the genetic modification
(a) is the GMO different from the recipient as far as survivability is concerned?
Yes (.) No (X) Not known (.)
Specify …

(b) is the GMO in any way different from the recipient as far as mode and/or rate of reproduction is concerned?
Yes (.) No (X) Unknown (.)
Specify …

(c) is the GMO in any way different from the recipient as far as dissemination is concerned?
Yes (.) No (X) Not known (.)
Specify …

(d) is the GMO in any way different from the recipient as far as pathogenicity is concerned?
Yes (.) No (X) Not known (.)
Specify …

2. Genetic stability of the genetically modified organism
See section A.3.(c)

3. Is the GMO significantly pathogenic or harmful in any way (including its extracellular products), either living or dead?
Yes (.) No (X) Unknown (.)
The GMOs are replicative-defective and are not considered pathogenic to the host or to non-target organisms.

(a) to which of the following organisms?
humans (.)
animals (.)
plants (.)
other …

(b) give the relevant information specified under Annex III A, point II(A)(11)(d) and II(C)(2)(i)
ChAd155-hIi-HBV:
For relevant information specified under Annex IIIA, point II(A)(11)(d), refer to sections B.7(b) and D.3.(b).
The GMO is not expected to have any toxic nor 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 study subjects 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. Intramuscular (IM) vaccination commonly precipitates a transient and self-limiting local inflammatory reaction. 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.

The proposed study is a First-Time-n-Human study, thus, safety and efficacy of the proposed vaccine regimen are unknown. However, clinical data from similar types of vaccine candidates are available that are supportive for the safe use of ChAd155-hIi-HBV vaccine in the proposed vaccination regimen.

ChAd3-based vaccines using other immunogens (e.g. HCV and Ebola) were evaluated in clinical trials in up to 2,800 subjects and were well tolerated. ChAd3-HCV administered as a prime followed by a booster dose of MVA-HCV vaccination was tested in 245 healthy subjects and 14 chronic HCV patients. Mild local and systemic reactions were observed that increased with dose but were short-lived (Barnes et al. 2012, Swadling et al. 2014). 270 subjects were vaccinated with a ChAd3-Ebola vaccine candidate in four Phase I clinical trials (doses ranged from 1x10e10 to 1x10e11 vp) where safety was carefully assessed.

In repeated dose toxicity studies in New Zealand white rabbits with vaccine candidates comprised of recombinant human adenoviruses Type 5 and Type 35 containing different transgenes including: HIV, Ebola and Marburg, hematology parameters that appeared to be impacted by adenovector delivery included hemoglobin, hematocrit, thrombocytes and mean thrombocyte volume (Sheets et al. 2008). In the Ebola Phase I studies in adults administered the ChAd3-Ebola Zaire candidate vaccine, transient decreases in thrombocyte counts were observed. These decreases occurred mostly on day 1 after vaccination and generally returned to baseline by day 7. Although most of these decreases remained within the normal range, the per protocol criteria for thrombocytopenia (i.e. thrombocyte count of < 150 x 103/µL) were met for 2.6% (7 out of 270) of the vaccinated subjects. None of the decreases in thrombocyte counts or the cases of thrombocytopenia was clinically significant (i.e. no clinical signs or symptoms suggestive of increased tendency to bleeding were reported in any of the subjects). Although the mechanism underlying these decreases currently remains unclear, it is well described in literature that, post intravenous administration, adenovirus activates platelets and induces platelet-leukocyte aggregate formation, causing an associated increase in platelet and leukocyte-derived microparticles (Othman et al. 2007)

The ChAd155-hIi-HBV includes a DNA sequence coding for CD74, also called the invariant chain (hIi). Since hIi is a self-antigen expressed throughout the immune system by B cells, activated T-cells, dendritic cells, monocytes and macrophages and widely expressed in the thymus, it should be highly tolerated. However, the risk that the ChAd155-hIi-HBV vaccine induces an immune response against the hIi and a potential immune-mediated disease cannot be entirely ruled out, and will be monitored closely during the conduct of the clinical trial.

During the natural course of the disease, viral clearance of HBV occurs through immunological mechanisms that may be associated with hepatitis flares, and in some rare instances, it can lead to fulminant hepatic failure. Hepatic damage is possibly triggered by inefficient T-cell control and recruitment of inflammatory cells (macrophages) during the disease progress. When the HBV-specific CD8+ T-cell response is unable to control virus replication, it may contribute to liver pathology, not only directly but also by causing the recruitment of non-virus-specific T-cells while, in the presence of an effective HBV-specific CD8+ T-cell response, inhibition of virus replication can be independent of liver damage (Maini et al. 2000).

Importantly, cytokines can mediate viral clearance without direct hepatocytes killing (Phillips et al. 2010). Also, several mechanisms are in place to control "exuberant" T-cell activation and protect from liver failure (IL-10, arginase, PD1 and other co-inhibitory pathways). The proposed vaccination strategy aims to induce a robust response in terms of antibodies, CD4+ and CD8+ T-cells to the selected antigens: robust antibody response and CD4+ T-cell response (but no CD8+ T-cell response) have already been observed in previous studies in CHB patients and no life-threatening hepatitis flares were reported (Vandepapelière et al. 2007).

Several types of vaccines have been tested in CHB patients including: recombinant proteins with or without adjuvants), immuno-complexes HBs-anti-HBs, DNA followed by MVA prime-boost vaccination and yeast-based vaccine expressing recombinant proteins. No safety issues were reported in these trials but the vaccines were generally ineffective in controlling the virus (Michel et al. 2015). When viral vectored-based vaccines using HCV immunogens were tested in chronic HCV patients, the safety profile was also acceptable, but the vaccine failed to induced virus-specific T-cell responses in HCV-infected patients (Swadling et al. 2016).

Considering the risk of hepatic failure that may be triggered by vaccine-induced immune response, this FTIH will be conducted in CHB adult (18-65 years of age) patients at low risk of flares, i.e. CHB patients under NA therapy since at least 30 months, well-controlled (HBV DNA undetectable and ALT under control since at least 24 months) and with no cirrhosis and no advanced fibrosis. In order to detect any potential safety issue, patients will be closely monitored for safety.

All available nonclinical data suggest that the ChAd155-hIi-HBV vaccine candidate has acceptable immunogenicity, biodistribution, tolerability/toxicity profiles for conducting the clinical trial.

Although human adenoviruses may cause more infections in immunocompromised population, as a replication-defective adenovirus, the GMO ChAd155-hIi-HBV is therefore not considered to be pathogenic in humans.

In addition, the GMO does not present a risk of integration or activation of latent provirus.

Since it is replication-defective, the GMO is not expected to display capacity for colonization. During the GMP manufacture of clinical lots over a period of several years, the Applicant has not observed a single RCA event during the routine quality control assessment for the presence of RCA in every batch of clinical material.

Finally, no antibiotic-resistance genes are expressed in the GMO further excluding antibiotic resistance patterns.

MVA-HBV:
Pathogenicity of the recombinant MVA-HBV does not differ from the parental MVA since the transgene is not expected to be hazardous nor trigger adverse effects.

Most authorities view the use of recombinant MVA constructs in clinical studies as belonging to the BSL1 classification of biological agents due to their limited pathogenicity and well-established safety record in humans (Goossens et al. 2013).

The attenuated MVA strain was developed for and used during the smallpox eradication campaign to vaccinate some 120,000 people in Germany who were considered at risk for vaccination with the vaccinia vaccine. The MVA vaccine did not produce serious adverse events in this at-risk group, and was associated only with minor local injection site reactions, fever and flu-like symptoms.

Finally, no toxic effects were observed during single and repeated dose GLP toxicity studies performed with the GMO.

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment

There are no techniques planned to detect and identify the GMOs in the environment in the context of the proposed clinical trial.

(b) Techniques used to identify the GMO

ChAd155-hIi-HBV:

The identity of ChAd155-hli-HBV is confirmed by full genome DNA sequencing. Identity testing of both the vector and the insert are performed as well at several stages during manufacture of the product using various methods including: PCR, restriction analyses and Western blot expression of the transgene.

MVA-HBV:

The identity of recombinant MVA-HBV vector is confirmed by DNA sequencing of the transgene. Identity of the GMO is also confirmed by PCR, where site-specific PCR primers must amplify DNA fragments of appropriate size to confirm the identity of the GMO.

F. Information relating to the release

1. Purpose of the release (including any significant potential environmental benefits that may be expected)

The purpose of the release is to evaluate the safety and immunogenicity and efficacy of escalating doses of the vaccine candidate in a FTIH Phase I clinical trial in CHB adult patients whom are at low risk of severe hepatitis exacerbation, under nucleos(t)ide analogue (NA) therapy. The ChAd155-hli-HBV and MVA-HBV have been developed to restore immunity to HBV, leading to clearance or reduction of HBsAg concentration, to allow patients to safely discontinue NA therapy without virological or clinical relapse. The study will enrol 148 patients and will last approximatively 4.5 years.

The GMOs will be released during a clinical study, at identified investigational sites under the responsibility of Principal Investigators, as part of an international, multicentre clinical trial.

The actual release involves the administration of the GMOs via the intramuscular injection route to study subjects. The release will take place in designated hospital or clinic rooms. The release will be performed by dedicated and trained personnel. Detailed instructions on how to

prevent contamination by the vaccine will be provided to all personnel involved in handling of the product. There are no significant environmental benefits expected following the GMO release during this clinical trial.

2. Is the site of the release different from the natural habitat or from the ecosystem in which the recipient or parental organism is regularly used, kept or found?

Yes (X) No (.)

If yes, specify …

ChAd155-hli-HBV:

Simian adenoviruses are not found naturally in the environment of the geographical locations surrounding the clinical study sites where the administration of the GMO will take place.

MVA-HBV:

Not applicable. The GMO and its parent MVA are not naturally found in the environment.

3. Information concerning the release and the surrounding area

(a) Geographical location (administrative region and where appropriate grid reference):

The ChAd155-hli-HBV and MVA-HBV GMOs 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

(b) Size of the site (m^2): … m^2

(i) actual release site (m^2): … m^2

No specific site size is required for the release. The release of the GMOs will take place in a designated standard-sized hospital or clinic examination room within each of the designated clinical institutions.

(ii) wider release site (m^2): … m^2

No wider release is expected.

(c) Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs), which could be affected:

Not applicable since the release will occur during a clinical trial held in hospital/clinical settings.

(d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO

Not applicable since the release will occur during a clinical trial held in hospital/clinical settings.

4. Method and amount of release

(a) Quantities of GMOs to be released:

ChAd155-hli-HBV:

In terms of total GMO release, considering the number of subjects in the ChAd155-hIi-HBV treatment cohort, the dosage administered, and the number of injections per subject, the total estimated quantity of GMO to be released across all clinical study sites, in all countries, during the conduct of the study, is equivalent to 5.72 x 10e12 ChAd155-hli-HBV viral particles.

MVA-HBV:

In terms of total GMO release, considering the number of subjects in the MVA-HBV treatment cohort, the dosage administered, and the number of injections per subject, the estimated quantity of GMO to be released across all clinical study sites, in all countries of the study, is equivalent to 2.81 x10e10 MVA-HBV pfu.

(b) Duration of the operation:

The duration of the GMOs release entails the period beginning with the first patient and first vaccination until the last patient last vaccination. Although the exact duration of the release will be dependent on study recruitment, the planned study is estimated to be approximately 30 months.

(c) Methods and procedures to avoid and/or minimise the spread of the GMOs beyond the site of the release

The GMO is intended for clinical use only according to the provisions of the clinical protocol. The amount of the GMOs supplied to the sites at any one time is limited to that needed for patient administration and access to the product is restricted to authorized personnel. The route of administration will minimise potential shedding from the patient.

The GMOs to be provided to clinical sites in sealed vials are appropriately labelled and packaged. The preparation and administration of the product is to be performed by trained personnel, under the responsibility of the investigator, according to a clinical protocol and respecting the rules of Good Clinical Practice (GCP).

The area used to prepare the GMOs for injection will be decontaminated before and after manipulation with standard disinfecting solution. All transfers of the GMO preparations should be undertaken using a closed container. Furthermore, clinical study personnel will follow the clinic or hospital policy recommended for handling of GMOs.

In case of accidental spillage, contaminated surfaces will be treated with appropriate disinfectants. Contaminated materials will be removed from the room and maintained in sealed containers or in special bags that are clearly labelled as biohazard medical waste.

5. Short description of average environmental conditions (weather, temperature, etc.)

Not applicable. All GMO administrations are to be performed in conventional hospital/clinical rooms at the clinical institutions listed.

6. Relevant data regarding previous releases carried out with the same GMO, if any, specially related to the potential environmental and human health impacts from the release.
ChAd155-hli-HBV:
Clinical studies conducted with similar recombinant GMO's containing other transgenes (e.g. malaria, Ebola, HCV) have not raised safety concerns. No significant adverse effects have been reported and the GMO appears to be generally safe and well tolerated. As this will be the first in human trial of the ChAd155-hli-HBV GMO, no data is available about the release of the proposed GMO.

MVA-HBV:
Clinical studies conducted with similar recombinant MVA GMO's containing other transgenes (e.g. malaria, Ebola, HCV, HIV) 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). As this will be the first in human trial of the MVA-HBV GMO, no data is available about the release of the proposed MVA-HBV GMO.

G. Interactions of the GMO with the environment and potential impact on the environment, if significantly different from the recipient or parent organism

1. Name of target organism (if applicable)

(i)	order and/or higher taxon (for animals)	…
(ii)	family name for plants	…
(iii)	genus	*Homo*
(iv)	species	*sapiens*
(v)	subspecies	…
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	Human

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)
The ChAd155-hli-HBV and MVA-HBV GMOs are recombinant viral vectors carrying relevant HBV antigens which are expected to mobilize both humoral and cellular arms of the immune response in the host. Refer to section C.6(c) for further details.

3. Any other potentially significant interactions with other organisms in the environment
The possibility of gene transfer to other species is minimal under the conditions of the proposed clinical release of the GMOs. The GMOs will be administered to subjects in a standard hospital/clinical room and is unlikely to come in contact with other animal species.

ChAd155-hli-HBV:
Further limiting any chance of gene transfer are the phenotypic characteristics of the ChAd155 vector in that it is replication-defective and as such is non-pathogenic.

In order for viral genes to be exchanged, to or from the GMO, to the genome of other wild type species of adenovirus, susceptible cells would need to be simultaneously infected with wild type simian adenovirus which is extremely unlikely. Although transmission of adenoviruses between species, particularly between humans and non-human primates is still not well established, possible horizontal transmission events between humans and NHPs of such viruses is most likely to occur at places with close physical contact between NHPs and humans, such as zoos and other animal facilities (Wevers et al. 2011). Furthermore, genetic information carried by the GMO remains epichromosomal in infected cells thus eliminating any risk of integration of the viral DNA into the host genome.

MVA-HBV:
Further limiting any chance of gene transfer are the phenotypic characteristics of the MVA in that it is replication deficient, has a severely limited host range, and has been shown to be non-virulent in animal models. In order for the viral genes encoded by the GMO to transfer into the genome of other species of poxviruses, susceptible cells would need to be simultaneously infected with pox virus and transduced by vector which is extremely unlikely. Furthermore the GMO MVA virus remains localized in the cell cytoplasm until lysis of the infected cell, and being replication-deficient is unable to generate infectious particles and spread to uninfected cells. These characteristics severely limit the potential for gene transfer from the MVA-HBV GMO to other organisms.

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?
Yes (.) No (X) Not known (.)
Give details
ChAd155-hli-HBV:
Compared to the parental simian adenovirus, the GMO has been modified to be replication defective, and there is no basis to believe that addition of the hli-HBV transgene to the GMO would promote any post-release selection for increased invasiveness.

MVA-HBV:
The parental MVA is not endemic in the human population. And there is no basis to believe that the addition of the HBV transgene to the GMO would promote any post-release selection for increased invasiveness.

5. Types of ecosystems to which the GMO could be disseminated from the site of release and in which it could become established
Due to the context of the proposed GMO release, where the GMOs are administered to subjects in an enclosed hospital or clinical examination room, it is unlikely the GMOs will come into contact with any non-target organisms in the ecosystem.

ChAd155-hli-HBV:
In the event of inadvertent administration to non-target organisms, further dissemination is unlikely because the ChAd155-hli-HBV GMO is unable to complete a viral replication cycle and as such is non-virulent and unable to disseminate in target or non-target organisms.

MVA-HBV:
In the event of inadvertent administration to non-target organisms, further dissemination is unlikely because MVA is unable to complete a viral replication cycle; it has a severely limited host range, and in several studies has shown it is non-virulent in animal models

(immune competent and immune compromised animals) as well as in primary human cell cultures.

6. Complete name of non-target organisms which (taking into account the nature of the receiving environment) may be unintentionally significantly harmed by the release of the GMO

(i) order and/or higher taxon (for animals) …
(ii) family name for plants …
(iii) genus …
(iv) species …
(v) subspecies …
(vi) strain …
(vii) cultivar/breeding line …
(viii) pathovar …
(ix) common name …

Not applicable. The possibility exists that clinical study staff may be injected by accident with the GMOs (e.g. needle-stick injury). As the administration of the vaccine will be performed by dedicated and trained medical personnel, this probability and the inherent risk associated with it are considered minimal. Secondary transmission to patients' family members is considered unlikely as well.

7. Likelihood of genetic exchange in vivo

(a) from the GMO to other organisms in the release ecosystem:
This is highly unlikely for the same reasons as described above in Section G.3

(b) from other organisms to the GMO:
This is highly unlikely for the same reasons as described above in Section G.3.

(c) likely consequences of gene transfer:
No data are available. However, toxicity studies ruled out any toxic or pathogenic effect of the GMOs.

8. Give references to relevant results (if available) from studies of the behaviour and characteristics of the GMO and its ecological impact carried out in stimulated natural environments (e.g. microcosms, etc.):
No data are available.

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)
Not applicable.

H. Information relating to monitoring

1. Methods for monitoring the GMOs
Monitoring the effects of the administered GMOs on subjects in the proposed release will be achieved using clinical assessments (e.g. physical examination, adverse event reporting), blood biochemistry and immunological assessments, as described in the protocol.

The safety assessment, for all subjects participating in the clinical trial will be made over duration of the proposed GMO release and will continue 24 months after the last injection.

No environmental or study personnel GMO monitoring is planned during the conduct of the clinical study.

2. Methods for monitoring ecosystem effects
No additional methods have been developed to monitor the effects of the GMOs in ecosystems.

Given the proposed conditions of clinical release of the GMOs, the limited viral shedding observed with the GMOs, and the non-spreading character of the replication-defective GMOs, there is little chance the GMOs will interact with non-target organisms in the environment. Based on these arguments no monitoring of the ecosystem is planned during the proposed clinical study.

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms
The probability for a transfer of the donated genetic material to other organisms is negligible (refer to section G.3. above). No additional methods will be performed to detect any transfer of genetic material from the GMO to others organisms during the proposed release.

4. Size of the monitoring area (m^2)
… m^2
Not applicable. The GMOs are administered only to study subjects by intramuscular injection in designated rooms at each clinical site.

5. Duration of the monitoring
See section H.1. Safety assessments will be performed all along the patient's participation in the clinical trial and up to study conclusion (week 120).

6. Frequency of the monitoring
According to the schedule provided in the clinical study protocol, subjects will be monitored routinely for safety and clinical outcome during planned visits throughout the period of the GMOs release and during a follow-up period after the last vaccination. 26 monitoring visits have been scheduled over the 2.5 years participation.

I. Information on post-release and waste treatment

1. Post-release treatment of the site
The hospital or clinic room(s) used to prepare and administer the GMOs will be cleaned with an appropriate disinfectant immediately after the administration according to institutional procedures.

In case of accidental spill or contamination, each contaminated surface should be treated following institutional procedures pertaining to biohazard products. The removal of contaminated material will be held in sealed containers or in special bags that are clearly labelled as biohazard medical waste.

2. Post-release treatment of the GMOs
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.

3. (a) Type and amount of waste generated
ChAd155-hli-HBV:
Based on the total number of ChAd155-hli-HBV vaccinations planned for all study cohorts, the total waste generated, in terms of clinical GMO material left unused in vials, for all study sites in all countries, is expected to be a total of 2.12 x 10e12 vp . Other generated waste will include material involved in the preparation and administration of the vaccinations (e.g. needles, syringes, packaging, and personal protective equipment).

MVA-HBV:
Based on the total number of MVA-HBV vaccinations planned for all study cohorts, the total waste generated, in terms of clinical GMO material left unused in vials, for all study sites in all countries, is not more than a total of 1.61 x 10e10 pfu. Other generated waste will include material involved in the preparation and administration of the vaccinations (e.g. needles, syringes, packaging, and personal protective equipment).

(b) Treatment of waste
Waste generated during the course of the study will either be destroyed on site, following institutional and clinical site procedures (e.g. autoclave, incineration or treated with sodium hypochlorite solution) by personnel trained in the disposal of biohazard waste, or will be returned to the sponsor for destruction.

J. Information on emergency response plans

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread
In case of accidental release (e.g. needle-stick injury or broken vial and spillage) the clinical study personnel will notify the principal investigator and others as required by institutional policy. All clinical study staff will be instructed on the procedures to implement in case of accidental release.

2. Methods for removal of the GMO(s) of the areas potentially affected
See Section J.1.

3. Methods for disposal or sanitation of plants, animals, soils, etc. that could be exposed during or after the spread
Not applicable.

4. Plans for protecting human health and the environment in the event of an undesirable effect
See Section J.1.

Patients included in the clinical trial will be monitored as provided by the protocol according to standards of GCP. Adverse events will be registered and reported according to detailed procedures in the protocol.

Due to the extensive procedural controls in place for the transport, storage, administration, disposal and monitoring of the administration of the GMOs, the risk of an accidental environmental release, or a resulting undesirable effect from such an accidental release, is considered very low.

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