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

In order to tick one or several possibilities, please use crosses (meaning x or X) into the space provided as (.)

A. General information

1. Details of notification

(a)	Member State of notification	Germany
(b)	Notification number	B/DE/19/PEI3520
(c)	Date of acknowledgement of notification	13/08/2018
(d)	Title of the project	

A Randomized, Placebo-Controlled, Phase 2 Study of HB-101, a Bivalent Cytomegalovirus (CMV) Vaccine, in CMV-Seronegative Recipient (R-) Patients Awaiting Kidney Transplantation from Living CMV-Seropositive Donors (D+).

…

(e) Proposed period of release — From /18-Jan-2019/ until /18-Sep-2020/

2. Notifier

Name of institution or company:

Hookipa Biotech AG
Helmut-Qualtinger-Gasse 2
1030 Vienna
Austria

3. GMO characterisation

(a) Indicate whether the GMO is a:

viroid (.)
RNA virus (X)
DNA virus (.)
bacterium (.)
fungus (.)
animal
- mammals (.)
- insect (.)
- fish (.)
- other animal (.)

specify phylum, class Arenavirus

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

Lymphocytic choriomeningitis virus (LCMV) was first isolated in the 1930s by Armstrong and Lillie from a monkey which developed lymphocytic choriomeningitis after monkey to monkey transmission of infectious material from a patient (Armstrong et al., 1934). LCMV Clone 13 is a variant of the parent Armstrong virus strain which was isolated from the spleen of carrier mice which sustained a persistent LCMV infection since birth. LCMV is the prototype member of the arenavirus family. It is an enveloped, bi-segmented, negative single-stranded ribonucleic acid (RNA) virus with an ambisense coding strategy. The genome of wildtype LCMV encodes four proteins essential for virus propagation: the surface glycoprotein (GP), the nucleoprotein (NP), the RNA-dependent RNA polymerase (L) and a zinc finger protein (Z) (Laposova et al, 2013).

The investigational agent (HB-101) is a bivalent vaccine that contains two replication deficient recombinant lymphocytic choriomeningitis virus (rLCMV) vectors based on the Armstrong-Clone 13 strain (HK1) wherein the open reading frame (ORF) encoding the LCMV glycoprotein, has been replaced with a gene encoding a truncated isoform of the envelope glycoprotein B (gB) or replaced with the tegument phosphoprotein pp65 (pp65) of human cytomegalovirus (HCMV), respectively.

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

None of the genetic modifications are anticipated to have any effect on the genetic stability of the GMO when compared to that of wild type virus, nor would they be expected to result in transfer or maintenance of genetic material in the environment (outside of the human subjects participating in the trial). The HCMV genes are not expected to confer any advantage to the GMO in terms of survival or selective pressure.

Genetic stability of the vector encoded transgenes:
Genetic stability of the premaster virus seed (PMS) vectors expressed transgenes were analyzed by passaging vectors in the production cell line (HEK293 expressing GP), followed by analysis of the HgB/nucleoprotein (NP) focus forming units (FFU) ratio by the double-immunofluorescence (DI) FFU assay.

The percentage of transgene expressing vectors at passage level corresponding to clinical material was 66% for HK1-Hpp65 and 87% for HK1-gB.

Identity/stability of the vector backbone (Pre master virus seed)

HK1-gB:
The viral plaque selected for preparation of the HK1-HgB master virus seed contains two silent mutations at nucleotide positions 423 and 1065 of gB, which do not alter the amino acid sequence at amino acid positions 141 and 355, respectively. Further, consensus sequencing of the whole coding sequence of the LCMV vector S- and L-genomic segments of HK1-HgB indicated three mutations at amino acid positions 258, 287 and 1271 of the polymerase (L) protein (F258L, S287N, I1271M). The mutations were verified to not cause measurable changes in the biological properties of the vector such as atypical vector growth kinetics in production cell lines or low antigen expression.

HK1-pp65:
No sequence deviations relative to the reference sequence were detected for the pp65 expressed transgene. Consensus sequencing of the whole coding sequence of the LCMV vector S- and L- genomic segments of the pp65 expressing PMS candidate was conducted and revealed a conservative valine to isoleucine exchange at position 451 in the NP protein. This exchange does not cause measurable changes in the biological properties of the vector, such as atypical vector growth kinetics in production cell lines or low vaccine antigen expression.

Reversion to replication competent virus (RCV):
The replication incompetent character of rLCMV vectors has been studied extensively and proven in qualified assays during the vector production process (Replication competent virus assay (RCV assay)) as well as in animal models.

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) NL, UK, BE, SE, DK, NO, FR, AT

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 (X) No (.)
   If yes:
   - Member State of notification United States of America (USA)
   - Notification number IND 17938

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

   The natural host of LCMV is the mouse, though it can infect other rodents, or accidentally (albeit rarely) be transmitted to primates and humans. It is a rare human pathogen, and with the rare exception of vertical transmission and transplant-associated transmission, there are, to Hookipa Biotech AG's (hereafter referred to as Hookipa Biotech) knowledge, no documented cases of human to human transmission. Non-replicating viral vectors of LCMV have been constructed by deletion of the gene encoding GP and the insertion of foreign genes in lieu thereof. Such recombinant, non-replicating vectors have been studied in mice and non-human primates where they have demonstrated the capacity to induce potent humoral and cellular immune responses.

The genetic modifications made for generation of HB-101 containing replication deficient vectors prohibit its amplification outside of a dedicated production cell line. Infectious progeny can only be formed in this cell line or analogously engineered cell lines. Consequently, the vectors cannot survive or replicate in the host and cannot disseminated. The replication incompetent design makes these vectors inherently safe for use.

Animal studies have demonstrated that the vector administered via intracranial route causes no apparent pathology in immunocompromised mice. Biodistribution studies in immunocompetent mice indicated that the vector material can be found in multiple tissues after immunization, but was not detectable in any tissues 56 to 90 days after inoculation. In addition, toxicity studies in rats supplement these safety data. Research studies using analogous vectors (encoding different antigens) were performed in guinea pigs, rabbits and non-human primates and no apparent toxicities were observed in any of these studies. Further, Hookipa Biotech conducted a Phase 1 healthy volunteer study (Study H-100-001) based on predecessor HB-101, which attested the vaccine a favourable safety profile.

The intended application of HB-101 is limited to a phase 2 clinical study enrolling a maximum of 150 consenting adult CMV seronegative (-) patients awaiting kidney transplant from a CMV seropositive (+) living donor, recruited globally from specified transplant centers. We estimate that 3 patients will participate in the study from Germany.

Hypothetical indirect effects of the release are limited to the consequences of the release of either attenuated or wild type LCMV following recombination in the recipient’s cells, an event considered highly unlikely, followed by shedding into the environment. In the unlikely event that such an exchange occurred, there is no reason to believe that the virus would be more pathogenic than the laboratory-adapted LCMV Armstrong-Clone 13 strain, and wild type LCMV is found in mouse populations around the globe thus is widely distributed in the environment. The potential risk associated with indirect administration of HB-101 is limited. Potential adverse effects are expected to be the same or most likely reduced as compared to those, which may be anticipated in patients receiving the treatment.

The composition of HB-101 particles is comparable to wild type LCMV and thus a similar tropism is expected. Hookipa Biotech does not anticipate that there is a route of transmission for HB-101, nor can the vector be disseminated, owing to the replication deficient character of the material and limited physical stability. Further, there is no evidence for human to human transmission of LCMV with the exception of transplacental infection of a foetus or transplantation of organs from infected donors.

In conclusion, overall the environmental risks associated with the deliberate release of HB-101 under the conditions of release proposed, and with the precautions and monitoring activities proposed, is considered negligible.

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

1. Recipient or parental organism characterisation:

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

(select one only)

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

other, specify …

2. Name

(i)	order and/or higher taxon (for animals)	…
(ii)	genus	Arenavirus
(iii)	species	Lymphocytic choriomeningitis virus
(iv)	subspecies	…
(v)	strain	Armstrong-Clone 13
(vi)	pathovar (biotype, ecotype, race, etc.)	…
(vii)	common name	Lymphocytic choriomeningitis virus

3. Geographical distribution of the organism

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

(b) Indigenous to, or otherwise established in, other EC countries:
(i) Yes (X)

If yes, indicate the type of ecosystem in which it is found:

Atlantic (X)
Mediteranean (X)
Boreal (X)
Alpine (X)
Continental (X)
Macaronesian (X)

(ii) No (.)
(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 Specific hosts are mouse and other rodents

(b) If the organism is an animal: natural habitat or usual agroecosystem:

Not applicable.

5. (a) Detection techniques

Commercially available PCR or ELISA Kits can be used to detect LCMV.

(b) Identification techniques

Commercially available PCR or ELISA Kits or PCR followed by consensus sequencing analysis.

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

In the European Union (EU) according to Directive 2000/54/EC on the protection of workers from risks related to exposure of biological agents at work, wild type LCMV can be classified as Risk Group 2 or Risk Group 3 biological agents, according to the strain used. Viscerotropic strains, such as Armstrong-Clone 13, are considered Risk Group 2 agents, while the neurotropic strains are classified as Risk Group 3 in the EU (EC Directive 2000/54/EC). However, a position statement issued by the Central Biosafety Commission (ZKBS) in Germany, on the risk assessment of laboratory strains of LCMV as donor or recipient organisms in genetic engineering operations, concluded that risk classification level 2 containment measures (BSL-2) are considered adequate when working with all laboratory strains of LCMV (Position Statement, ZKBS, March 2009). The ZKBS also suggests that a risk assessment of new isolates of LCMV, as donor and recipient organisms for genetic engineering operations, should be assessed on a case-by-case basis. Therefore, based on the above ZKBS assessment, an internal literature review and longstanding experience working

with LCMV in animal models Hookipa Biotech fully agree with the German ZKBS, and support their conclusions on the classification of LCMV donor strains.

This replication-incompetent vector material is classified as a BSL-1 agent by the responsible national authorities for GMOs under Austrian license number for work with the described agent BMG-76110/0052-II/B/15/2012.

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

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

If yes:

(a) to which of the following organisms:

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

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

The natural host of LCMV is the house mouse (*Mus musculus*) but other rodents can be alternative reservoirs (Tagliapietra et al., 2009; Ledesma et al., 2009). In mouse carrier colonies, the virus maintains itself over generations by transplacental transmission and does not cause any clinical disease in its natural host. An acute infection in adult mice is controlled by a vigorous cellular immune response whereas mice infected in utero or shortly after birth become immunologically tolerant to the virus and develop life-long persistent LCMV infection. The mouse model is routinely used to investigate LCMV pathogenesis and the large amount of data published renders the mouse the best described animal model with regards to LCMV. It has been an excellent model for studying viral persistence, immunological tolerance and immunopathogenesis and represents the best experimental system for defining immune responses to chronic infection. Further, mice represent the most faithful and sensitive animal model to recapitulate choriomeningitis induced by LCMV, which is the only clinically significant complication of LCMV infection in immunocompetent human adults.

Further, a broad range of mammalian species are permissive to LCMV infection whereas the course of the disease and severity of the clinical symptoms are species specific.

Humans

Lymphocytic choriomeningitis virus is a seldom human pathogen and with the rare exception of vertical transmission and transplant associated transmission, there are to the sponsor's knowledge, no documented cases of human to human transmission. Congenital infection of the human foetus can occur when the mother is infected during pregnancy. Such vertical transmission can result in clinical disease of the unborn child, including malformations of the central nervous system. In humans, LCMV can cause a variety of symptoms from common malaise to meningitis, which is rare. In all events, postnatal patients virtually always recover without sequelae. Congenital infection more frequently results in clinical disease (Bonthius et

al., 2012). Seroprevalence in humans is very low on most continents and geographic regions and does not normally exceed 5% of the human population (Welsh and Seedhom, 2008).

Other mammals:
Neither guinea pigs, nor rabbits, dogs or non-human primates consistently show CNS pathogenesis after infection with LCMV. Rats have been used as a model to study LCMV pathogenesis in the CNS too, but data published on LCMV parenteral infections in rats is scarce. The available reports on LCMV pathogenesis in hamsters and the corresponding parameters of virus-host balance are unclear and somewhat conflicting data have been published. Guinea pigs do not reproduce any of the signs of CNS disease occasionally seen in humans with LCMV infection. Instead, LCMV-infected guinea pigs can develop a systemic inflammatory condition with high mortality. The severity of clinical signs of LCMV infection in guinea pigs and the corresponding lethality in this species are dependent on the virus strain used. Except rare cases of fever, rabbits and dogs show no clinical signs after LCMV infection and only very limited data about LCMV infection in these animal models is available. Infection of non-human primate models (cynomolgus and rhesus macaque) with the LCMV strain WE causing haemorrhagic fever and death within about two weeks. Conversely, symptoms of self-limiting choriomeningitis as occasionally seen in humans, are uncommon in non-human primates. Unlike in rodents, persistent LCMV infection has not been documented in human or non-human primates.

8. Information concerning reproduction

(a) Generation time in natural ecosystems:

Wild type LCMV replication rate in the natural host (*Mus musculus*) is approximately 4-6 hours (Bocharov et al., 2004).

HB-101 vectors are replication defective and cannot form infectious progeny in the natural ecosystem.

(b) Generation time in the ecosystem where the release will take place:

All vaccines will be administered to subjects within the designated clinical trial sites. HB-101 vectors are replication defective and cannot form infectious progeny in any ecosystem (only in the vector production cell line).

(c) Way of reproduction: Sexual NA Asexual NA

(c) Factors affecting reproduction:

LCMV is a live virus, which requires a host cell for replication.

The recombinant vectors used here, rLCMV, are propagation incompetent in non-complementing cell lines. The specific complementing cell line is not provided.

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

(b) relevant factors affecting survivability:

LCMV is a membrane enveloped virus and is thus expected to be unstable and lose significant amounts of infectivity upon exposure to room temperature and drying. Factors influencing virus stability are temperature (resulting in higher stability at low temperature), pH and buffer conditions. Hookipa Biotech did not test stability of wildtype LCMV virus however, very high concentrations of vector material stored in a stabilizing buffer system at room temperature are reduced to very low / undetectable quantities within 2 weeks (i.e. >100.000 fold decrease in titer).

10. (a) Ways of dissemination

Wild type LCMV is naturally spread by the common house mouse, *Mus musculus*, which can become persistently infected transplacentally and maintain virus in their blood and persistently shed virus in their excretions. Chronically infected female mice usually transmit the infection to their offspring (vertical transmission), which in turn become chronically infected, too.

Transmission from rodent carrier mice to humans commonly occur through bites and putatively also upon direct mucosal exposure of humans to the infectious excretions of persistently infected mice.

(b) Factors affecting dissemination

In mouse carrier colonies, the virus maintains itself over generations by transplacental transmission and does not cause any clinical disease in its natural host. An acute infection in adult mice is controlled by a vigorous cellular immune response whereas mice infected in-utero or shortly after birth develop a chronic LCMV infection.

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/../../…

The predecessor HB-101 formulation containing identical GMO material was used in a phase 1 clinical trial in Belgium and followed the contained use procedure (notification number: SBB 219 2016/0220).

Deliberate release not previously notified.

C. **Information relating to the genetic modification**

1. Type of the genetic modification

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

2. Intended outcome of the genetic modification

The deletion of the essential viral surface protein GP from the genome of LCMV prevents the vector from propagating in cells other than engineered packaging cell lines. Therefore, infection of the individual target cell is a self-limiting one-time event and the number of cells affected correlates with the dose administered.

One vector expresses a truncated isoform of human cytomegalovirus glycoprotein gB (HCMV gB), the second vector expresses HCMV phosphoprotein 65 (HCMV pp65). The vectors infect target cells in the vaccine recipient, replicate the genome and express the encoded vector proteins and vaccine antigens, which are presented to B- and T- cells to elicit an immune response. The gB antigen was designed to be transported and anchored on the surface of vector-infected cells, and pp65 is an intrinsically intracellular protein. The antigen gB elicits neutralising antibodies (humoral response), whereas pp65 is a strong inducer of cytotoxic T cells (cellular response). The vectors are incapable of propagating in the recipient.

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 (.) No (X)

If no, go straight to question 5.

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

(a) Type of vector

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

(b) Identity of the vector

(c) Host range of the vector

…

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

Yes (.) No (.)

antibiotic resistance (.)
other, specify …

Indication of which antibiotic resistance gene is inserted
…

(e) Constituent fragments of the vector
…

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

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

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

The GMO is generated *de novo* using a plasmid based vector rescue system encoding the (glycoprotein-deleted) sequence of LCMV strain Armstrong Clone 13. These plasmids encode the vector genome and an auxiliary factor (LCMV NP). Plasmids encoding for the LCMV S-Segment were genetically modified by introducing the coding sequence of HCMV gB or HCMV pp65 instead of the LCMV surface protein GP.

These plasmids were transfected in a dedicated vector production cell line in which the vector RNA genome is transcribed via endogenous polymerase I ultimately resulting in formation of rLCMV vector particles. Using GP proteins expressed by the vector production cell line, these rLCMV vector particles can replicate and spread. This is only possible in the production cell line and does not need any component of the plasmid system. Respective plasmids used for rescue degrade over time and are not part of the vaccine product.

6. Composition of the insert

(a) Composition of the insert

Deleted genetic material:
Hookipa Biotech HK-1-based vectors have been rendered replication-incompetent by deletion of the LCMV surface protein GP from the viral genome.

In wild type LCMV, GP is synthesized as a precursor GP-C, which is post-translationally cleaved into GP-1 and GP-2 subunits, and these remain non-covalently associated on the virion surface. GP-2 is membrane-anchored with an extracellular stalk-like domain, on which sits the GP-1 globular head that serves as receptor-binding unit. The GP-1/GP-2 complex is responsible for binding of the cellular receptor on target cells, and after internalization it mediates membrane fusion.

Inserted genetic material:
HCMV is a large complex virus encoding at least 200 proteins. Many of these proteins serve critical functions during the infection of host cells and in immune evasion and pathogenesis. Therefore, it is generally conceded that it would be beneficial to have more than one protein target in a vaccine against which to activate both humoral (neutralizing antibodies) and cellular responses in the host with the goals of pre-exposure prophylaxis, prevention of reactivation, and therapeutic activity.

Hookipa Biotech has selected two HCMV proteins to be expressed by LCMV vectors for generation of HCMV-specific neutralizing antibodies and T-cell responses, both of the cytotoxic CD8+ and of the helper CD4+ subset i.e. the HCMV proteins gB and pp65.

gB:
HCMV Glycoprotein B is the membrane fusion protein of HCMV (Sharma et al., 2013) and is expressed as a single polypeptide chain that is cleaved by furin into two domains of 58 kDa and 116 kDa that are covalently linked by disulphide bonds (Britt et al., 1986; Vey et al., 1995). gB together with proteins gH/gL are required for HCMV receptor binding and cell fusion, whereas gB acts as fusion protein of the virus. Importantly, viruses of the herpesvirus family (such as HCMV) require multiple membrane protein complexes for infectivity, and individual glycoproteins are inert. Thus, functional pseudotyping of LCMV vectors by herpesvirus glycoproteins does not occur which has been tested and confirmed with dedicated assays analysis formation of replication competent virus (RCV assays) in the context of the manufacturing process as well as in animal models.

Neutralizing antibodies to HCMV are directed towards the glycoproteins involved in viral attachment and entry. Glycoprotein B (gB) and gH elicit antibodies that neutralize HCMV in fibroblast cells. All HCMV seropositive individuals have antibodies against gB. A phase 2 randomized double blind placebo controlled study in seronegative women who received 3 doses of recombinant gB protein with MF59 adjuvant showed 50 % efficacy in protection against HCMV infections (Pass et al., 2009). The vaccine also reduced the duration of viremia and number of days of antiviral treatments in transplant recipients (Griffiths et al., 2011).

Pp65:
HCMV tegument proteins, such as pp65, pp71, pp150, and pp28, are localized between viral lipid membrane and capsid protein. They comprise more than half of the total protein mass found in HCMV infectious virions and play an important role in all steps of the viral life cycle including entry, gene expression, evasion assembly, and egress. The most abundant tegument protein is pp65, and it is a dominant protein target for both humoral

(non-neutralizing antibodies) and, especially, cellular immune responses. Pp65 has been incorporated in several different vaccine candidates tested in clinical trials, including poxvirus vectors, alphavirus replicons, and plasmid DNA vaccines.

(b) Source of each constituent part of the insert

All insert sequences have been chemically synthesized and are based on publically available reference sequences.

Specifically, vector encoded gB is based on HCMV strain Merlin (Genbank ref AY446894) and the respective open reading frame underwent codon optimization for human cell expression. Vector encoded pp65 (UL83) is based on HCMV strain AD169 (Genbank acc. No: P06725) and was also codon optimized for human cells.

(c) Intended function of each constituent part of the insert in the GMO

Upon vaccination with HB-101, the heterologous HB-101 antigens gB and pp65 are expressed in infected cells. The gB antigen was designed to be transported and anchored on the surface of vector-infected cells, and pp65 is an intrinsically intracellular protein. The gB antigen is included in the vaccine to elicit neutralizing antibodies (humoral response), which prevents HCMV fusion with the cell surface and entry into the cell. It also induces a cellular immune response, consisting of both CD4+ helper cells and CD8+ cytotoxic T cells. The pp65 antigen is a strong inducer of cytotoxic T cells (cellular response) and promotes clearance of HCMV infected cells.

(d) Location of the insert in the host organism

- on a free plasmid (.)
- integrated in the chromosome (.)
- other, specify …

The insert is integrated in the rLCMV vector genome. The open reading frame (ORF) encoding the LCMV GP, has been replaced with a gene encoding a truncated isoform of the envelope glycoprotein B (gB) or replaced with the tegument phosphoprotein pp65 (pp65) of HCMV.

(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:

viroid (.)
RNA virus (.)
DNA virus (X)
bacterium (.)
fungus (.)
animal

- mammals (.)
- insect (.)
- fish (.)
- other animal (.)
  (specify phylum, class) …

other, specify …

2. Complete name

(i)	order and/or higher taxon (for animals)	Herpesvirales
(ii)	family name for plants	Herpesviruses
(iii)	genus	Cytomegalovirus
(iv)	species	Human cytomegalovirus
(v)	subspecies	…
(vi)	strain	Merlin and AD169
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	Human CMV (HCMV)

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:

(b) to which of the following organisms:

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

(b) are the donated sequences involved in any way to the pathogenic or harmful properties of the organism

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

If yes, give the relevant information under Annex III A, point II(A)(11)(d):

Contribution of gB and pp65 to pathogenicity of HCMV

gB together with proteins gH/gL are required for HCMV receptor binding and cell fusion, whereas it appears that gB acts as fusion protein of the virus. Importantly, viruses of the herpesvirus family (such as HCMV) require multiple membrane protein complexes for infectivity, and individual glycoproteins are inert.

HCMV tegument proteins, such as pp65, pp71, pp150, and pp28, are localized between viral lipid membrane and capsid protein. Together, they comprise more than half of total proteins found in HCMV infectious virions and play an important role in all steps of the viral life cycle including entry, gene expression, evasion assembly, and egress. The most abundant tegument protein is pp65, and it is a dominant protein target for both humoral (non-neutralizing antibodies) and, especially, cellular immune responses. Pp65 has been

incorporated in several different vaccine candidates tested in clinical trials, including poxvirus vectors, alphavirus replicons, and plasmid DNA vaccines.

Pathogenicity of HCMV

Cytomegalovirus are species specific, thus pathogenicity of human CMV is restricted to humans only.

HCMV (also known as human herpesvirus 5 or HHV-5) is a virus of the betaherpesvirinae subfamily. The virus is acquired by contact with bodily fluids, with the most frequent transmission via the oropharyngeal and genital tract, although transmission can also occur through breast milk, blood transfusions or organ transplants (Ross and Boppana, 2005; Mocarski et al., 2007). In healthy individuals, primary infection is usually mild or asymptomatic, though acute infection can cause mononucleosis-like syndrome. Following primary infection, HCMV establishes a latent state, reactivating when there are changes in immune status (Mocarski et al., 2007).

Depending on study population, the reported seroprevalence is 50% to 85% in adults in the United States (CDC, 2015; Staras et al. 2006), 70% in Europe (Lopo et al., 2011; Hyde et al., 2010) and up to 95% in developing countries (Cannon et al., 2010). In Belgium, the seroprevalence in women of childbearing age was 30 and 54% in two separate studies conducted in Flanders and Brussels, respectively (Francisse et al., 2009; Leuriden et al., 2012).

In immune-suppressed persons like transplant patients, primary infection, reinfection or reactivation may cause severe disease such as pneumonitis, retinitis, hepatitis, nephritis, and encephalitis (Snydman, 1999; Johansson et al., 2010). Antiviral prophylactic strategies are limited by toxicities, drug–drug interactions and development of antiviral resistance (Kotton et al., 2013). Vaccination-mediated prevention of HCMV infection in solid organ and stem cell transplant patients, particularly in seronegative patients receiving solid organ transplants from seropositive donors, and in seropositive stem cell transplant recipients, represents a significant medical need.

The most important health consequence of HCMV at the public health level is congenital infection of the foetus, which may result from primary infection, reinfection, or reactivation during pregnancy. The risk of congenital infection is significantly higher in immunologically naïve women undergoing primary infection. In the US and Europe the prevalence of congenital CMV approximates 6.4 cases/1000 live births (60,000 cases/year) (CDC, 2015). Amongst congenitally infected infants, 4% die and 17–19% of the survivors will have permanent neurological sequelae, including microcephaly, hearing loss, motor deficits, cerebral palsy, mental retardation, seizures, ocular abnormalities and learning disabilities (Boppana et al., 1997; Kylat et al., 2006; Krause et al., 2014; CDC, 2015). Prevention of congenital HCMV infection is widely recognised as a high priority for new vaccine development.

Immunity acquired through natural infection helps contain HCMV in a latent state and reduces the clinical consequences of reinfections and reactivations. Neutralising antibodies against the virus contribute to protection. This has been demonstrated by protection of the foetus by transplacental maternal antibody and by passive transfer of immunoglobulin containing HCMV antibodies to transplant patients. The effect of passive immunoglobulin in prevention of congenital infection and resulting disease remains less clear and is still under

investigation. T cell mediated immunity has been shown to also prevent reactivation and to aid in recovery from HCMV infection, with multiple supporting lines of evidence, including adoptive transfer of cytotoxic T cells to transplant recipients (Riddell et al., 1992).

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

Cytomegalovirus (*Herpesviridae*) is listed in Annex III of Directive 2000/54/EC (which repealed and superseded Directive 90/679/EEC). Cytomegalovirus is listed as a Risk Group 2 infection hazard classification.

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

Specify …

The genetic modifications made for generation of HB-101 containing replication deficient vectors prohibit its replication outside of a dedicated production cell line. Infectious progeny can only be formed in this cell line or analogously engineered cell lines. The specific complementing cell line is not provided. Consequently the vectors are non-pathogenic and cannot survive in the host and cannot disseminated.

(b) is the GMO in any way different from the recipient as far as mode and/or rate of reproduction is concerned?

Yes (X) No (.) Unknown (.)

Specify

See answer to E 1(a) above.

(c) is the GMO in any way different from the recipient as far as dissemination is concerned?

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

Specify

See answer to E 1(a) above.

(d) is the GMO in any way different from the recipient as far as pathogenicity is concerned?

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

Specify

See answer to E 1(a) above.

2. Genetic stability of the genetically modified organism

See response provided for Section A.3(c) above.

None of the genetic modifications are anticipated to have any effect on the genetic stability of the GMO when compared to that of wild type virus, nor would they be expected to result in transfer or maintenance of genetic material in the environment (outside of the human subjects participating in the trial). The HCMV genes are not expected to confer any advantage to the GMO in terms of survival or selective pressure. As the mode of attenuation of rLCMV vectors is deletion of an essential gene (GP) for the viral life cycle, adaptive/reverting mutations restoring the replication competence cannot occur.

3. Is the GMO significantly pathogenic or harmful in any way (including its extracellular products), either living or dead?
Yes () No (X) Unknown (.)

(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)

The potential for adverse events caused by replication-deficient rLCMV vaccines is virtually excluded because the vector cannot form infectious progeny and spread. Cytopathology is not observed when human cells (HEK293) are infected with rLCMV vectors.

Animal studies have demonstrated that the vector administered via intracranial route causes no apparent pathology in immunocompromised mice. Biodistribution studies in immunocompetent mice indicated that the vector material can be found in multiple tissues after immunization, but was not detectable in any tissues 56 to 90 days after inoculation. In addition, toxicity studies in rats supplement these safety data. Research studies using analogous vectors (encoding different antigens) where performed in guinea pigs, rabbits and non-human primates and no apparent toxicities were observed in any of these studies. Further, Hookipa Biotech conducted a Phase 1 healthy volunteer study (Study H-100-001) based on predecessor HB-101, which attested the vaccine a favourable safety profile.

There are no reports indicating that LCMV or any other arenavirus are oncogenic. Arenavirus RNA transcription occurs in the cytoplasm of the infected cell, and there is no potential for integration into human host cell chromosomes. Thus, the use of rLCMV in humans has no oncogenic risk and there is no risk for integration into the human host cell genetic material. Both Klenerman et al. (Klenerman et al., 1997) as well as Geuking et al. (Geuking et al., 2009) report independently that complementary DNA (cDNA) formation and integration of LCMV sequences can occur, but is restricted to mice and hamster and their derived cell lines,

i.e. to the natural host species of LCMV, which harbour a murine retrotransposon accountable for the reverse transcription of LCMV RNA. Neither human nor monkey, dog or cow cell lines supported the process of cDNA formation/integration, despite being highly permissive to LCMV replication, and despite some of these cells expressing high levels of endogenous retrotranscriptase. Mice and hamster accidentally infected with HB-101 would not be exposed to a higher risk of integration of LCMV sequences in their genome than mice infected with wild type LCMV, which is globally endemic. In contrast, due to the replication incompetent, single round infectious character of HB-101 vectors, only a limited number of cells would be infected which dramatically reduces the likelihood of cDNA integration in cells of these animals compared to wild type virus infection.

<u>Risk of formation of replication competent viruses (RCV):</u>
In some instances foreign envelope proteins have the potential to be substituted for LCMV GP protein in the vector genome that would permit assembly of a functional envelope. Such pseudotyped viruses would be replication competent but still strictly confined to a range of host cells with such putative heterologous envelope protein pseudotyping capacity. Human cells do not normally have such pseudotyping capacity. Only under the hypothetical assumption that the HCMV transgenes in HK1-gB or HK1-pp65 were replaced by a heterologous viral glycoprotein with pseudotyping capacity, a replication-competent virus would result. Such hypothetical recombination events would be at least equally unlikely to occur as outlined for the hypothetical recombination of rLCMV vector genomes with the LCMV GP expressed in producer cells. Even in the highly unlikely event of such recombination to occur, the resulting virus would be highly attenuated. When the GP ORF of LCMV was experimentally substituted with the ORF of the G protein (VSVG) of vesicular stomatitis virus (VSV), the virus was able to replicate in normal cells (Pinschewer et al., 2004). These studies have demonstrated that a VSVG recombinant LCMV was highly attenuated with in vitro titers 10 to 100 fold lower than wild type LCMV (Pinschewer et al, 2004; Bergthaler et al, 2006). Further, the VSVG recombinant virus elicited overt viremia only in very highly immunocompromised mice deficient in recombination activation gene 1 (RAG1), thus lacking T cells and, B cells, and additionally lacking the type I interferon receptor. Moreover, the ability to confer heterologous enveloped virions with infectivity is a very unique feature of select viral glycoproteins and specifically of VSVG. Conversely, most other viruses including those of the herpesvirus family require multiple membrane protein complexes for infectivity, and individual glycoproteins are inert. Thus, functional pseudotyping by herpesvirus glycoproteins is extremely unlikely to occur. As the HB 101 vectors do not contain GP in their genome, pseudotyped LCMV would require integration of genetic material for a compatible envelope protein from an exogenous virus. This would require co-infection of one cell by the vaccine vector and a heterologous virus and two recombination events at precise sites in the vector genome within a single replication step. In the very unlikely event that such an exchange would occur, there is no reason to believe that the virus would be more pathogenic than the parent LCMV Armstrong-Clone 13.

The potential for pseudotyped viruses in the drug product is controlled as part of master virus seed (MVS) release testing and routine manufacture of the drug substances through testing for replication competent viruses.Presence of RCV is tested at the harvest step after vector production is complete, and represents a stage that is the worst-case scenario for RCV, if it were to be present. Recombination events resulting from propagation of HK-1-gB or -pp65 in the complementing HEK293-GP cell line to yield a replication competent LCMV are extremely unlikely as i) negative strand RNA viruses seldom recombine, ii) two recombination events in fairly precisely defined nucleotide stretches of the vector genome would have to take place during one single replication cycle of the viral RNA dependent RNA polymerase in order to reconstitute a replication-competent genome, and iii) hypothetical RNA recombination intermediates, resulting from only one of these recombination events, would lack a functional viral promoter and thus could not be read or amplified by the viral polymerase. This excludes the possibility that replication-competent LCMV could result from two independent recombination events in sequential rounds of RNA amplification.

Additionally, emergence of hybrid virus in a vector-infected subject who would become infected with wild type LCMV is extremely unlikely. In this case, only a reassortment step with wild type LCMV would be needed to yield a replication competent virus. However, the resulting virus would be composed of LCMV Armstrong-Clone 13 encoding the surface protein of wild type LCMV virus, which would be the virus that the subject was already infected with and would not be thought to yield a more infectious or pathogenic virus than the wild type.

Risks associated with expression of HCMV transgenes:

Glycoprotein B is the membrane fusion protein of HCMV (Sharma et al., 2013) and is expressed as a single polypeptide chain that is cleaved by furin into two domains of 58 kDa and 116 kDa that are covalently linked by disulphide bonds (Britt et al., 1986; Vey et al., 1995). Glycoprotein B is targeted by neutralizing antibodies, and four antigenic domains containing the corresponding epitopes have been identified (Utz et al., 1989; Meyer et al., 1990). Phosphoprotein pp65 is an autophosphorylated kinase (Somogyi et al., 1990) and is the most abundant protein in HCMV particles and is delivered to the target cell upon infection, where it interferes with the interferon response (Varnum et al., 2004; Li et al., 2013). Live, attenuated HCMV vaccines, DNA vaccines expressing HCMV genes, and alphavirus and poxvirus vectors encoding gB and pp65 (or isoforms thereof) have been brought into clinical trials and so far, no safety signals were reported. In addition, translation of HCMV proteins by rLCMV vectors is transitory, due to replication deficiency of the vector and clearance of vector infected cells by the immune system of an infected host.

Safety of HB101 in humans:

The safety profile of HB-101 is based upon data from 24 healthy male and 30 healthy female subjects aged between 18 and 45 years inclusive in study H-100-001. Following three administrations of HB-101 at 2.6×10^8, 2.6×10^7, or 2.6×10^6 FFU, pain at the site of injection was the most frequently reported local solicited symptom. Malaise, fatigue, and generalised myalgia were the most frequently reported general solicited symptoms. Fever was rarely reported. The majority of the general solicited symptoms were considered related to HB-101.

Outside of the host rLCMV material is expected to be susceptible to common disinfectants, such as sodium hypochlorite, aldehydes, sodium hydroxide, formaldehyde and peracetic acid.

HB-101 does not present specific resistance to antibiotics. The vector does not contain any gene that confers resistance to antibiotics.

Currently, there are no prophylactic vaccines available and an effective antiviral therapy has not been established. Given the usually flu-like manifestation of infection and the benign course of the (comparably rare) CNS manifestations (choriomeningitis, meningoencephalitis) symptomatic therapy is sufficient. As the material is derived from a virus, antibiotics are ineffective and should not be administered without further cause. Ribavirin is effective *in vitro*, and may be effective for treatment of LCMV *in vivo*; other antivirals have not been tested.

4. Description of identification and detection methods

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

   No environmental detection measures are planned.

   (b) Techniques used to identify the GMO

   No direct GMO detection measures are planned.

F. Information relating to the release

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

   The investigational product HB-101 is being developed as a vaccine for the prevention of clinically significant CMV infection, defined as the occurrence of either CMV end-organ disease, or initiation of anti-CMV pre-emptive therapy based on documented CMV viremia and the clinical condition of the patient.

   The intended application of HB-101 is limited to a phase 2 clinical study titled "A Randomized, Placebo-Controlled, Phase 2 Study of HB-101, a Bivalent Cytomegalovirus (CMV) Vaccine, in CMV-Seronegative Recipient (R-) Patients Awaiting Kidney Transplantation from Living CMV-Seropositive Donors (D+)."

   The administration of HB-101 will occur at approximately 25 to 40 sites globally.

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 …

   HB-101 will be released in a hospital or clinic. LCMV is indigenous to EC countries (see answer to B (3) above).

3. Information concerning the release and the surrounding area

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

The release will be conducted at the following clinical study sites in Germany

Prof. Dr. Oliver Witzke
Universitätsklinikum Essen
Klinik für Nephrologie und Klinik für Infektiologie
Hufelandstr. 55
45147 Essen
Germany

(b) Size of the site (m^2): … m^2
   (i) actual release site (m^2): Not applicable
   (ii) wider release site (m^2): Not applicable

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

Not applicable.

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

Sections (b) – (d) above are not considered relevant in this case. Environmental release of HB-101 is not intended beyond the treatment of trial subjects. Treatment will be on an outpatient basis and therefore proximity to internationally recognized biotopes or protected areas (including drinking water reservoirs) cannot be specified as the movement of treated subjects will not be restricted.

Wild type LCMV is not known to be involved in environmental processes and therefore HB-101 is not anticipated to be involved in environment processes.

4. Method and amount of release

(a) Quantities of GMOs to be released:

It is the intent of Study H-100-002 to administer three doses of study drug HB-101 (or placebo) prior to transplantation and within proximity to the time of transplantation.

The volume of administration of one dose of study drug is 1.0 mL.

(b) Duration of the operation:

HB-101 is intended for intramuscular (IM) administration and is ready-to-use.

The vaccine will be prepared at the hospital pharmacy by an unblinded pharmacist or designee per instructions in the pharmacy manual, independent of the clinical staff, and delivered to the clinical team for blinded administration.

The HB-101 vaccine vials are removed from the ultra-low freezer and are placed at room temperature to thaw for 15 minutes. Trace amounts of small product-derived particles may be observed in the vaccine. HB-101 will be drawn into the syringe using a filter needle. After replacing the filter needle by a syringe needle, the vaccine will be administered via the intramuscular route in a hospital setting, followed by a 60-minute observation period.
60-minute observation period.

HB-101 prepared syringe can be stored at 2°C to 8°C for up to 4 hours until administration.

Hookipa Biotech AG will provide the sites with a syringe and a needle for product administration, as well as a second compatible needle with integral filter to be used to initially draw the product into the syringe.

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

The material is considered to be non-hazardous, Category B, for ground or air transport.

- LCMV is not listed as a category A agent by the IATA.
- The disease pattern induced does not meet the criteria for inclusion into category A.

Transport of the material should be in closed, leak proof, break resistant containers, lined with absorbent material and labelled “biological substance, category B” as well as “UN 3373” according to the “IATA Guidance Document Infectious Substances.”

At the participating clinical trial sites all the medical professionals involved in all steps of the clinical trial are qualified and specifically trained on the protocol and the related procedures to minimize any risk of unintended exposure.

Established routine practices for dealing with potentially biohazardous materials are in place along with protective equipment including laboratory coats and gloves. Appropriate procedures are established for collection, processing and transportation of clinical supplies per the study protocol and, site procedures.

All monitoring activities will be reported and archived. In addition, monitoring visits will be documented at the investigational site by signature and date on the study-specific monitoring log. No monitoring of unintended recipients is planned and is not considered necessary.

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

HB-101 will be administered at clinical sites in an enclosed facility at ambient room temperature.

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.

Hookipa Biotech completed a Phase 1 healthy volunteer study (Study H-100-001) of predecessor HB-101 (encoding pp65 and a truncated gB of HCMV) in Belgium (EudraCT Number 2016-000632-17) according to Directive 2009/41/EC of 6 May 2009 on the contained use of genetically modified micro-organisms.

In brief, Hookipa Biotech observed neutralizing antibodies formed against the gB antigen after 2 or 3 vaccinations, a favourable safety profile, CMV neutralizing antibodies after 3 vaccinations on a par with previously studied vaccines, and gB- and pp65- specific T cell responses previously shown to correlate with protection (in adoptive T cell transfer studies). All three doses of the HB-101 candidate vaccine were safe and well-tolerated for all three administrations. There was a trend of increasing reactogenicity with increasing dose. However, there was no significant cumulative reactogenicity. The long term safety of HB-101 was confirmed, no SAEs were reported. No AE led to discontinuation of test article administration or discontinuation of subjects.

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)	Primate
(ii)	family name for plants	…
(iii)	genus	*Homo*
(iv)	species	*Homo 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)

   HB-101 is being developed as a vaccine to prevent clinically significant CMV infection, defined as the occurrence of either CMV end-organ disease, or initiation of anti CMV pre-emptive therapy based on documented CMV viremia and the clinical condition of the patient. The target population of HB-101 in the proposed phase 2 study is recipients of solid organ transplantation. The HB-101 vectors infect target cells in the vaccine recipient, replicate the genome, and express the encoded vector proteins and vaccine antigens, which are presented to B- and T-cells to elicit an immune response in the vaccinee.

3. Any other potentially significant interactions with other organisms in the environment

   No potentially significant interactions with other organisms in the environment are predicted.

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?

   Yes (.) No (X) Not known (.)
   Give details…

   The genetic modifications made for generation of HB-101 containing replication deficient vectors prohibit its replication outside of a dedicated production cell line. Infectious progeny can only be formed in this cell line. The specific complementing cell line is not provided. Consequently, the vectors are not anticipated to possess increased competitiveness or invasiveness.

5. Types of ecosystems to which the GMO could be disseminated from the site of release and in which it could become established

   HB-101 cannot form infectious progeny unless propagated in genetically engineered production cell lines. This prohibits spread of these GMOs in the environment/free nature. Formation of RCV is extremely unlikely to occur and was not detected in the HK1-HgB or HK1-Hpp65 virus seed banks nor in the harvests of the GMP grade bioreactor runs (which were performed for generation of the clinical trial material) in a qualified RCV assay.

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 …

7. Likelihood of genetic exchange in vivo

   (a) from the GMO to other organisms in the release ecosystem:

   Negligible.

   (b) from other organisms to the GMO:

   Negligible.

   (c) likely consequences of gene transfer:

   Hookipa Biotech does not expect significant harm to any species in case of unintended release and therefore the consequence of any potential gene transfer is considered negligible.

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 specific studies have been conducted regarding transmission of HB-101 between humans or animals and on the ecological impact of the vector in simulated natural environments.

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)

   HB-101 is not anticipated to have any interactions with biogeochemical processes.

H. Information relating to monitoring

1. Methods for monitoring the GMOs

   No specific direct monitoring for the GMO is currently planned for the study.

2. Methods for monitoring ecosystem effects

   Not applicable.

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms

   No monitoring of other organisms is planned.

4. Size of the monitoring area (m^2)

   Not applicable.

5. Duration of the monitoring

   Not applicable.

6. Frequency of the monitoring

   Not applicable.

I. Information on post-release and waste treatment

1. Post-release treatment of the site

   Appropriate routine cleaning procedures, practices and waste disposal procedures for potentially infective materials are in place. All used material are regarded as medical waste.

   Spills and leaks: Allow aerosols to settle, wear protective clothing, contain any spills and decontaminate using a solution of sodium hypochlorite (bleach) or other viricidal disinfectant. Volume of bleach or disinfectant should not be less than 10% of the spill volume. Allow enough time for neutralization according to choice of disinfectant. Absorb spill using suitable absorbent materials to wipe.

   Environmental precautions: Do not allow product to enter drains or municipal waste stream without inactivation or decontamination.

   Susceptibility to disinfectants: Biological rLCMV material is expected to be susceptible to common disinfectants, such as sodium hypochlorite, aldehydes, sodium hydroxide, formaldehyde, peracetic acid.

2. Post-release treatment of the GMOs

   Proper precautions and biological containment should be taken when handling viral material:

- o Personal protective equipment should be worn as needed, including e.g. lab coats and gloves.
- o Wash hands and other exposed areas thoroughly with mild soap and water before eating or drinking.
- o Disinfect and wash contaminated clothing before reuse.

Handling: Keep tightly closed when not in use.
Storage procedures: Suitable for most general chemical storage areas.
Specific end uses: For use in humans (individuals participating in clinical trial)

3. (a) Type and amount of waste generated

Empty vials and used vials and the used delivery system components (injection needle and syringe), gauzes and personal protective equipment (gloves, lab coat) and components used for collecting body fluids samples after administration.

3. (b) Treatment of waste

Decontaminate all materials or unused product for disposal by steam sterilization.

J. Information on emergency response plans

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread

Several measures have been implemented at the sites to mitigate the impact of any spillage or breakages during administration at the sites:

- Qualification of site personnel and vaccine administrators, and
- First aid and control measures to address exposure and contact following spillage or breakage

The likelihood of secondary exposure to close contacts of patients who receive the treatment is considered negligible. HB-101 cannot replicate in the human body, and shedding through saliva, urine, faeces and semen is expected to be low level if any, transient, and non-infectious. In the clinical trial protocol, patients are required to use highly effective contraception from the time period between signing of the informed consent and up to twelve months after the last dose of study drug or up to completion of the study, whichever is longer.

2. Methods for removal of the GMO(s) of the areas potentially affected

Biological rLCMV material is expected to be susceptible to common disinfectants, such as sodium hypochlorite, aldehydes, sodium hydroxide, formaldehyde and peracetic acid.

In the event of a spill incident allow aerosols to settle, wear protective clothing, contain any spills and decontaminate using a solution of sodium hypochlorite (bleach) or other viricidal disinfectant. The volume of bleach or disinfectant should not be less than 10% of the spill

volume. Allow enough time for neutralization according to choice of disinfectant. Absorb spill using suitable absorbent material to wipe.

3. Methods for disposal or sanitation of plants, animals, soils, etc. that could be exposed during or after the spread

Decontamination of plants, (non-human) animals and soils will not be required.

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

The patients participating in the clinical trial will be monitored throughout treatment. All adverse events occurring during the study period must be reported per reporting requirements mandated by applicable regulatory authorities.

All clinical monitoring activities, to ensure the safety of patients, will be reported and archived. In addition, monitoring visits will be documented at the investigational site by signature and date on the study-specific monitoring log.

No specific direct monitoring for the GMO is currently planned for the study. No monitoring for detecting transfer of the donated genetic material from the GMO to other organisms is planned. No monitoring of unintended recipients is planned and is not considered necessary.

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