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	**BE**
(b)	Notification number	B/BE/20/BVW2
(c)	Date of acknowledgement of notification	20/Feb/2020
(d)	Title of the project: A Phase 1b/2, Randomised, Placebo-controlled, Dose-ranging Study to Evaluate Safety, Tolerability and Immunogenicity of a modified Chimpanzee Adenovirus (ChAdOx1)-vectored Multigenotype High Risk Human Papillomavirus (hrHPV) Vaccine and Modified Vaccinia Ankara (MVA)-vectored Multigenotype hrHPV Vaccine in Women with Low-grade HPV-related Cervical Lesions	
(e)	Proposed period of release	From 08-Jun-2020 until 31-March-2021

2. Notifier

Name of institution or company:

Vaccitech Limited
Schroedinger Building
Oxford Science Park,
Heatley Road
Oxford OX4 4GE

3. GMO characterization

- ChAdOx1-HPV
  - Parental organism: the replication-competent simian-derived ChAdY25 adenovirus isolate from which the engineered vector backbone is derived
  - Recipient organism: the "empty" (i.e. without the transgene) replication-defective ChAdOx1 simian-derived adenovirus vector backbone
  - Donor organism: the organism(s) from which sequences encoded by the GMO are derived: Human Papillomavirus (HPV).
- MVA-HPV
  - Parental organism: the replication-competent dermal vaccinia strain Chorioallantois Vaccinia virus Ankara (CVA) from which the parental viral MVA vector is derived
  - Recipient organism: the "empty" (i.e. without the transgene) replication-deficient Modified Vaccinia Ankara (MVA) parental viral vector
  - Donor organism: the organism(s) from which sequences encoded by the GMO are derived: Human Papillomavirus (HPV).

(a) Indicate whether the GMO is a:

- ChAdOx1-HPV and MVA-HPV

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)

- ChAdOx1-HPV

ChAdOx1-HPV is a recombinant replication-incompetent simian (chimpanzee-derived) adenoviral vector derived from serotype Y25, encoding 59 gene segments from HPV proteins E1, E2, E4, E5, E6 and E7 from genotypes 16, 18, 31, 52, 53 and 58; the segments are joined end-to-end and the resulting insert is a transgene of 4082 base pairs encoding a fusion protein of 1255 amino acids.

Adenoviruses are classified by the EMA as non-integrating. The ChAdOx1 vaccine vector was derived from the ChAdY25 after it was genetically modified and incapacitated for replication, by deletion of the essential E1 genes (Dicks, 2012); the non-essential E3 gene is also deleted. The genetically modified ChAdOx1 parent viral vector for ChAdOx1-HPV is not capable of genetic integration into the host. The adenoviral DNA will only replicate in permissive cells which provide the essential viral replication E1 region in trans e.g. Human Embryonic Kidney 293 (HEK293) cells (with which there are only short overlapping sequences, making the likelihood of recombination very low). ChAdOx1-HPV is a non-integrative virus and following infection of the target human host cell, ChAdOx1-HPV DNA localises in the cell nucleus but does not integrate its DNA into the host cell genome. Integration of adenovirus DNA into the host cell genome has been observed only as an extremely rare event in some human primary cell line cultures. It remains transiently episomal until the cell is destroyed.

The HPV transgene is a synthetic antigen sequence derived from consensus regions, not related to pathogenicity, from thousands of strains of HPV.

- MVA-HPV

Orthopoxviruses are non-integrating. The MVA vaccine vector was derived from the replication-competent dermal vaccinia strain Chorioallantois Vaccinia virus Ankara (CVA) and has been attenuated by more than 570 serial passages in primary cultured Chicken Embryo Fibroblasts (CEF). These mutations have rendered the MVA virus highly attenuated and unable to productively replicate in most mammalian cell lines, including primary human cells and most transformed

human cell lines. MVA is a non-integrative virus, following infection of the target human host cell, it remains exclusively in the cytoplasm, therefore, its DNA remains outside the cell nucleus eliminating any risk of integration of the viral DNA into the host genome.

The HPV transgene is a synthetic antigen sequence derived from consensus regions, not related to pathogenicity, from thousands of strains of HPV.

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

- ChAdOx1-HPV

  The probability of ChAdOx1 reversion is negligible as for homologous recombination to occur it would require co-localisation with a wild-type adenovirus, but ChAdOx1 homologues only circulate in chimpanzees. In addition, the likelihood of recombination with a wild-type human adenovirus is negligible, since there is not enough DNA sequence homology in the E1 region to allow for this event to occur. During growth of ChAdOx1 up to 10^{14} viral particles in the (HEK293) cell-line, no replication-competent virus has been identified, despite the cell-line expressing the adenoviral E1 genes (IMPD-ChAdOx1).

  The genetic structure of ChAdOx1-HPV 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 vector insert. All genetic characterisation analyses on tested products showed conformity to theoretical 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 ChAdOx1 viral vector and the HPV insertion region of the production cell could arise. Although the risk of occurrence of this event is considered very low, the ChAdOx1-HPV material (MVS and DS) is tested for the presence of RCA using a well-established assay (specification: <1 RCA / $3x10^{10}$ vp). The confirmation of absence of RCA is a DS release test and has been demonstrated in all batches of ChAdOx1-HPV and all other ChAdOx1 GMP material manufactured to date.

- MVA-HPV

  The genetic stability of the MVA-HPV viral vaccine clinical vector 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. Analytical measures include the determination of infectious titre in permissive cell culture, DNA sequencing of the transgene, restriction analysis and identity and purity testing by PCR amplification of specified target sequences. We have also confirmed the genetic stability of MVA-HPV by whole-genome sequencing after 10 viral passages in cell culture. In summary, testing performed at different stages of the production process provides phenotypic and genotypic verification of the genetic stability of the clinical viral vector (MVA-HPV) as compared to reference standards.

The probability of the MVA vector reversion to CVA is highly improbable, as there is no known poxvirus able to complement MVA to generate a replication-competent virus; furthermore, the extensive passaging in CEF has resulted in the loss of roughly 15% (30 kbp) of the original genetic information. Spontaneous reversion of MVA to replication-competent CVA virus has never been documented, despite extensive use of MVA as a viral vector.

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

   Yes () No (x)

   GMO will be released in GB under "Contained Use"

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

   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

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

- <u>ChAdOx1-HPV</u>
  The proposed study is a FIH clinical trial being conducted within specific clinical study sites only. The GMO will only be applied by trained staff at the study sites. Furthermore, once administered no additional release (e.g. via shedding) is expected. The following features indicate that it is highly unlikely that clinical trial will have any impact on other humans, flora or fauna, whether in close or far proximity to where the GMO is administered:

  - *Controlled manufacturing processes:*
    Each vaccine is produced under good manufacturing practice (GMP) conditions with the handling of live material in appropriate facilities with proper containment measures. Potential release of GMO is thereby controlled, waste material is inactivated and incinerated, using single use equipment as much as possible; this avoids unnecessary release of GM material into the environment.

  - *Absence of replication competent adenovirus (RCA):*
    ChAdOx1-HPV is incapable of replicating outside a host cell as it is engineered to be replication-incompetent. The ChAdOx1-HPV vaccine is tested for the presence of RCA during different steps of the manufacturing process.

- *Clinical study site management:*
  The release of the GMO will take place during the conduct of a controlled clinical trial at a restricted number of centres, experienced and trained in the handling of GMOs. The vaccines will be handled according to the hospital procedures for GMOs; this includes receipt and storage of the vaccine and disposal as hazardous waste via certified vendors. A Pharmacy Manual for the clinical trial will be provided which specifies all the instructions for storage, dilution, preparation and administration of the vaccines and all relevant staff will be trained in these requirements and the hospital GMO procedures. Personal Protective equipment will be utilised comprising gloves aprons/gowns and eye protection. Any spills will be cleaned with 70% IPA to which the vectors are susceptible and a spill-kit will accompany the transport of prepared syringes to the clinical trial participants; the syringes will have been sealed with a Luer lock prior to transport and placed in a labelled GMO-transport box. The administration of the vaccine will be performed within designated clinical study sites. All these measures will ensure that dissemination and inadvertent transmission of the virus is minimised, even in the unlikely event of accidental exposure.

- *Route of administration in the clinical study:*
  A small volume and viral titre will be administered via IM to the participants, so it is unlikely to leak from the injection site after needle removal. As an extra precaution, the site is cleaned with a standard alcohol wipe following injection, which would inactivate any virus that had leaked onto the skin. A dressing is then applied on the injection site and the participant remains at the clinical trial centre for at least 30 minutes, at which point the dressing is removed and the injection site will be dry. It is, therefore, highly unlikely that the GMO will come into contact with the environment.

- *Lack of toxicity:*
  A GLP single-dose toxicity study was conducted in CD-1 mice dosed via the intramuscular route with ChAdOx1-HPV dose levels approximating to the maximum clinical dose to be used in this clinical trial. There were no significant toxicological findings in animals sacrificed at 2 days and 2 weeks after dosing or in recovery groups sacrificed 4 weeks after cessation of dosing.

- *No integration in host genome / stability:*
  ChAdOx1 is a non-integrative virus, so it does not integrate into the host cell genome. Subgroup E adenoviruses enter cells via the Coxsackievirus-adenovirus receptor (CAR); once it has entered these cells, it is replication-incompetent and exists episomally with no capacity of gene transfer to the genome of the host cell. They possess a stable virion, allowing inserts of foreign genes to remain intact and they can infect many different cell types. Lack of prolonged transgene expression has made replication incompetent adenoviruses attractive as viral vectors for vaccine development.

- *Limited biodistribution:*
  Although no biodistribution studies have been conducted with a ChAdOx1-vectored vaccine, they have been performed by the University of Oxford with three recombinant viral vectored vaccines based on E1, E3-deleted simian adenovirus (AdCh63-ME-TRAP and AdCh63-MSP-1 for malaria and AdCh3NSmut for hepatitis C), as well as one human adenovirus 6 vectored vaccine. These studies, conducted on

BALB/c mice, have shown limited spread to other tissues and no evidence of shedding, as the virus vector does not replicate and remains mainly localized to the site of injection.

- *Absence of homologous viruses:*
  The risk of occurrence of the formation of RCA from homologous recombination between the ChAdOx1 vector and its naturally occurring homologue is considered to be negligible; firstly, for homologous recombination to occur it requires co-localisation with a wild-type adenovirus, but ChAdOx1 homologues only circulate in chimpanzees. In addition, the likelihood of recombination with a wild-type human adenovirus is negligible, since there is not enough DNA sequence homology to allow for this event to occur.

- *Clinical experience indicating low risk of viral shedding:*
  Replication-incompetent adenoviruses have been used extensively in clinical trials, either through direct administration or cell therapy strategies (contained in the cells). Following IM administration of a similar E1, E3-deleted simian adenovirus AdCh3NSmut, expressing a hepatitis C virus transgene, in both a chimpanzee study and a clinical trial (EudraCT Number: 2007-004259-12), no viral vector shedding was detected in urine or throat swabs.

- MVA-HPV

  The proposed study is a FIH clinical trial being conducted within specific clinical study sites only. While there is no available data on the environmental impact of the release of MVA-HPV, there is a large experience with trials with MVA-based investigational medicinal products. The following features indicate that it is highly unlikely that clinical trial will have any impact on other humans, flora or fauna, whether in close or far proximity to where the GMO is administered:

  - *Controlled manufacturing processes:*
    Each vaccine is produced under good manufacturing practice (GMP) conditions with the handling of live material in appropriate facilities with proper containment measures. Potential release of GMO is thereby controlled, waste material is inactivated and incinerated, using single use equipment as much as possible; this avoids unnecessary release of GM material into the environment.

  - *Absence of wild type and replication competent virus:*
    MVA-HPV is engineered to be replication-deficient and has a highly restricted host range. It does not replicate in most mammalian cells, including humans.
    Testing of viral vectors using PCR-based assays and other approved methods are used to differentiate between human pathogenic Vaccinia viruses and attenuated MVA strains. They are also used to confirm the identity of the specific viral vaccine vector. The release criteria for this particular viral vector is the confirmation of sequence specificity of the vector and the absence of any other contaminating vectors.

  - *Clinical study site management:*
    The release of the GMO will take place during the conduct of a controlled clinical trial; the administration of the vaccine will be performed within designated clinical study sites. Clinical study staff at these sites will have been trained in the preparation, administration and disposal of the GMO according to the instructions in the Pharmacy Manual specific to the clinical trial and the study site SOPs. All these measures will ensure that dissemination and inadvertent transmission of the virus is minimised, even

in the unlikely event of accidental exposure. All GMO materials used during the preparation and administration of the GMO will be treated as biohazard waste and disposed of by certified vendors.

- *Route of administration in the clinical study:*
  A small volume and viral titre will be administered via IM to the participants, so it is unlikely to leak from the injection site after needle removal. As an extra precaution, the site is cleaned with a standard alcohol wipe following injection, which would inactivate any virus that had leaked onto the skin. A dressing is then applied on the injection site and the participant remains at the clinical trial centre for at least 30 minutes, at which point the dressing is removed and the injection site will be dry. It is, therefore, highly unlikely that the GMO will come into contact with the environment.

- *Lack of toxicity:*
  A GLP single-dose toxicity study was conducted in CD-1 mice dosed via the intramuscular route with MVA-HPV dose levels approximating to the maximum clinical dose to be used in this clinical trial. There were no significant toxicological findings in animals sacrificed at 2 days and 2 weeks after dosing or in recovery groups sacrificed 4 weeks after cessation of dosing.

- *No integration in host genome / stability:*
  MVA is a non-integrative virus; it remains exclusively in the cytoplasm after entering a cell, thus its DNA remains outside of the cell nucleus.
  MVA is a double-stranded DNA virus, and as with all orthopoxviruses, encodes its own DNA polymerase that provides genetic proofreading; this results in typically low rates of mutation from one passage to the next.

- *Limited biodistribution:*
  Several biodistribution studies have been performed with other MVA-vectored vaccines (MVA85A [tuberculosis], MVA-ME-TRAP and MVA-MSP1 [malaria] by the University of Oxford and MVA-MERS [Middle East Respiratory Syndrome]) (Langenmayer[1, 2], 2018), in which mice were vaccinated either by intradermal or IM injection. These studies have shown no viral shedding and limited spread to other tissues, as the virus vector remains mainly localized to the site of injection. Similar biodistribution is expected for MVA-HPV.

- *Absence of homologous viruses:*
  The risk of homologous recombination is negligible as the parental Vaccinia virus no longer exists since the eradication of the smallpox virus and there are no known pox viruses able to complement MVA to generate a replication-competent virus. Spontaneous reversion of MVA to replication-competent virus has never been documented, despite extensive use of MVA as a viral vector. The HPV antigen transgene segment in MVA-HPV is unable to reverse the replication-deficient genotype of the MVA vector.

- *Clinical experience indicating low risk of viral shedding:*
  MVA-vectored vaccines have been used extensively in clinical trials with no reports indicating shedding of the vaccine following administration. In one study reporting the results of a Phase I immunotherapy with a MVA expressing human MUC1, urine samples collected 4 h post-injection and on day 8 appeared to be negative for the presence of vector sequences (Rochlitz, 2003).

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)

- ChAdOx1Y25 and MVA

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

specify phylum, class …

2. Name

- ChAdOx1Y25

(i)	order and/or higher taxon (for animals)	Adenoviridae
(ii)	genus	Mastadenovirus
(iii)	species	Simian adenovirus
(iv)	subspecies	Subgroup C
(v)	strain	Serotype Y25
(vi)	pathovar (biotype, ecotype, race, etc.)	…
(vii)	common name	ChAdY25

The parental ChAdY25 strain is a wild-type chimpanzee adenovirus.

The recipient is the adenoviral genome (replication-incompetent) of ChAdOx1 in the form of a bacterial artificial chromosome (BAC) in order to allow for replication of the DNA construct in bacteria. The E1 and E3 loci were deleted from the adenoviral genome to render the adenovirus replication-incompetent, and the endogenous E4Orf6/7 was replaced with E4Orf6/7 from Human adenovirus 5 (HAd5) in order to improve productivity in human embryonic kidney (HEK293)-based producer cell lines.

- MVA

(i)	order and/or higher taxon (for animals)	Poxviridae/Chordopoxviridae
(ii)	genus	Orthopoxvirus
(iii)	species	Vaccinia virus
(iv)	subspecies	
(v)	strain	Chorioallantois Vaccinia virus Ankara pathovar
(vi)	pathovar (biotype, ecotype, race, etc.)	…
(vii)	common name	CVA

The MVA virus is a highly attenuated strain of Vaccinia virus originally developed as a smallpox vaccine. The virus has been derived from the replication-competent dermal vaccinia strain Chorioallantois Vaccinia virus Ankara (CVA) and has been attenuated by more than 570 serial passages in primary cultured Chicken Embryo Fibroblasts (CEF). This passaging has resulted in many mutations in the MVA virus genome.). These mutations have rendered the MVA virus highly attenuated and unable to productively replicate in most mammalian cell lines, including primary human cells and most transformed human cell lines.

3. Geographical distribution of the organisms

- ChAdOx1Y25

(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)
(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)

- MVA (CVA)

(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)
(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

- ChAdY25

water	(.)
soil, free-living	(.)
soil in association with plant-root systems	(.)
in association with plant leaf/stem systems	(.)
other, specify	(x) chimpanzee

Adenoviruses have a worldwide distribution. Wild-type adenoviruses have been detected in waters around the world, including waste water, river water, drinking water, oceans and swimming pools. Humans and animals are the natural reservoirs for wild-type adenoviruses. The natural host of the ChAdY25 adenovirus is the chimpanzee. The parental adenovirus is not found in natural ecosystem outside of its natural host.
The recipient is a replication-incompetent chimpanzee adenovirus modified in the laboratory. It is not found in natural ecosystems. It is grown in E1-complementing human embryonic kidney (HEK-293) based cell lines dedicated to the propagation of viruses with deletions of key genes for replication, such as the E1.

- MVA

water	(.)
soil, free-living	(.)
soil in association with plant-root systems	(.)
in association with plant leaf/stem systems	(.)
other, specify	(x) not found in natural ecosystems

CVA no longer exists, since the eradication of the smallpox virus. MVA is a recombinant attenuated live vaccinia virus with an extremely limited host range. It is not found in natural ecosystems. It replicates well in avian cells (chicken embryo fibroblasts or CEF) and baby hamster, but poorly in most mammalian cells (Mayr, 1975; 1978; Drexler, 1998) and it is unable to spread in normal human cells.

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

5. Detection & identification techniques

(a) Detection techniques

- ChAdY25
Adenoviruses are detected by PCR according to generic sequences of chimpanzee adenovirus or specific for serotype Y25. They can also be detected with immunocytochemistry assays using anti-hexon antibodies.

- MVA
The identity of MVA can be confirmed by PCR based on the absence of genes deleted from the wildtype vaccinia virus, specific from the MVA strain. MVA virus infectivity is measured by the average of 3 independent titrations in chicken embryo fibroblasts. The virus titre is expressed in plaque forming units per milliliter (pfu/mL).

(b) Identification techniques
As per 5(a).

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

- ChAdY25
  Human adenovirus is considered as a group 2 biological agent (BSL2) 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.
  The recipient recombinant ChAdOx1 vector has not been classified according to the EC Directive 2000/54/EC. Competent Authorities, such as the UK Health & Safety Executive (HSE), have previously accepted the outcome of Risk Assessments that classify ChAdOx1, and other ChAdOx vectors, as biohazard Risk Category 1 (BSL1), as it is a highly attenuated virus strain that is replication-incompetent in human cells and can only form infectious particles in E1-complemented HEK293 cells; it is, therefore, unable to cause human infection or disease.

- MVA
  The human Vaccinia virus is classified as a group 2 biological agent (BSL2) according to Directive 2000/54/EC.
  The recombinant MVA strain has not been classified according to the EEC directive: however, most Competent Authorities view MVA as belonging to hazard group BSL1, since it is a highly attenuated strain of Vaccinia virus 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, 2013).

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

- ChAdY25
  The original parental organism ChAdY25 is pathogenic to chimpanzees and could cause “cold-like” symptoms, bladder infections or diarrhoea. Such infections are normally mild. Treatment is supportive for the signs and symptoms, which are usually transient. The virus has not been isolated from humans but there is evidence of neutralising antibodies to other ChAd viruses in those caring for, or exposed to,

chimpanzees, suggesting that humans are permissible to infection with ChAds. However, those exposed are reportedly clinically asymptomatic.

Human adenoviruses commonly cause asymptomatic infections in human, although they can also cause respiratory tract infections, gastrointestinal infections and eye infections (or discomfort) of varying severity depending upon the serotype (Wold, 2013; Athanasopoulos, 2017). They are more common in children and in the immunocompromised population. The incubation period varies from 1 to 10 days. The majority of the population is seropositive for more than one sub-species of adenovirus and can rapidly produce adenovirus neutralising antibodies.
Normally, the virus is infected through the respiratory tract or the eyes through aerosols produced by infected people. Most infections are mild in nature. Adenoviruses are rarely integrated into the genome of host cells and do not persist in lymphoid tissues. Adenovirus infections in non-human primates (NHP) are also predominantly mild. Chimpanzee adenoviruses, including ChAdOx1 are increasingly used in clinical trials as an advantageous alternative to human adenoviruses because of their great ability to generate an immune response, the possibility of inserting longer sequences and the lack of immunity against the vector due to the no previous exposure. These vectors, like ChAdOx1, are usually replication-incompetent, however safety trials do exist which utilise replication-competent human adenoviral vectors (Gurwith, 2013). Currently, Adenovirus Type 4 and Type 7 vaccine is approved in the US for immunisation of military populations; this is a live unattenuated vaccine which is shed in faecal matter (FDA Prescribing Information, 2019).

- MVA
  The parental vaccinia virus was originally used as a vaccine for smallpox. The vaccinia virus infection is very mild and usually asymptomatic in healthy individuals but can cause a mild rash and fever. However, the vaccine sometimes caused complications and side effects, and the likelihood of this happening was significantly higher in immunocompromised persons. The MVA was then developed and used as a vaccine against smallpox in the 1970s to the end of the eradication campaign in 1980; in a vaccination programme in 120,000 people, it did not produce any serious adverse reaction. Since then, a non-replicating smallpox vaccine has developed and manufactured by Bavarian Nordic (MVA – Bavarian Nordic Live) and was authorised in the EU in 2013 under the tradename Imvanex for active immunization against smallpox disease in adults since 2013, in case there is ever an outbreak of the disease in the future. Numerous other MVA-based vaccines have been tested in clinical trials (Goossens, 2013), without any serious adverse reactions.

8. Information concerning reproduction

(a) Generation time in natural ecosystems:
ChAdOx1 is replication-incompetent.

MVA replicates well in avian cells (CEF) and baby hamster kidney cells, but poorly in most mammalian cells (Mayr, 1978; Drexler, 1998) and it is unable to spread in normal human cells.

(b) Generation time in the ecosystem where the release will take place:
Not Applicable – the ChAdOx1-HPV and MVA-HPV vaccines will not replicate after injection.

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

(d) 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 for ChAdOx1 and MVA

(b) relevant factors affecting survivability:

- ChAdOx1
  While adenoviruses can survive for up to 8 weeks on environmental surfaces at ambient temperatures, ChAdOx1-HPV is replication-incompetent and is not expected to survive, multiply or disperse following its release during the proposed clinical study. ChAdOx1-HPV will be administered by intramuscular injection. With this route of administration, studies show their limited spread to other tissues, and no shedding, as the virus vector remains mainly localised to the site of injection. 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
  MVA has high environmental stability with high resistance to drying up to 39 weeks at 6.7% moisture at 4^{o}C; it also has increased temperature tolerance compared to other viruses (Goossens, 2013), but can be inactivated by steam sterilisation. MVA does not replicate in human cells and there have been no reports that other MVA vectored vaccines distribute significantly beyond the injection site when administered via the IM route. Poxviruses have a low content of lipids in their envelope, so are not very sensitive to organic solvents; however, they are quite susceptible to a variety of chemicals, such as formaldehyde, glutaraldehyde, ethanol and isopropanol (Verheurst, 2012).

  The MVA virus cannot persist for long periods in the environment, as it is highly attenuated and has an extremely restricted host range for infectivity. It will not replicate in the targeted human host cell; MVA cannot form complete viral particles,

therefore cannot form the structures necessary to survive more than temporally within the targeted host and the environment.

10. Dissemination

(a) Ways of dissemination

- ChAdOx1
  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 organism. The minimal infectious dose of adenovirus is 150 plaque forming units (pfu) when administered intra-nasally. Adenoviruses may also be spread via the faecal-oral route.

  There are no reports that adenovirus vectors, including ChAdOx1 administered in clinical trials, have been shed into the environment (Wold, 2013).

- MVA
  Vaccinia viruses are disseminated through excreta, skin pock lesions and contamination; however, there is no evidence that the vaccine distributes beyond the site of injection to excretory organs; distribution is notably limited when given via the IM route, which also eliminates the development of skin pock lesions.

(b) Factors affecting dissemination
Generally, dose, route of administration, formation of aerosols and proximity of susceptible uninfected hosts could all affect dissemination for both ChAdOx1 and MVA.

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)

There have been no previous deliberate releases of ChAdOx1-HPV and MVA-HPV in Belgium. However, there have been previous deliberate releases of vaccines containing both the ChAdOx1 and MVA vectors as listed in F.7.

No vaccine-related Serious Adverse Events (SAEs) have been reported in any of these studies and all vaccines have shown an acceptable safety profile.

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 expected biological effect of both vaccines is an antigen-specific robust T-cell response comprising both CD4+ and CD8+ T-cells.

- ChAdOx1-HPV
  The transgene for the viruses comprises 59 gene segments from HPV proteins E1, E2, E4, E5, E6 and E7 from genotypes 16, 18, 31, 52, 53 and 58. Segments range from 9-55

amino acids in length and are joined end to end. The transgene encodes for six early proteins that are conserved across the important hrHPV genotypes. The sequence was codon optimised to the codon bias of Homo sapiens genes in order to have a higher level of expression. A highly efficient leader sequence was added at the 5' end of the transgene: this drives the protein into the ER and enhances expression and immunogenicity (Kou, 2017; Luo, 2008); a Kozak sequence was added to improve the initiation of translation.

The vector is the adenoviral genome (replication-incompetent) of ChAdOx1 in the form of a bacterial artificial chromosome (BAC) in order to allow for replication of the DNA construct in bacteria. The adenoviral genome was modified as described in Dicks, 2012. Succinctly, the E1 and E3 loci were deleted to render the adenovirus replication-incompetent, and the endogenous E4Orf6/7 was replaced with E4Orf6/7 from Human adenovirus 5 (HAd5) in order to improve productivity in HEK293-based producer cell lines.

- MVA-HPV
  The antigen expression cassette of MVA-HPV consists of an F11 promoter, a synthetic antigen sequence derived from consensus regions from thousands of strains of hrHPV and an early poxviral termination signal (TTTTTAT). The transgene is not toxic and does not confer any advantage to the clinical viral vector in terms of viral vector survival or replication. The HPV sequence does not alter the transmission route or host range of the MVA viral vector. A highly efficient leader sequence was added, at the N terminus of the transgene, which drives the protein into the endoplasmic reticulum and enhances expression and immunogenicity (Kou, 2017; Luo, 2008).

3. Vector use

(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.

The GM vaccines are free of the vectors that were used to construct them, only the sequence of interest remain.

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

(a) Type of vector

For ChAdOx1 and MVA

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

(b) Identity of the vector

For ChAdOx1: ChAdOx1 BAC

For MVA: MVA-mCherry

(c) Host range of the vector

- ChAdOx1
  ChAdOx1 BAC will replicate in laboratory strains of *E. coli* bacteria; final adenovirus ChAdOx1 can only replicate in E1-complemented HEK293 cell lines.

- MVA
  MVA-mCherry and the final recipient MVA vector are replication-deficient; it will only replicate efficiently in avian cell lines.

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

Yes (X) No (.)

- ChAdOx1
  The E1 and E3 regions of the ChAdOx1 genome have been deleted and the native E4 region has been modified by replacement of the ChAdY25 E4Orf6/7 sequence with the hAd5 E4Orf6/7 gene to facilitate packaging of the ChAdOx1 virus in the HEK293 cell line.

- MVA
  The specific MVA phenotype is strong attenuation and a highly restrictive host range; its replication is only possible in permissive cells e.g. CEF.

antibiotic resistance (.)
other, specify …

Indication of which antibiotic resistance gene is inserted

- ChAdOx1
  None

- MVA
  Plasmids used for construction of the recipient MVA vector system bear antibiotic resistance genes; however, the MVA-HPV viral vector does not encompass any of these resistance genes.

(e) Constituent fragments of the vector

- ChAdOx1
  Virus genome (replication-deficient) and HPV antigen expression cassette

- MVA
  Virus genome (attenuated) and HPV antigen expression cassette

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

(i)	transformation	(X)
(ii)	electroporation	(.)
(iii)	macroinjection	(.)
(iv)	microinjection	(.)
(v)	infection	(X)

(vi) other, specify in vitro recombination…

- ChAdOx1
  The HPV immunogen and mammalian promotor sequence (CMV) was cloned into the Invitrogen Gateway system entry plasmid pENTR4 to create pENTR4-HPV. A homologous recombination reaction was performed between the ChAdOx1 viral vector backbone, in the form of a Bacterial Artificial Chromosome (BAC) and the pENTR4-HPV.

- MVA
  The MVAeGFP shuttle plasmid vector encoding the synthetic HPV antigen is transfected into chicken embryonic fibroblasts which are simultaneously transduced with a parental MVA-mCherry viral vector.

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 ChAdOx1 and MVA-HPV

(a) Composition of the insert
The transgene comprises 59 gene segments from HPV proteins E1, E2, E4, E5, E6 and E7 from genotypes 16, 18, 31, 52, 53 and 58. Segments range from 9-55 amino acids in length and are joined end to end.

A highly efficient leader sequence was added at the 5' end of the transgene: this drives the protein into the ER and enhances expression and immunogenicity (Kou, 2017; Luo, 2008).

(b) Source of each constituent part of the insert
The insert comprises the cytomegalovirus (CMV) immediate early promoter with enhancer, the HPV coding sequence, and the polyadenylation sequence from the bovine growth hormone (BGH) gene. The HPV protein segments contained in the HPV antigens are derived from the human papilloma virus.

(c) Intended function of each constituent part of the insert in the GMO
The protein fragments contained in the HPV antigens are expected to induce an antigen-specific robust T-cell response comprising both CD4+ and CD8+ T-cells.

The leader sequence added at the 5' end of the transgene drives the protein into the ER and enhances expression and immunogenicity (Kou, 2017; Luo, 2008).

(d) Location of the insert in the host organism

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

Integrated at the E1 locus of the viral vector (ChAdOx1), integrated into the F11 locus of the MVA vector

(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)	Papillomaviridae
(ii)	family name for plants	…
(iii)	genus	Alpha-Papillomavirus
(iv)	species	Humanpapillomavirus
(v)	subspecies	…
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	…

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	(.)
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 …

Human papillomaviruses have been classified as Risk Class 2 pathogen for humans by the Belgian Competent Authority. https://www.biosafety.be/sites/default/files/h_a_virus.pdf.

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 (X) No (.) Unknown (.)

Specify …

ChAdOx1-HPV and MVA-HPV is replication-incompetent.

(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 (.)

(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)

- ChAdOx1
  ChAdOx1-HPV cannot replicate. The inability to replicate from the originally transduced cells prevents it from spreading to other cells, which completely changes the pathogenicity (Dicks, 2012).

- MVA
  MVA causes no known human disease or pathology. The MVA was used as a vaccine against smallpox in the 1970s at the end of the eradication campaign in 120.000 people without serious adverse reactions. MVA-HPV maintains the same characteristics of pathogenicity as MVA. The effects are limited to those arising from the initial transduction of cells at the injection site.

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment
See next point.

(b) Techniques used to identify the GMO

- ChAdOx1
  ChAdOx1-HPV viral vaccine vector is identified by standard molecular biology techniques e.g. PCR, DNA sequencing.

- MVA
  Testing of viral vectors using PCR-based assays and other approved methods is used to differentiate between human pathogenic Vaccinia viruses and attenuated MVA strains. They are also used to confirm the identity of the specific viral vaccine vector. The release criteria for MVA-HPV is the confirmation of sequence specificity of the vector and the absence of any other contaminating vectors.

F. Information relating to the release

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

The primary purpose of the proposed clinical trial is to study the safety, tolerability and immunogenicity of ChAdOx1-HPV and MVA-HPV as a treatment for persistent HPV infection, when given in a prime-boost vaccination regime (ChAdOx1-HPV vaccine, followed by MVA-HPV-1 vaccine 28 days later). Three different doses of ChAdOx1-HPV will be tested: 2×10^8 vp, 2×10^9 vp and 2×10^{10} vp; and two different doses of MVA-HPV: 1×10^7 pfu and 1×10^8 pfu.

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 …

- ChAdOx1
  ChAdOx1 does not exist naturally in the ecosystem. The natural host of the parental ChAdY25 wild-type adenovirus is the chimpanzee. ChAdY25 is not found in the natural ecosystem outside its natural host.

- MVA
  MVA does not exist naturally in the ecosystem; the parental CVA virus no longer exists.

3. Information concerning the release and the surrounding area

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

The preparation and administration of ChAdOx1-HPV and MVA-HPV will be conducted at four hospitals in Belgium:

Site 201
Department of Gynaecology & Gynaecological oncology
Universitair Ziekenhuis Brussel
Laarbeeklaan 101, 1090 Jette, Belgium

Site 202
Center for Vaccinology (CEVAC)
Universitair Ziekenhuis Gent
Corneel Heymanslaan 10, 9000 Gent, Belgium

Site 204
Department of Gynaecology & Obstetrics
Hôpital Erasme
Lenniksebaan 808, 1070 Brussel, Belgium

Site 205
Department of Gynaecology & Gynaecological oncology
Universitair Ziekenhuis Antwerpen
Wilrijkstraat 10, 2650 Edegem, Belgium

(b) Size of the site (m2): … m2
(i) actual release site (m^2): … m^2
(ii) wider release site (m^2): … m^2

Not applicable.

In the main phase of this clinical trial 64 participants in Belgium will each receive one injection of ChAdOx1-HPV and one injection of MVA-HPV.

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

Not applicable – any effect on such areas is not considered possible due to this clinical trial. Even in case the vaccines would be released in such an area, the consequences would be nil given the biological containment.

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

Not applicable – any effect on flora and fauna is not considered possible due to this clinical trial.

4. Method and amount of release

(a) Quantities of GMOs to be released:

The total amount of GMOs to be released in Belgium is calculated as follows:

ChAdOx1-HPV

Dose Group	Total Number of participants		Number of vaccinations	Total quantity administered
	Lead-in phase (not being conducted at Belgian sites)	Main phase		
2 x 10^8 vp	0	3	3	(3 x 0.25 mL) = 0.75 mL
2 x 10^9 vp	0	8	8	(8 x 0.25 mL) = 2 mL
2 x 10^{10} vp	0	11	11	(11 x 0.25mL) = 2.75mL
Total ChAdOx1-HPV released				5.5 mL

MVA-HPV

Dose Group	Total Number of participants		Number of vaccinations	Total quantity administered
	Lead-in phase (not being conducted at Belgian sites)	Main phase		
1 x 10^7 pfu	0	11	11	(11 x 0.5 mL) = 5.5 mL
1 x 10^8 pfu	0	11	11	(11 x 0.5 mL) = 5.5 mL
Total MVA-HPV released				11 mL

(b) Duration of the operation:
Each participant will receive two vaccinations: Each vaccination will take a few seconds; participants will remain in the treatment centre for at least 30 minutes following each vaccination. Participants in Belgium will return to the centre at months 3, 6 and 12 for follow-up assessments.

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

The GM vaccines are only used within the four treatment centres. As no shedding is expected, release beyond the trial centres is deemed highly unlikely.

All preparation (including dilution of lower doses) and administration is conducted by suitably qualified unblinded health professionals, fully trained according to the study-specific Pharmacy Manual and the study site SOPs relevant to the handling of GMO materials.

Preparation of the vaccinations is conducted within a biosafety cabinet. All doses are prepared by first placing all required items (sterile vials, syringes, needles, Luer Locks, alcohol swabs and resealable plastic bags) on a stainless steel tray, spraying with 70% IPA and allowing to dry. The doses are then drawn up, including required dilutions according to the instructions in the Pharmacy Manual, using the dilution kits provided.

Before a vaccine is transported for administration to the participant, a Self-Righting Luer Lock Tip Cap is secured tightly on the end of the syringe to avoid spillage; the vaccine is then placed in a sterile plastic bag which is sealed and placed inside a second sealed bag. This is then placed in a transport pack which is labelled “GMO” and remains in this until reaching the site of administration. A commercially available spill kit will accompany all vaccines that are transported to participants for administration. Any spillages will be cleaned up according to the study site SOPs. All residual vaccine in used vials will be placed in designated, labelled sharps bins with used vials, needles and dilution kits and disposed of as biohazardous GMO waste.

Injections are given via the intramuscular route, to avoid leakage via the needle track, in a single designated room within the treatment centre. Study personnel will utilise PPE as standard good clinical practice; this consists of gloves, eye protection and an apron or laboratory coat/gown during the preparation and administration. Training will be given on full use of PPE to ensure that no contamination occurs during the process. All disposable PPE will be dealt with as biohazardous waste, according to the study site SOPs. Other items will be sent for cleaning in suitable biohazard containers.

Following injection, the vaccination site will be cleaned with a standard alcohol wipe and then covered with a sterile, occlusive dressing to absorb any leakage via the needle track and so minimise dissemination into the environment. The dressing will be removed approximately 10 minutes after vaccination and the participant will remain in the clinic for at least a further 20 minutes, by which time the injection site will be completely dry. The dressing will be discarded as GMO waste in accordance with the study site SOPs.

All GMO waste, including empty vials, will be destroyed by certified vendors. Prior to being sent for destruction, vials, syringes, needles, dressings and contents of the dilution

kits will be placed in relevant, labelled biohazard containers, as will all plastic gowns and disposable gloves worn by those handling the vaccines in any way. All biohazard containers will be labelled as containing GMO waste and will be transported outside of clinical areas to designated areas prior to destruction by certified vendors. A chain of custody form will be completed at each site that will track which personnel conducted each activity related to handling of the GMO, as well as the time and details of the activity; these activities will include receipt, storage, preparation, administration, disposal on site. The Form is included as an Annex of the Pharmacy Manual. Waste management records will also be maintained.

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

   Not applicable. All vaccinations will take place inside the hospital centres.

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.

   There have been no previous deliberate releases of ChAdOx1-HPV and MVA-HPV. However, there were previous deliberate releases of vaccines containing both the ChAdOx1 and MVA vectors:

   - ChAdOx1

     B/ES/18/20: A Phase I, randomized, double-blind, placebo-controlled safety, tolerability and immunogenicity study of candidate HIV-1 vaccines DNA.HTI, MVA.HTI and ChAdOx1.HTI in early treated HIV-1 positive individuals (AELIX-002). 23-Oct-18

     B/ES/18/21: Phase IIa, randomized, double blind, placebo-controlled, first-in-human study of the safety, tolerability and immunogenicity of candidate vaccines MVA.HTI and ChAdOx1.HTI alone or administered sequentially with GS-9620 in HIV-1positive patients treated early (AELIX-003). 23-Oct-18

   - MVA

     There have been 25 deliberate releases of vaccines containing the MVA vector in the EU since 2003. Those that have occurred in the last 5 years include the two trials listed above, for ChAdOx1, and:

     B/DE/17/PEI3056: Phase I clinical trial of MVA-based recombinant vaccine (MVA-MERS-S) encoding Middle East Respiratory Syndrome coronavirus spike protein. 26 Feb 2018

     B/ES/16/11: A phase I randomized, placebo-controlled trial, to evaluate the safety, tolerability and immunogenicity of experimental HIV-1 vaccines, DNA.HTI and MVA.HTI administered in HIV-1 negative volunteer adults (Aelix-001). 22 Nov 2016

     There have been no reports of shedding, or serious adverse reactions from these clinical trials.

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) Primates

(ii)	family name for plants	Hominidae
(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)

   Development of a significant level of HPV-specific immune cells (T-cells), which are maintained long-term, directed against the consensus regions in the synthetic HPV transgene.

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

   The possibility of gene transfer to other species is negligible during the conduct of this clinical trial, as it is considered that the vaccines will not distribute beyond the vaccination site and are highly unlikely to shed; any vaccine that leaks from the injection site will be cleaned with a standard alcohol wipe and by the time the participant leaves the study centre, the site will be completely dry. Furthermore, ChAdOx1 is replication-incompetent and MVA-HPV does not replicate in mammalian species, so the survival of the vaccines will be limited to transfected cells within the host. Neither vaccine transfers its genome to the host cell. Both vaccines encode a small portion of synthetic antigen sequence originally identified in HPV, however the vectors do not produce any harmful elements of the HPV virus.

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

   Yes (.) No (x) Not known (.)

   Give details

   - ChAdOx1

     As ChAdOx1 is replication-incompetent, it will have reduced competitiveness and invasiveness compared to the parental ChAdY25 virus; furthermore, ChAdOx1 homologues only circulate in chimpanzees, which are not native to Belgium. The likelihood of recombination with a wild-type human adenovirus is negligible, since there is not enough DNA sequence homology in the E1 region to allow for this event to occur.

   - MVA

     MVA is replication-deficient; it has a highly limited host range and cannot replicate in mammalian cells; the parental CVA no longer exists and there is no known pox virus able to complement MVA to generate a replication-competent virus and spontaneous reversion of MVA to replication-competent virus has never been documented; it is thus highly unlikely that MVA-HPV could develop increased competitiveness and invasiveness compared to the parental virus.

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

   The genetic modification of the ChAdOx1-HPV vaccine has rendered it replication-incompetent and the engineered MVA-HPV vaccine cannot replicate in mammalian cells. Furthermore, the containment measures being implemented in this clinical trial mean that it is

highly unlikely that either vaccine will be released into the ecosystem; they can also not be disseminated from the place of release. Both MVA and ChAdOx1-vectored vaccines have been used in clinical trials, with no reports of dissemination beyond the site of release.

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

Not applicable.

(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:
Highly unlikely – please refer to G.3.

(b) from other organisms to the GMO:
The possibility of ChAdOx1-HPV reverting to a replication-competent virus due to co-localisation with a wild-type adenovirus is negligible since there is not enough DNA sequence homology in the E1 region to allow for this event to occur.

(c) likely consequences of gene transfer:

None – both ChAdOx1-HPV and MVA-HPV are non-pathogenic.

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.):

None 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

ChAdOx1-HPV and MVA-HPV will be administered in a controlled clinical trial via intramuscular injection. No significant biodistribution of either vaccine is expected, as detailed in Section 7. No environmental monitoring of the clinical sites for the release of ChAdOx1-HPV and/or MVA-HPV is planned during the conduct of the study.

Monitoring of the functional effects resulting from GMO vaccination will be performed to assess the antigen-specific immunity from blood samples collected in an immunogenicity analysis conducted on at least 60 participants during the main phase. Safety assessments will also be made on all participants.

Immunogenicity Sampling in the Main Phase:

Main Phase											
Sample type	**Visits**										
	Screening	**Day 0**	**Day 1**	**Day 7**	**Day 28**	**Day 29**	**Day 35**	**Month 3**	**Month 6**	**Month 9**	**Month 12**
PBMC		X			X		X	X			X
Whole blood sample		X	X		X	X	X				X
Serum		X			X		X	X			X
Cytobrush samples							X		X		X

Immunogenicity analysis will include, but not be limited to:

- Blood:
  - Definition of HPV-specific CD4+ and CD8+ within T cell subsets by phenotypic markers of activation, differentiation and memory
  - IFN-γ ELISPOT assays to determine the breadth of HPV-specific T cell responses (defined by number of peptide pools or individual peptides recognised)
  - recognition of overlapping HPV peptide pools using ICS for multiple cytokines including, and not limited to IFN-γ
  - analysis by multiparameter flow cytometry to obtain frequencies of HPV-specific T cells within CD4+ and CD8+ T cell subsets
  - mean/median fluorescence intensity of cytokine signals determined to provide a composite assessment of HPV-specific T cell avidity
  - RNA extraction and RNA sequencing
- Cytobrush:
  - Extraction of cervical mononuclear cells for analysis of innate and adaptive immune response by techniques including, but not limited to ELISPOT, multiparameter flow cytometry and RNA sequencing

Safety assessments will include:

- Monitoring of participants in clinic for 30 minutes post-injection (in case of anaphylaxis or immediate allergic reaction).
- Solicited adverse events (vaccination site reactions and systemic adverse events) will be assessed at the following times
  - Pre- and post-vaccination (at the end of the 30-minute observation period)
  - Daily for 3 days post-vaccination
  - Days 7 and 35
- Unsolicited adverse events
  - From date of Informed Consent until study completion (all Serious Adverse Events will be monitored until resolution or the participant discontinues from the study); all participants will be provided with a contact number for the clinic to report any potential adverse effects that occur outside of assessments at the study site
- Laboratory safety tests may be performed in any participant if the Investigator deems them to be necessary to fully evaluate an adverse event

2. Methods for monitoring ecosystem effects

No monitoring ecosystem effects is planned.

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

Not planned.
ChAdOx1-HPV is replication-incompetent and exists episomally in the host cell. MVA-HPV cannot replicate in mammalian cells and has a highly restricted host range. It exists in the cytoplasm of the cell. Therefore, it is not expected that either vaccine will transfer its genome to the host or other cells.

4. Size of the monitoring area (m2)

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

The site will undergo cleaning according to the site SOPs for GMOs. All waste will be treated as biohazardous material and disposed of by certified vendors.

2. Post-release treatment of the GMOs

Participants will remain at the site for at least 30 minutes post-injection, by which time the injection site will be completely dry. All GMO waste will be disposed of as detailed in Section 3.(b) below. Unused vials are returned to the manufacturer.

3. Waste

(a) Type and amount of waste generated

Minimal waste will be generated following the preparation and administration of the vaccines by intramuscular injection. This will consist of aprons, cartons, vials, swabs, needles, dilution kits and syringes only. Protective eyewear and laboratory coats/gowns will be cleaned and re-packaged according to the relevant site SOPs.

- For each participant: one pack of ChAdOx1-HPV (carton + vial) (or placebo) + one pack of MVA-HPV (carton and vial) (or placebo) + 4 needles + 2 syringes + 1-2 sterile, occlusive dressings
- For each participant receiving doses of 2×10^8 vp or 2×10^9 vp ChAdOx1-HPV and doses of 1×10^7 pfu MVA-HPV : 1 kit per dose
- For each member of staff handling, preparing and administering vaccines: disposable PPE.

(b) Treatment of waste

All GMO waste, including empty vials, will be destroyed by certified vendors. Prior to being sent for destruction, cartons, vials, syringes, needles, dressings and contents of the

dilution kits will be placed in relevant biohazard containers, as will all disposable PPE worn by those handling the vaccines in any way:

- Soft waste bins: cartons, dressings, gowns, gloves
- Sharps bins: vials, syringes, needles, dilution kits

All biohazard containers will be labelled as containing GMO waste and will be transported outside of clinical areas prior to destruction by certified vendors.

J. Information on emergency response plans

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

   All staff will be trained on dealing with accidental spillages of the vaccine according to the study site SOPs.

   Accidental spillages will be reported accordingly. The spillage and relevant areas will also be cleaned and monitored according to the SOPs and staff will remove all protective clothing and undertake appropriate cleaning procedures prior to leaving the spillage zone.

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

   No contact with plants, animals or soil with ChAdOx1 is anticipated as the participant will leave the hospital with a dry injection site and no viral shedding is expected to occur, as detailed in Section 7.

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

   No contact with plants, animals or soil with ChAdOx1 is anticipated as the participant will leave the hospital with a dry injection site and no viral shedding is expected to occur, as detailed in Section 7.

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

   The proposed clinical trial is a FIH for this prime-boost vaccination treatment. No serious adverse effects to the ChAdOx1 or MVA vectors used in other vaccines that have been tested in clinical trials have been identified to date. It is, however, recognised that anaphylactic reactions to vaccines are possible. All participants will be observed at the study site for at least 30 minutes post-vaccination. Resuscitation drugs and equipment necessary to treat acute anaphylactic reactions will be available and a doctor trained to recognise and treat anaphylaxis will be present in the clinic during the entire vaccination procedure and post-vaccination observation period.

   A follow-up safety assessment will be conducted 24-48 hours post-vaccination and all participants will be provided with a contact number for the clinic to report any potential adverse effects that occur outside of this assessment, in order that any medical treatment can be provided as quickly as possible.

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