Common application form for viral vectors contained in investigational medicinal products for human use[1]

Note 1: This application form can be used for submissions in the following jurisdictions: Austria, Belgium, Croatia, Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Luxembourg, the Netherlands, Romania, and Spain.

Note 2: The application form must be accompanied by the SNIF (summary notification information format for notifications concerning the deliberate release into the environment of genetically modified organisms for purposes other than for placing on the market)[2] in the case of submissions that are made under Directive 2001/18/EC.

Document history	Publication date	Description of main changes
Version 1	October 2019	Not applicable

[1] This document has not been adopted by the European Commission and, therefore, it does not contain the official position of the European Commission.

[2] Council Decision 2002/813/EC establishing, pursuant to Directive 2001/18/EC of the European Parliament and of the Council, the summary notification information format for notifications concerning the deliberate release into the environment of genetically modified organisms for purposes other than for placing on the market (OJ L 280,18. 10. 2002, p. 62).

1. Introduction

Clinical trials conducted in the EU with investigational medicinal products that contain or consist of genetically modified organisms ("GMOs"[3]) must comply with the legislation governing the authorization of clinical trials. [4]

Clinical trials with medicinal products that contain or consist of GMOs must also comply with applicable requirements under Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms[5] ("deliberate release framework") and/or under Directive 2009/41/EC on the contained use of genetically modified micro-organisms ("contained use framework"). [6]

This application form implements the requirements of the Directive 2009/41/EC and of the Directive 2001/18/EC, as adapted to the specific characteristics of viral vectors contained in investigational medicinal products for human use.

This is an application form for medicinal products for human use that contain or consist of viral vectors (hereafter referred to as "clinical vectors"). Specific application forms developed for certain category of medicinal products prevail over this application. For example, developers of CAR T-cells should use the *common application form for clinical research with human cells genetically modified by means of retro/lentiviral vector*. Likewise, developers of AAVs should use the *common application form for investigational medicinal products for human use that contain or consist of AAV vectors*. Finally, in case the application concerns an investigational medicinal product that has already been granted a marketing authorisation, the *submission form for use in case of clinical trials with authorised medicinal products* should be used. [7]

The application form has been endorsed by Austria, Belgium, Croatia, Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Luxembourg, the Netherlands, Romania, and Spain and may be used for submissions to these Member States.

2. Explanatory notes

The common application form is without prejudice to consultation requirements that exist under Directive 2001/18/EC.

In addition, certain national requirements may need to be considered by developers of medicinal products before they submit the application form to the relevant competent authorities:

[3] Throughout this document, the term "GMO" should be understood as covering both genetically modified organisms as defined under Article 2(2) of Directive 2001/18/EC, and genetically modified micro-organisms within the meaning of Article 2(b) of Directive 2009/41/EC.

[4] Regulation (EU) No 536/2014 of the European Parliament and of the Council of 16 April 2014 on clinical trials on medicinal products for human use and repealing Directive 2001/20/EC, (OJ L158, 27. 5. 2014, p. 1). Until the Regulation applies, Directive 2001/20/EC is applicable (Directive 2001/20/EC of the European Parliament and of the Council of 4 April 2001 on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use, OJ L121,1. 5. 2001, p. 34).

[5] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC (OJ L 106, 17. 4. 2001, p. 1).

[6] Directive 2009/41/EC of the European Parliament and of the Council of 6 May 2009 on the contained use of genetically modified micro-organisms (OJ L 125, 21. 5. 2009, p. 75).

[7] The specific application/submission forms referred to in this paragraph are only applicable in the countries that have endorsed them.

Austria:

Applicants should send separate submissions in case there are multiple sites concerned in Austria (including clinical premises, laboratories in which activities with GMOs are carried out, locations of storage of the investigational medicinal product and location of storage of samples from clinical trial subjects that contain GMOs).

Further information is available at:

Belgium:

The common application form should be part of a biosafety dossier submitted by each of the clinical sites where the investigational medicinal product will be administered. However, one person (*e.g.* the sponsor) can be empowered by the concerned sites to submit all the necessary notifications, provided that the person responsible for the activity is clearly indicated in the form.

More information on procedural requirements and forms for the three regions is available at: https://www. biosafety. be/content/contained-use-gmos-andor-pathogenic-organisms-notification- procedures.

Czech Republic:

Each clinical site as well as other institutions where the activities with GMOs will take place (*e.g.* laboratories that are not premises of one of the clinical sites) should submit a separate notification for deliberate release or for contained use, as appropriate. However, one person (*e.g.* the sponsor) can be empowered by the concerned sites/institutions to submit all the necessary notifications.

France:

For investigational medicinal products that are assessed under the contained use framework, applicants should send separate submissions in case there are multiple sites concerned in France.

Italy:

For investigational medicinal products that are assessed under the contained use framework, each clinical site (including clinical premises, laboratories in which activities with GMOs are carried out, locations of storage of the investigational medicinal product and location of storage of samples from clinical trial subjects that contain GMOs) should submit a separate notification. However, one person (*e.g.* the sponsor) can be empowered by the concerned sites/institutions to submit all the necessary notifications.

It is stressed that, in case the submission is made by a third party on behalf of the site, the responsibilities of the site holders and users concerned (as set out under Legislative Decree n. 206/2001) remain unchanged.

The Netherlands:

More information on national procedural requirements and forms is available at: https://www. loketgentherapie. nl/en/viral-vectors

COMMON APPLICATION FORM FOR VIRAL VECTORS CONTAINED IN INVESTIGATIONAL MEDICINAL PRODUCTS FOR HUMAN USE

SECTION 1 – ADMINISTRATIVE INFORMATION

1.1 Identification of the applicant.

Organisation Name:	Vaccitech Limited
Address Details:	The Oxford Science Park, The Schrodinger Building, Oxford OX4 4GE, UK
Contact person:	Vicky Wheeler
Telephone No:	+44 (0)1865 818808
Email Address:	vicky.wheeler@vaccitech.co.uk

1.2 Identification of the sponsor (to the extent that is different from the applicant).

Organisation Name:	
Address Details:	
Contact person:	
Telephone No:	
Email Address:	

1.3 Identification of the manufacturer of the clinical vector.

Organisation Name:	**ChAdOx1-HPV:** **MVA-HPV:**	Batavia Biosciences ABL Europe
Manufacturing location:	**ChAdOx1-HPV:** **MVA-HPV:**	Bioscience Park Leiden Zernikedreef 16 2333 CL Leiden The Netherlands 4, rue Laurent Fries 67 400, Illkirch France

SECTION 2 –INFORMATION RELATING TO THE INVESTIGATIONAL MEDICINAL PRODUCT

A. Virus from which the clinical vector was derived (parental virus).

A.1 Characterisation

2.1. Which virus was used as the parental virus in the construction of the clinical vector?

ChAdOx1-HPV:

Scientific name: Family: Adenovirudae Genus: Mastadenovirus
Strain and isolate: Species: Chimpanzee adenovirus, serotype Y25
Other names (*e.g.* commercial name):…….
Biosafety classification[8]: European Union Class 2 GMO
Parental virus attenuated: Yes [X] No [ ]

MVA-HPV:

Scientific name: Family: Poxviridae; Genus: Orthopoxvirus; Species: Vaccinia virus
Strain and isolate: Modified Vaccinia Ankara (MVA)
Other names (*e.g.* commercial name):…….
Biosafety classification[8]: European Union Class 1 GMO
Parental virus attenuated: Yes [X] No [ ]

2.2. Phenotypic and Genetic Markers.

Briefly describe the most relevant phenotypic and genetic markers of the parental virus, including information on the viral genome size and the packaging limit of the parental virus.

ChAdOx1-HPV:

The parental ChAdY25 is a wild-type adenovirus strain originally isolated from the faecal matter of chimpanzees (Hillis, 1969). ChAdY25 was obtained from William Hillis, John Hopkins University of Medicine where the viral genome had been sequenced and found to be phylogenically related to the Human Adenovirus E (HAdV-4) species of virus (Dicks, 2012). The ChAdOx1 vaccine vector was then derived from ChAdY25 after it was genetically modified and incapacitated for replication. The complete sequence of its genome is available from GenBank no. JN254802, as well as the phylogenetic relationship with other serotypes (by comparison of hexon and fibre proteins) and the main components of the capsid exposed on the virion surface.

The ChAdOx1 viral vector genome is ~35Kb; compared to the parental virus it has deletion of the E1 and E3 gene sequences (early viral gene sequences which are indispensable for viral replication) and a modified E4 region to optimise growth rate and yield in human cell lines. The E1 region has been replaced with the transgene expression cassette containing the cytomegalovirus (CMV) promotor to facilitate high levels of transgene expression in mammalian cells. The transgene is inserted downstream of the CMV promoter; the packaging limit for any transgene is ~8Kb.

[8] Explain if the classification varies between different territories in which the clinical trial will take place.

MVA-HPV:

The 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). The viral genome size is 180 kb; the packaging limit for transgenes is 20 kb.

The passaging has resulted in many mutations in the MVA virus genome, as well as six major deletions (Del-I, -II, -III, -IV, -V, and -VI) comprising 24,688 nucleotides. In addition, shorter deletions, insertions and point mutations have occurred in the genome, resulting in gene fragmentation, truncation, short internal deletions, and amino acid exchanges. This has resulted in the loss of roughly 15% (30 kbp) of the original genetic information of CVA. The deletions include a number of genes that contribute to viral evasion from host immune responses and that determine the virus host range (Meisinger-Henschel, 2010). 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. Following infection by MVA, non-infectious immature virions and abnormal particles are produced but infectious particles are not.

It is considered that the six major deletions in the MVA genome as well as further genes, gene truncations, or gene mutations are the major determinants governing the limited MVA host -range and avirulence.

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

2.3. What is the host range of the parental virus?

Describe the hosts in which the parental virus naturally occurs, also including hosts that serve as a reservoir. For each possible host, indicate the tissue and cell tropism.

If natural hosts of the parental virus include humans, provide available information about the seroprevalence in the EU.

ChAdOx1-HPV:

Chimpanzees are the natural reservoir for ChAdY25. It can replicate efficiently in chimpanzees; otherwise, the host range for this virus is not well characterised. The parental wild-type ChAdY25 virus phylogenically groups with the Adenovirus subgroup E viruses. Subgroup E adenoviruses enter cells via the Coxsackievirus-adenovirus receptor (CAR) and so have tissue tropism towards tissue cells exhibiting these receptors, such as those lining the respiratory tract. ChAdY25 has not been isolated from humans, therefore no tissue tropism can be documented. In chimpanzees it is possible that ChAdY25 has similar tissue tropism to other subgroup E adenoviruses.

MVA-HPV:

Natural reservoir:
Vaccinia viruses are a member of the Orthopoxvirus genus of viruses. Vertebrates, including mammals and humans, and arthropods all serve as natural hosts to this genus of wild-type virus. Vaccinia virus is closely related to the cowpox virus, and the two were originally

considered to be the same entity.

The Modified Vaccinia Ankara strain of the viral vector used for the vaccine vector of MVA--HPV was originally derived from the Chorioallantois Vaccinia Ankara virus (CVA). CVA was successively cultivated and passaged for over 500 passages in primary Chicken Embryonic Fibroblasts (CEF). This resulted in major attenuation and host range restriction of the virus. In particular, the host range for replication became highly restricted to avian cells when compared to wild-type Vaccinia virus strains (Mayr, 1975, 1978; Werner, 1980). Adapted to growth in avian cells, MVA has lost the ability to replicate in mammalian hosts and lacks many of the genes that the Orthopoxviruses use to conquer their host (cell) environment.

MVA does not exist in the natural habitat and has no natural reservoir. It is a highly attenuated virus that must be maintained frozen or in primary cell culture within a controlled laboratory environment. The Vaccinia virus no longer exists within the natural habitat since the successful completion of the smallpox eradication vaccination campaign.

Tissue tropism:
MVA is an enveloped virus, for which the precise pathway (receptor) that it uses to enter cells is largely unknown. There is limited evidence that heparin sulphate proteoglycans on the cell surface might be involved in its ability to enter cells; this may confer the viral vector the potential to enter (infect) a wide variety of cell types, despite the subsequent lack of ability to replicate (REF).

However, despite this, studies have demonstrated preferential tropism towards cells and tissues of the immune system (Altenburg, 2017): *In vitro* studies performed with human peripheral blood mononuclear cells and mouse lung explants, demonstrated that MVA predominantly infected antigen presenting cells. Subsequent *in vivo* experiments performed in mice, ferrets and non-human primates indicated that preferential targeting of dendritic cells and alveolar macrophages was observed after respiratory administration. Following intramuscular (IM) injection, MVA was detected in interdigitating cells between myocytes, but also in myocytes themselves.

Seroprevalence in EU:
Reports of human poxvirus infections have been rare since smallpox eradication. The main Orthopoxviruses that can infect humans include Cowpox virus (Europe), Varicella virus and Monkeypox virus (Congo and USA), and Vaccinia Virus (India), which was the vaccine strain used for smallpox eradication (Nagasse-Sugahara, 2004). The Cowpox and Vaccinia viruses cause similar local lesions in humans upon contact with an infected animal. Any reported cases are usually observed in workers involved in the husbandry of cows and buffaloes, or laboratory personnel handling the virus. A study of 1076 cattle in Europe revealed a very low seroprevalence of Cowpox virus of <0.7% (Crouch, 1995).

2.4. Zoonotic potential of the parental virus. [9]

If humans are not natural hosts of the parental virus, provide information on the zoonotic potential of the parental virus. Describe also the natural geographic distribution of the parental virus and indicate if the parental virus is endemic in the EU.

ChAdOx1-HPV:

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

[9] This Section needs not be filled in case of replication incompetent clinical viral vectors.

swimming pools. Humans and animals are the natural reservoirs for wild-type adenoviruses.

There is evidence that wild-type Chimpanzee adenoviruses have zoonotic potential, as those caring for captive chimpanzees have been found to have antibodies to ChAd viruses, although there have been no reports of virus isolation or clinical symptoms in any of these individuals (Xiang, 2006).

The natural host of the ChAdY25 wild-type adenovirus is the chimpanzee. The host is restricted to central Africa in the wild, but is also held in captivity worldwide. Chimpanzee adenoviruses are not indigenous to the EU.

The genetically modified ChAdOx1 virus was derived from ChAdY25 and was genetically modified to make it replication-incompetent (Hillis, 1969; Dicks, 2012). The ChAdOx1 parent viral vector for ChAdOx1-HPV is a laboratory-derived strain capable of transducing human cells but, as it is replication incompetent and does not replicate in human cells (except those which are laboratory cell lines that provide essential viral vector replication elements in *trans,* and with which there is poor sequence homology, making the likelihood of recombination very low), then there is no natural reservoir or host for this viral vector.

MVA-HPV:

The Orthopoxvirus family of viruses have zoonotic potential; there is evidence of disease transmission between various mammals; cattle, primates and humans. The CVA parental viral vaccine has significant homology with the cowpox virus and was produced from skin lesion material after intracutaneous inoculation of calves and donkeys. A sample of this virus was taken to Germany in 1953 and amplified on bovine skin before being used as the initial vaccine for smallpox in this country. Following some adverse effects in immunocompromised recipients, the vaccine was subsequently extensively passaged and titrated on chicken embryo fibroblasts (CEF), following which its replication potential and ability to initiate clinical symptoms became severely impaired.

Worldwide studies of the geographic prevalence of the Cowpox virus are lacking. However, it is likely to have worldwide distribution. Studies suggests that bank and field voles and wood mice are the main reservoir hosts of Cowpox virus in Europe, but Cowpox virus is of low seroprevalence (Crouch, 1995).

The original CVA parental virus is not used in the production of MVA-HPV. The MVA viral vectors are significantly attenuated; as such, they are unable to spread disease and are not prevalent in the wild.

2.5. Replication properties of the parental virus.

Provide information about the replication of the parental virus. Indicate where replication takes place (cell nucleus, cytoplasma). Is the parental virus capable of establishing latency in the natural host?
What are the sequence elements involved in the reactivation process?

Provide also any available information on the potential for homologous/non-homologous genomic recombination occurring in nature between viral genomes of the parental virus and related strains or members of the same viral (sub)family.

ChAdOx1-HPV:

ChAdY25 parental adenovirus is a replication-competent DNA virus that can replicate

efficiently in chimpanzees but does not establish latency in the natural host i. e. it is not inherently capable of integration into the host cell genome. However, ChAdY25 was not used directly in the construction and manufacture of the ChAdOx1-HPV viral vector vaccine. The adenoviral genome ChAdY25 was modified as described by Dicks, 2012 to create the ChAdOx1 adenoviral vector; the E1 and E3 loci were deleted to render the adenovirus replication-incompetent, and the endogenous chimpanzee adenoviral E4*Orf6/7* was replaced with E4*Orf6/7* from Human Adenovirus 5 (HAd5) in order to improve the yield of the ChAdOx1 viral vaccine vector in the Human Embryonic Kidney based producer cell line (HEK293). The genetically modified ChAdOx1 parent viral vector for ChAdOx1-HPV is also not inherently 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. HEK293 cells (with which there are is poor sequence homology, making the likelihood of homologous recombination very low) and the viral DNA remains transiently episomal in the nucleus of the host cell.

Only Chimpanzee adenoviruses could transfer genetic material to modified ChAdY25 or potentially to ChAdOx1-HPV. Moreover, adenoviruses are infectious agents of the respiratory and gastrointestinal tracts, whereas the replication incompetent ChAdOx1-HPV vector will be administered IM. The parental virus was isolated from the faeces of a chimpanzee and genetically modified. It is engineered to be replication-incompetent, so that the likelihood of any recombination with any wild-type adenovirus is negligible. Replication-incompetent adenoviruses have been administered to thousands of clinical trial participants and there have been no reports of recombination.

MVA-HPV:

Orthopox viruses do not integrate into the host genome, therefore latency within the host is extremely unlikely. The MVA virus has been generated from the CVA strain by serial passage in chicken cells for over 570 passages. This passaging has resulted in many mutations in the parental virus genome, as well as six major deletions, resulting in the loss of roughly 15% (30 kbp) of the original genetic information. Deletions include those of a number of genes that contribute to viral evasion from host immune responses and of those that determine the host range potential of the viral vector (Meisinger-Henschel, 2010). These mutations have rendered the MVA virus highly attenuated and unable to productively infect most mammalian cell lines. It replicates very poorly, if at all, in most mammalian cell types, including primary human cells and most transformed human cell lines. Following infection by MVA, non--infectious immature virions and abnormal particles are produced but infectious particles are not. As the parental MVA virus does not exist in nature, recombination between the parental virus and related strains cannot occur.

Numerous MVA-based vaccines have been tested in clinical trials (Goossens, 2013). Following very wide usage of the Smallpox vaccine, the disease was officially declared eradicated in 1980. 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.

A.2 Pathogenicity

2.6. What are the pathogenic properties of the parental virus and what are the available treatment methods?

Describe any pathogenic properties of the parental virus. Where relevant, provide information on pathogenic properties of the parental virus in vulnerable groups such as immunosuppressed individuals, pregnant women and small children. Describe the symptoms caused by the parental virus. Indicate also if therapeutic/prophylactic treatments exist to treat/prevent such an infection.

ChAdOx1-HPV:

The original parental organism is ChAdY25, which 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, 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. ChAdOx-1 was originally derived from ChAdY25 but wild-type ChAdY25 was not used directly in the construction and manufacture of the ChAdOx1-HPV viral vector vaccine. ChAdOx1 is replication-incompetent and, as such, is non-pathogenic and unlikely to produce clinical symptoms due to infection.

Chimpanzee adenoviruses are increasingly used in clinical trials and have excellent safety profiles. These vectors are usually replication-deficient;, 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 immunization of military populations; this is a live unattenuated vaccine which is shed in faecal matter (FDA Prescribing Information, 2019).

MVA-HPV:

During the Smallpox eradication campaign, vaccination with the parental Vaccinia virus (CVA) resulted in some adverse effects that occurred at a higher frequency in immunosuppressed persons. This included limited skin lesions, fever and malaise. Therefore, in order to reduce the frequency of these events occurring during vaccination, the attenuated MVA strain was developed, as described above. This was subsequently used to vaccinate ~ 120,000 people in Germany, who were previously considered at higher risk of the adverse effects with the original CVA vaccinia virus vaccine. Results demonstrated that the attenuated MVA vaccine was well tolerated with any adverse reactions reported being mild local injection site reactions, transient fever and flu-like symptoms, which resolved spontaneously. MVA vaccines have been demonstrated to have an acceptable safety profile in humans in many trials to date.

2.7. Provide relevant data on attenuation and biological restrictions of the parental virus.

If the parental virus is an attenuated/restricted virus, the basis for attenuation/restriction should be described. Describe the conditions (steps) needed for reversion of the attenuation/restriction and the factors that may affect reversion.

ChAdOx1-HPV:

Adenoviruses, such as the parental ChAdY25 Chimpanzee adenovirus have been shown to have extremely low levels of integration and do not naturally stably transfer genetic material.

Chimpanzee Adenovirus isolate Y25 (ChAdY25) is engineered to be replication-incompetent by deletion of the essential E1 genes (Dicks, 2012). The non-essential E3 gene is also deleted. It is manufactured in the well-established HEK293 cell line supplemented with the adenoviral E1 genes. HEK293 is a cell line derived from human embryonic kidney cells grown in tissue culture.

The probability of ChAdOx1 reversion is negligible for a few reasons:

(i) For homologous recombination to occur it would require co-localisation with a wild-type adenovirus, but ChAdOx1 homologues only circulate in chimpanzees.
(ii) 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.
(iii) ChAdOx1 is a non-integrative virus, which means that viral DNA does not integrate into the host cell genome following transduction.
(iv) During growth of ChAdOx1 up to 10^{14} viral particles, no replication-competent virus has been identified, despite the presence of the E1 gene in the actual cell line used (IMPD-ChAdOx1-HPV).

MVA-HPV:

MVA is a highly attenuated strain of the viral vector originally developed from CVA, as described above. The attenuated genotype of MVA no longer encodes many of the known poxviral immune evasion and virulence factors.

The probability of MVA reversion back to CVA is highly negligible:

(i) For homologous recombination to occur it requires co-localization and the likelihood of this is extremely low.
(ii) The extensive attenuation process (> 500 successive cell culture passages) has resulted in the loss of roughly 15% (~30Kb) of the parental genome, and there is no known poxvirus able to complement MVA to generate a replication-competent virus; spontaneous reversion of MVA to replication competent CVA virus has never been documented, despite extensive use of MVA as a viral vector.
(iii) MVA is a non-integrative virus, following infection of the target human host cell, it remains exclusively in the cytoplasm, its DNA remains outside the cell nucleus, thus eliminating any risk of integration of the viral DNA into the host genome.
(iv) The HPV antigen transgene segment in MVA-HPV is unable to reverse the replication deficient genotype of the MVA vector.

A.3. Ability to colonise

2.8. What are the transmission routes of the parental virus?

Describe possible transmission routes of the virus. Provide information on viral shedding including asymptomatic shedding of the parental virus. In the case of vector-borne viruses (e.g. arbo viruses), indicate the geographic location of the vector.

ChAdOx1-HPV:

Based on the known transmission of adenoviruses, the route of transmission is likely to be directly through the conjunctiva or the respiratory tract. ChAdY25 was originally isolated from chimp faeces and could, therefore, also be conceivably transmitted through the faecal/oral route. Adenoviruses are not vector-borne.

The natural host of the ChAdY25 wild-type adenovirus is the chimpanzee. The host is restricted to central Africa in the wild, but chimps are also held in captivity worldwide. Chimpanzee adenoviruses are not indigenous to the EU and do not replicate efficiently in non-primate cells. Human cells expressing the CAR are permissive to transduction with ChAdOx1 parental vector used as the basis of ChAdOx1-HPV. The ChAdOx1 viral vector is therefore expected to enter cells of the respiratory tract and other cell types expressing the CAR. However, ChAdOx1 is a non-replicating viral vector (it can only replicate in laboratory lines of cells which supply the viral E1 deleted gene in *trans,* with which there are is poor sequence homology, making the likelihood of recombination very low). Moreover, the HPV antigen gene cassette is located in the place of the deleted E1 gene, which precludes the generation of a replication-competent HPV-containing ChAdOx1 by recombination even if a cell transduced with ChAdOx1-HPV were to be infected with a ChAd shed from a chimpanzee. Therefore, the E1 deletion and host range restriction constraints mean that ChAdOx1 viral vectors cannot spread and cannot shed any infectious material from the host.

This is a First-in-Human (FIH) study being conducted on ChAdOx1-HPV. The vector ChAdOx1 has been administered in other clinical studies of vaccines including Middle East Respiratory Syndrome, malaria, influenza tuberculosis, chikungunya disease and prostate cancer e.g. https://clinicaltrials. gov/ct2/show/NCT03203421 and the safety databases for these vaccines have not generated any safety signals to date.

No biodistribution studies have been conducted specifically with a ChAdOx1-vectored vaccine. However, studies have been performed by the University of Oxford on three recombinant viral vectored vaccines based on E1, E3-deleted simian adenovirus C63 and C3 (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 viral DNA dispersion and only limited spread to other tissues as the virus vector DNA remains mainly localised to the site of injection. As ChAdOx1-HPV will be administered *via* IM injection any biodistribution/release of the non-infectious DNA is expected to be insignificant and, as such, represents a negligible risk to the environment.

MVA-HPV:

The original parental CVA can be transmitted between humans and across species. The transmission is through close contact with infected cattle e.g. between those contacting infected cattle through milking, by dermal contact or through aerosol droplets. However, CVA is not used in the production of MVA-HPV; an MVA-mCherry parental viral vector in combination with an MVA shuttle plasmid is used. These viral vectors are highly host restricted and propagation is only possible in avian cells, it is therefore not possible to

transmit MVA as it is replication-deficient in mammalian cells (outside of the laboratory setting).

The natural host for CVA is rodents, shedding is likely to occur through faeces and saliva droplets. Shedding of replication-competent CVA is not possible as it is not used in the production of the MVA-HPV viral vector vaccine. MVA is replication-deficient due to a failure in virus particle assembly (except in avian cells) and therefore only replication--deficient particles could conceivably be shed.

MVA is not a vector-borne virus.

The MVA viral vectors are significantly attenuated; as such it is unable to spread disease and is not prevalent in the wild.

2.9. Can the parental virus survive outside the host?

Describe all survival options and the survival time of the parental virus under optimal environmental conditions, and describe the factors that may be of influence.

ChAdOx1-HPV:

The parental virus ChAdY25 was not used directly in the construction and manufacture of ChAdOx1-HPV. However, in general adenoviruses can survive for up to 8 weeks on environmental surfaces at ambient temperatures. Adenoviruses are resistant to lipid disinfectants because they are non-enveloped but are inactivated by many common chemical agents (e.g. 70% isopropyl alcohol (IPA), sodium hypochlorite, ethyl alcohol, 2% glutaraldehyde and 0. 25% sodium dodecyl sulphate). The virus is also susceptible to inactivation by heat and autoclaving at 121°C for 15 minutes.

MVA-HPV:

MVA has high environmental stability with high resistance to drying up to 39 weeks at 6.7% moisture at 4 °C; it also has increased temperature tolerance compared to other viruses (Goossens, 2013), but can be inactivated by steam sterilisation. 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.

B. Genetic modification and manufacturing of the clinical vector.

2.10. Provide a brief description of the manufacturing process of the clinical vector.

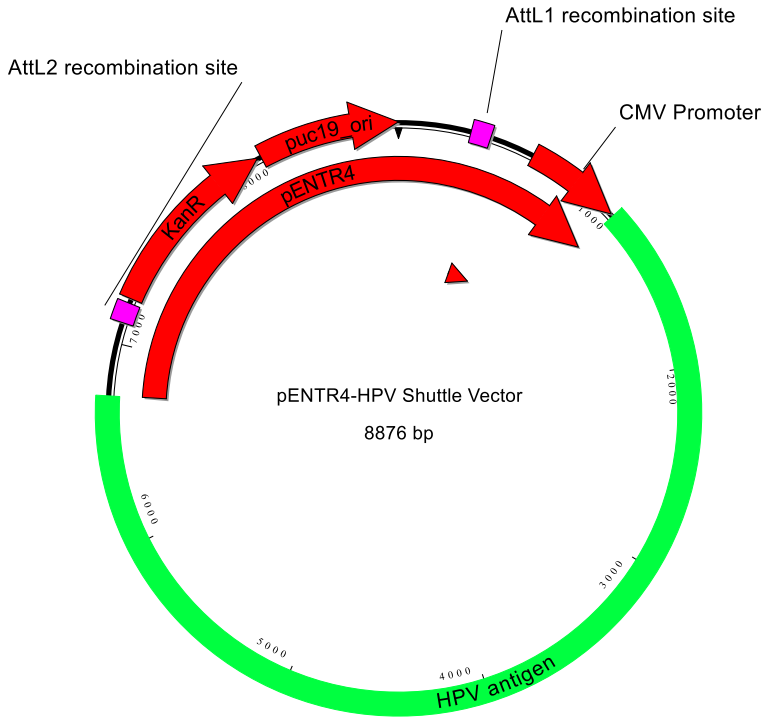
Answer this question preferably by using a diagram that describes the various production steps.

When using plasmids for the manufacturing of the clinical vector, clear maps of the plasmids showing all the constituent parts of the vector should be provided (i. e. in addition to the "transgene plasmid", all other plasmids such as helper, packaging and pseudotyping plasmids should be described). Explain if there are overlapping sequences in the plasmids.

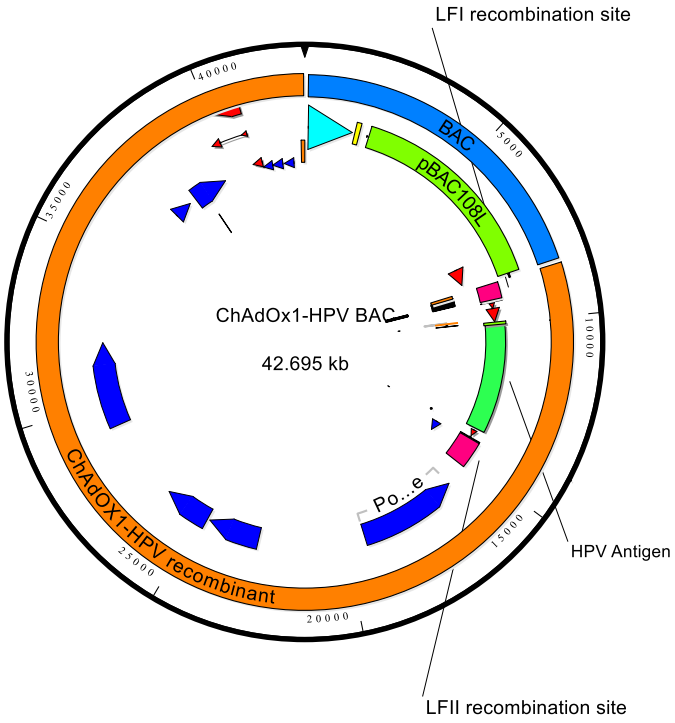
Confidential information should be provided in an annex, together with the reasons why it is considered confidential. The Section of the application form to which the information refers should be clearly identified. When confidentially is claimed, a summary that can be made public should be provided in this section.

ChAdOx1-HPV:

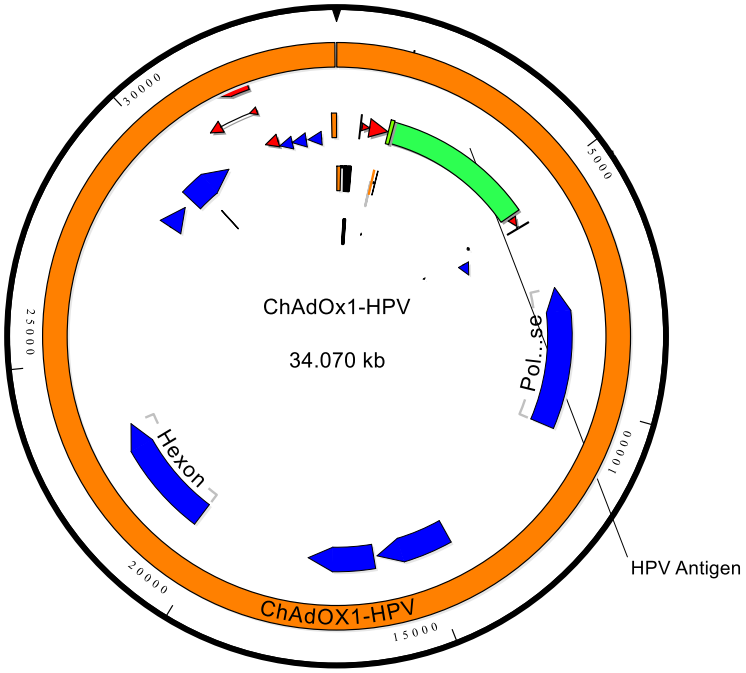
Step 1: Generate pENTR4-HPV shuttle



Step 2: Recombination of ChAdOx1-HPV into BAC



Step 3: Linearise ChAdOX1-HPV recombinant and transfect into HEK293 packaging cell line (E1 *in trans*) for viral vector packaging



The HPV immunogen and mammalian promotor sequence (CMV) was cloned into the Invitrogen Gateway system entry plasmid; pENTR4 to create pENTR4-HPV (Step 1 above). A homologous recombination reaction was performed between the ChAdOx1 viral vector backbone (destination vector, in the form of a Bacterial Artificial Chromosome (BAC)) and the pENTR4-HPV. The resulting recombinants (Step 2 above) were transformed into bacteria and the ChAdOx1-HPV recombinants were selected using standard molecular biology techniques (antibiotic selection). Bacterial clones with the desired ChAdOX1-HPV vector (as determined by standard methods e.g. PCR) were propagated to produce sufficient material to isolate the recombinant BAC by standard methods e.g. column purification. The ChAdOX1-HPV was separated from the BAC by restriction digest and the linearised ChAdOX1-HPV was then transfected into the viral producer cell line (HEK293; Step 3). Following transfection, the cells displaying cytopathic effect (and therefore indicating successful viral vector production) were harvested and used for clonal expansion of the ChAdOX-1 viral vector to ensure single virion isolation. The single virion isolate was then used to passage the virus through HEK293 cells to produce a larger scale propagation of virus ready for purification of the ChAdOX1-HPV viral vector vaccine. The final vaccine product was then purified by established methods, e.g. Ultra-Diafiltration and Anion Exchange Chromatography before formulation into the final drug product, as outlined, below.

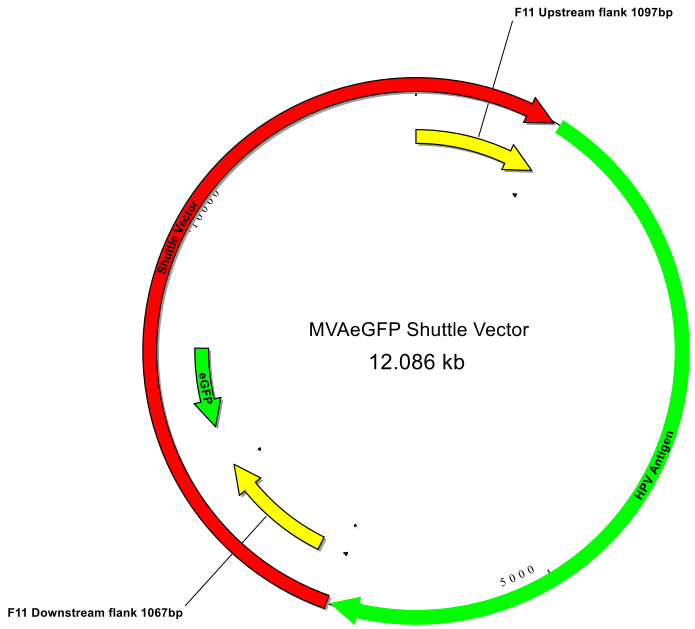
Step of Operation
293 Cell Culture
Virus infection propagation
Harvest & Lysis DNase digestion Clarification by centrifugation
Clarification by depth filtration and 0.22µm filtration
Concentration by UF/DF 1 AEX / Membrane UF Diafiltered Bulk Final Formulation in Buffer 0.2 µm filtration
Bulk Drug Substance Filling in bags

MVA-HPV:

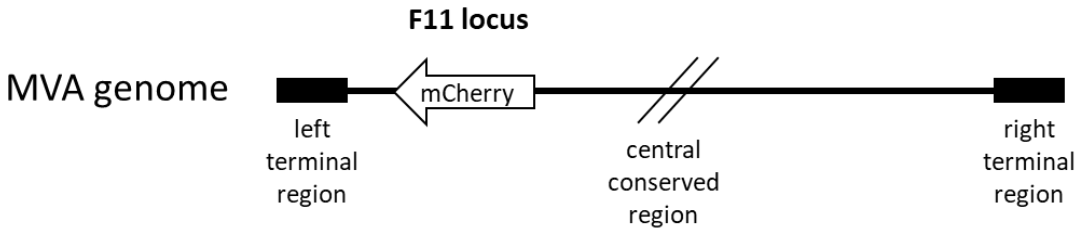
The MVAeGFP shuttle plasmid vector (A) encoding the synthetic HPV antigen is transfected into chicken embryonic fibroblasts which are simultaneously transduced with (B) a parental MVA-mCherry viral vector.

The resulting homologous recombination between (A) and (B) *via* the F11 recombination sites result in the integration of the shuttle plasmid into the MVA-mCherry genome (C). Plaque picking assays are subsequently performed and repeated a number of times to ensure that a single virion isolate is identified which represents the MVA viral vector with the HPV antigen encoded. Further passaging and plaque-picking is then performed to select for 'markerless' virus which has successfully recombined to remove the mCherry, eGFP and plasmid sequences (D). The MVA-HPV viral vaccine vector is then produced to scale by serial propagation in CEF.

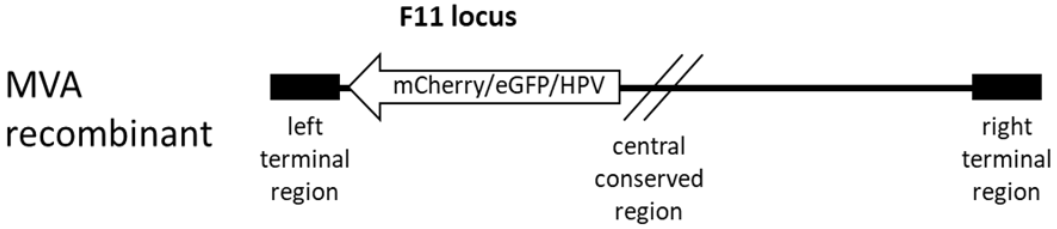
A)



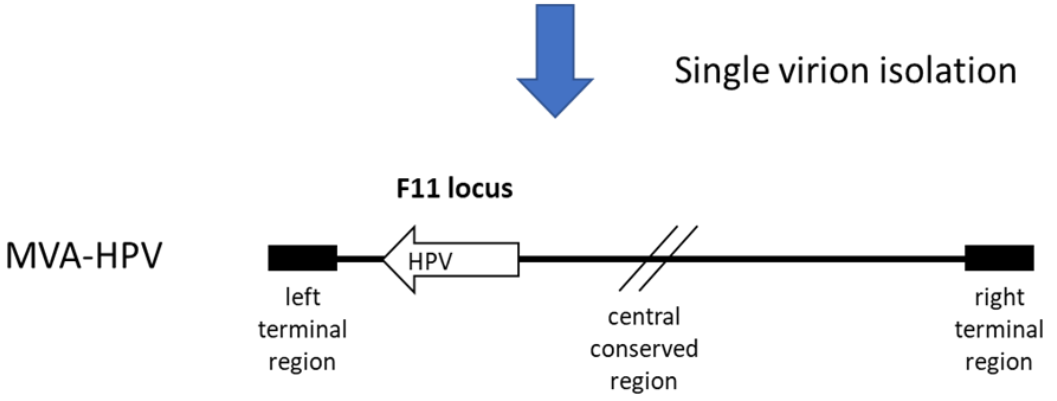
B)



C)



D)



Manufacturing Process Summary

Step of Operation
Embryo Digestion with Trypsin and Cell Disruption
Cell Seeding in Cell Factories
Infection of Cell Factories with Seed Virus Stock and Incubation
Lysis of Infected Cells
Depth Filtration
Tangential Flow Filtration
Diafiltration to Produce Purified Bulk

2.11. Describe the characteristics of the cell lines in which the clinical vector is produced. Also indicate which of the genetic components of the cell could possibly cause complementation or recombination.

The characteristics of all cell lines used and eventual modifications of the cell genome should be explained. Describe the cell types concerned as well as their origin (e.g. human kidney, epithelial cells). The possibility of the genetic material in the cells/cell lines causing a certain interaction with the clinical vector, such as by complementation or recombination should be discussed.

Explain if there is a risk of clinical vector modification by trans-complementing sequences. Provide also a description of the identity of these sequences. This can be done on the basis of bio-informatic analysis, such as sequence analysis, alignments or phylogenetic analysis.

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ChAdOx1-HPV:

HEK293 is a well-established cell line that was generated in 1973 by transfection of cultures of normal human embryonic kidney cells with sheared Adenovirus type 5 DNA. The cells were cultured by Van der Eb, under laboratory conditions to create a stable transfected cell line. In HEK293, the Ad5 DNA encodes the E1 adenoviral early gene; as the viral vector ChAdOx1 is E1 deleted, the cell line, therefore, provides this viral gene element in *trans* and so enables the formation of infectious recombinant adenovirus particles; however, there are only short homologous sequences, making the likelihood of recombination very low. The adenoviral vector DNA is not integrated into the host cell and exists episomally, further negating the possibility of recombination to produce replication-competent viral particles. This is also mitigated by serial virion isolation protocols, to ensure viral clonality. The supplemented E1 region is a human adenoviral region and the cells do not host any wild-type Chimpanzee adenovirus, this precludes recombination of the ChAdOx1-HPV back to the original ChAdY25 virus.

MVA-HPV:

MVA-HPV is grown and manufactured in primary cultures of Chicken Embryonic Fibroblasts derived from embryonated chicken eggs. These eggs are obtained from Valo Biomedica and conform to the necessary Quality Standards required for producing vaccines for human use e.g. European Pharmacopoeia Chapter 5. 2. 2 (Chicken flocks free from specified pathogens for the production and quality control of vaccines). The cells are isolated and maintained from certified Specific Pathogen Free (SPF) chicken flocks and therefore the likelihood of complementation or recombination between other viruses is mitigated. The cells are grown for a limited number of passages in a controlled laboratory environment (with GMP compliance). As such, all reagents and materials used are from fully tested, certified and controlled sources. MVA-HPV, like the parental MVA virus, has a host-range restriction to avian cells and consequently does not need trans-complementation by the cells for growth. This, and the cytoplasmic location of MVA replication, precludes the possibility of clinical vector modification by trans-complementing sequences.

2.12. Contaminating replication-competent virus.

For replication-deficient and conditionally replication-competent clinical vectors, strategies to avoid the generation of replication-competent virus (RCV) should be described. Test methods for detection of replication-competent virus should be described, including information on the specificity and sensitivity thereof. Data from RCV testing at different manufacturing steps should be provided (e.g. virus seed bank, final product). Release criteria with regard to RCV testing should be specified.

Confidential information should be provided in an annex, together with the reasons why it is considered confidential. The Section of the application form to which the information refers should be clearly identified. When confidentially is claimed, a summary that can be made public should be provided in this section.

ChAdOx1-HPV:

A potential hazard for any gene transfer viral vector is a recombination event between the GMO and its naturally occurring homologue that results in either the transfer of the transgene to the wild-type virus, or the reverse i. e. the transfer of essential genomic elements from the wild-type homologue to the vector, both of which could result in a return to virulence of the vector.

The following factors are considered to ensure that the generation of replication-competent adenovirus does not occur:

(i) Adenoviruses, such as the parental ChAdY25 Chimpanzee adenovirus have extremely low levels of integration and do not naturally stably transfer genetic material.
(ii) The Chimpanzee adenovirus isolate Y25 (ChAdY25) was engineered to be replication -deficient by deletion of the essential E1 genes (Dicks, 2012). The E3 gene is also deleted to increase the space available for insertion of transgene expression cassettes. The original ChAdY25 is not used in the process of ChAdOX1-HPV production.
(iii) The ChAdOX1-HPV is generated and propagated only supplemented in well-established and fully characterised producer cell lines HEK-293. These cells do not host the Chimpanzee adenovirus.
(iv) The HEK293 cell line expresses the human adenoviral E1 region in *trans* and does not express the parental ChAd E1 or the human Ad5 E4 gene that has been introduced into ChAdOx1.
(v) The recombination with a wild-type human adenovirus is negligible, since the degree of DNA sequence homology is too low to allow for this event to occur.
(vi) The ChAdOx1-HPV material (master virus stock [MVS] and drug substance [DS]) is tested for the presence of replication-competent adenovirus (RCA) using a well -established *in vitro* assay (specification: <1 RCA / 3 x 10^{10} viral vector particles). 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:

A potential hazard for any gene transfer of the viral vector is a recombination event between the attenuated viral vector and a naturally occurring homologue. This could result in either the transfer of the transgene to the wild-type virus, or the reverse, the transfer of essential genomic elements from the wild-type homologue to the vector resulting in a return to virulence of the vector. The probability of MVA reversion is negligible for the

reasons outlined in Section 2. 7. In addition, the viral vaccine vectors are produced under quality standards using GMP compliant processes.

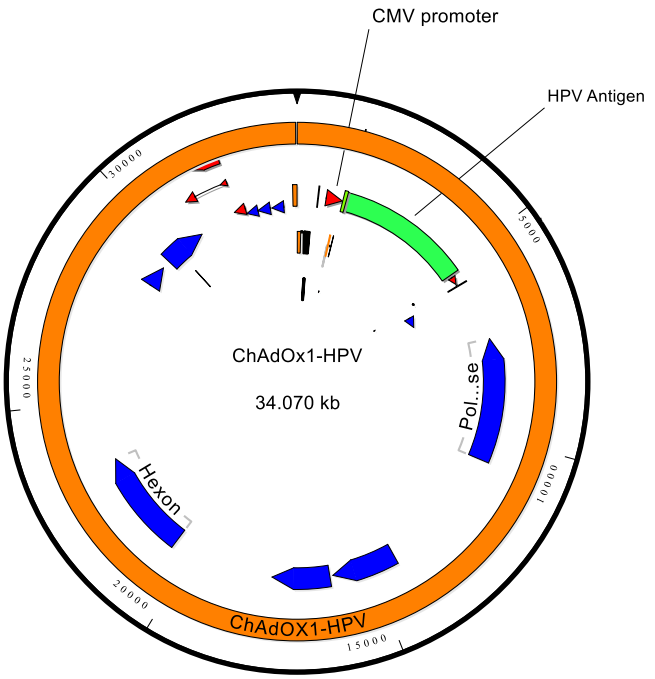
Extensive testing of the viral vectors using PCR-based assays and other approved methods are used to differentiate between human pathogenic Vaccinia viruses and attenuated MVA strains. They are also used to confirm the identity of the specific viral vaccine vector. The release criteria for this particular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

C. Clinical vector

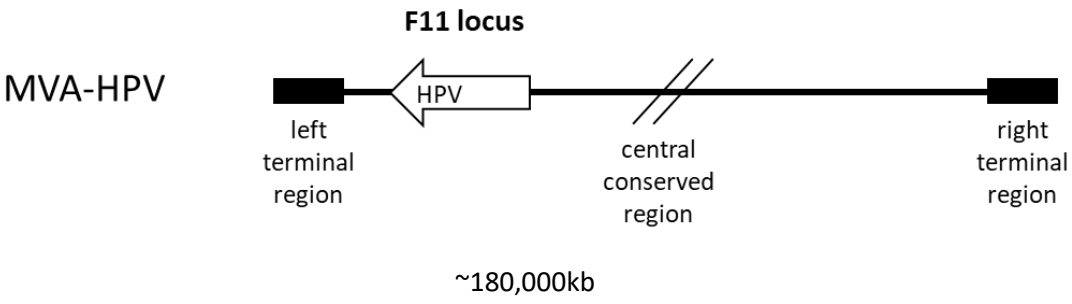
2.13. Provide a diagram ('map') of the clinical vector.

Confidential information should be provided in an annex, together with the reasons why it is considered confidential. The Section of the application form to which the information refers should be clearly identified. When confidentially is claimed, a summary that can be made public should be provided in this section.

ChAdOx1-HPV:



MVA-HPV:



2.14. Molecular characterisation of the clinical vector(s).

Provide the annotated sequence of the complete genome (i. e. indicate the location of the sequences encoding the transgene expression cassette(s) and its regulatory elements). As a minimum, the sequence of the elements that could affect the replication ability, host range, tropism, ability to survive outside the host, route of transmission or pathogenic potential of the clinical vector should be provided.

Describe in what way the clinical vector deviates from the parental virus at the level of molecular characterisation

Available data supporting genetic stability of the clinical vector should be provided. Deviations should be discussed, in particular the biological significance thereof.

ChAdOx1-HPV:

ChAdY25 E1 and E3 gene regions of the parental virus were deleted. The deletion of ChAdY25 E1 to create ChAdOx1 precludes viral replication, except in the case of supplementation of the E1 function in *trans*.

The full ChAdY25 E4*Orf6/7* sequence was deleted and replaced with the Human Adenovirus type 5 E4*Orf6/7* gene to facilitate packaging of the ChAdOx1 virus in the HEK293 cell line.

To generate the ChAdOX-1-HPV vector, the E1 viral gene region is replaced with a CMV promoter, the HPV antigens sequence and a poly(A) sequence (Dicks, 2012):

Cytomegalovirus (CMV) immediate early promoter sequence:

CCTTTCACTCATTAGATGCATGTCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCC
AACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTC
CATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCA
TATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGT
ACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGG
TGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGT
CTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGAACCAAAATCAACGGGACTTTCCAAAATGT
CGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAA
GCAGAGCTCTCCCTATCAGTGATAGAGATCTCCCTATCAGTGATAGAGATCGTCGACGAGCTCGT
TTAGTGAACCGTCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCG
GGACCGATCCAGCCTCCGGT

HPV antigen sequence:

This is confidential information and is included in Annex 1.

Bovine growth hormone (BGH) polyadenylation (polyA) sequence:

CTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGG
TGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCA
TTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCA
GGCATGCTGGGGATGCGGTGGGCTCTATGG

These sequences replace the E1 viral sequence and constitute the antigen expression cassette, which facilitates gene expression of antigen sequence and primes protective immunogenicity in the host.

ChAdOx1 is a chimpanzee adenovirus. Parental adenoviruses are genetically stable. ChAdOx1,

encoding various antigenic sequences have been used in several trials to date, generating good safety data. There is no reason to believe that ChAdOx1-HPV will behave differently to any of these other ChAdOx1 viral vaccine vectors in this respect.

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 digest pattern and DNA sequencing of the vector insert). All genetic characterisation analyses on the tested products showed conformity to theoretical sequences.

Throughout development, the genetic stability of ChAdOx1-HPV has been assessed and demonstrated by analytical testing from the primary virus seed (PVS), to the master virus seed (MVS), and at different stages during the manufacture of clinical material. Specifically:

(i) Identity and purity testing is performed by PCR at the harvest step of the MVS and drug product.
(ii) PCR is used to confirm the identity of the viral vector backbone (ChAdOx-1).
(iii) Genetic characterisation by DNA sequencing of the antigen cassette is performed on the MVS stock.

The stability of ChAdOx1-HPV has been additionally demonstrated by the making of ten serial passages beyond the pre-MVS stock that was used to make the MVS stock in order to expand the virus stock past the level to be used in large scale manufacturing: the genetic sequence of the virus was shown to be stable by PCR and sequence analysis.

All steps of the manufacture of the recombinant ChAdOx1-HPV vaccine will be conducted using current Good Manufacturing Practices (cGMP) based on a seed lot system. Clinical batches of ChAdOx1-HPV vaccine will be produced from a cGMP seed lot.

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

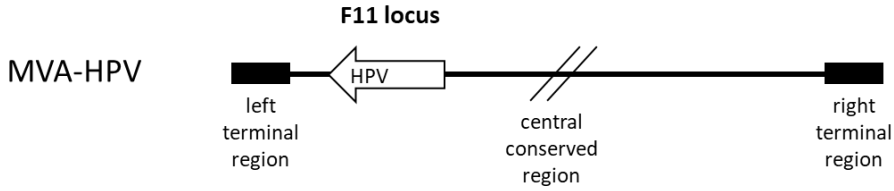
MVA-HPV:

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.

Genetic stability of the MVA-HPV viral vaccine clinical vector has been assessed and demonstrated by means of ten passages of the pre-master seed virus stock. The tenth passage was shown to be identical to the pre-master seed virus stock when analysed by PCR analysis of insertion site flanking regions, the antigen expression cassette, and four regions of the MVA backbone. The antigen expression cassette, including the promoter, of the Passage 10 virus stock was sequenced and shown to be correct. In addition, PCR analysis was used to demonstrate that the product remained free from contamination with the mCherry parental virus, as well as from wild-type MVA. Analytical testing was also performed throughout development starting from the primary virus seed (PVS), to the master virus seed (MVS), and at different stages during the manufacture of clinical material. All steps of the manufacture of the recombinant MVA-HPV vaccine are conducted using current Good Manufacturing Practices (cGMP) based on a seed lot system. Assessments include identity, purity and potency. 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. 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.

In addition, the recipient MVA-mCherry strain which was used to generate the recombinant MVA-HPV was shown to be homogeneous by next-generation sequencing, proving that the MVA strain is not polyclonal and does not contain any variants that may differ in their attenuation profile.



The entire genome sequence of MVA is 178 kb in length (Antoine, 2006). The insertion of the antigen expression cassette has no effect on host range or virulence of the MVA vector.

HPV antigen sequence:

This is confidential information and is included in Annex 1.

Bovine growth hormone (BGH) polyadenylation (polyA) sequence:

CTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGC
CACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATT
CTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTG
GGGATGCGGTGGGCTCTATGG

2.15. Describe the coding genes and the regulatory sequences present in the clinical vector backbone and in the DNA inserted. *A full description must be provided of the inserted or deleted genetic material, also discussing the functions of the sequences, for example:*

- *Expression cassette, including promoter, terminator, and enhancer sequences.*
- *Transgene: e.g. is the expressed product toxic or otherwise harmful to humans (other than the clinical trial subject) or other hosts? Does the transgene provide an advantage for replication/ survival of the clinical vector (vis-à-vis parental virus) or alter the transmission route?*
- *Whether the DNA inserted into the clinical vector contains elements of which the origin or function is unknown.*
- *Whether the clinical vector contains elements that are not specifically intended for the therapeutic functions.*

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ChAdOx1-HPV:

The antigen expression cassette of ChAdOx1-HPV consists of a CMV promoter (modified to contain a tetracycline repressive element), a synthetic antigen sequence derived from Human Papilloma Virus (HPV) and a polyadenylation sequence.

The transgene expressed in ChAdOx1-HPV is a synthetic antigen sequence derived from HPV. 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 ChAdOx1 viral vector.

The origin of the sequence for the HPV synthetic antigen is derived using consensus regions of genomes of HPV strains, and contains no naturally-occurring HPV genes.

The therapeutic potential of ChAdOx1-HPV is conferred through expression of the HPV antigen under the control of the CMV promoter. The remaining elements, such as a tetracycline repressive element, which are not specifically intended for therapeutic function in the ChAdOx1-HPV viral vaccine vector, are elements which facilitate the replication and packaging of ChAdOx1-HPV viral vector during manufacture in permissive cell lines. However, these upstream cis-acting viral elements are not expressed in the clinical product, and not replicated due to the ChAdOx1 vector being replication incompetent.

MVA-HPV:

The antigen expression cassette of MVA-HPV consists of an F11 promoter, a synthetic antigen sequence derived from Human Papilloma Virus (HPV) and an early poxviral termination signal (TTTTTAT).

The transgene expressed in MVA-HPV is a synthetic antigen sequence derived from consensus regions from thousands of strains of Human Papilloma Virus (HPV). 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).

The origin of the sequence for the HPV synthetic antigen is derived from consensus regions from thousands of strains of HPV.

Although HPV viruses are known to be pathogenic, the gene sequences extracted from them which are compiled into the MVA vector do not encode any known harmful, pathological, oncogenic or allergenic products. Furthermore, they are not known to alter the infectivity, toxicity, virulence or antigenicity of the MVA vector.

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, which is confirmed by sequence analysis of the MVA-HPV genome. Therefore, the probability of transfer of any antibiotic sequence to the target human host is effectively zero.

The therapeutic potential of MVA-HPV is conferred through expression of the HPV antigen under the control of the F11 promoter. The remaining elements, which are not specifically intended for therapeutic function in the MVA-HPV viral vaccine vector, are elements which facilitate the replication and packaging of MVA-HPV viral vector during manufacture in permissive avian cell lines.

2.16. Differences between the biological profile of the clinical vector and the parental virus.

Indicate whether the clinical vector particles are pseudotyped and whether the envelope is provided in trans.

Explain differences that exist between the clinical vector and the parental virus regarding:

- *Host range, including host specificity and the tissue and cell tropism.*

- *Transmission route.*
- *Pathogenic properties. Where relevant, consider potential effects in common population and in vulnerable groups such as immunosuppressed individuals, pregnant women, small children, or any other group with a higher risk.*
- *Ability to survive outside the host. If available, provide data on the loss of infectivity of the clinical vector on different materials or in liquids (e.g. waste water).*

Confidential information should be provided in an annex, together with the reasons why it is considered confidential. The Section of the application form to which the information refers should be clearly identified. When confidentially is claimed, a summary that can be made public should be provided in this section.

ChAdOx1-HPV:

The parental simian-derived ChAdY25 adenovirus is a replication-competent adenoviral isolate from which the engineered vector backbone ChAdOx1 is derived; ChAdOx1 has been engineered to be replication incompetent by deletion of the essential E1 genes (Dicks, 2012). The E3 gene is also deleted. The ChAd E4Orf6/7h has been replaced with human Ad5 E4Orf6/7; the E1 deletion renders the virus nonreplicating (except under laboratory conditions) but the addition of the human Ad5 E4Orf6/7 improves the viral growth characteristics of the ChAdOx1 vector in the HEK293 permissive cell line.

The changes to ChAdY25 mean that the viral vector ChAdOx1 will no longer replicate in chimpanzee cells (or any other primary cells), and the pathogenicity of the virus is, therefore, prevented.

The ChAdOx1 vector has not been pseudotyped; the virus structural proteins are the same as the wild-type ChAdY25 on which the ChAdOx1 vector is based. The changes made to the viral vector replication capability are not expected to affect the tissue and cellular tropism; the viral vector may still be capable of transducing cells expressing the CAR and thus it is also possible for the viral vector to infect tissues such as those lining the respiratory tract. However, since the virus cannot replicate it is highly unlikely to produce clinical symptoms typically associated with an adenovirus infection. As the viral vector will be injected IM, its expression is likely to be primarily restricted to the site of administration e.g. muscle cells.

The ChAdOx1 viral vector has been used with excellent safety in numerous human clinical trials to date, with no indication of vector-related adverse effects.

As the ChAdOx1-HPV viral vector is replication-incompetent it is also very unlikely to cause any adverse effects. The ChAdOx1-HPV viral vector encodes a small portion of synthetic antigen sequence, originally identified in HPV, however the vector does not produce any harmful elements of the HPV virus. Both ChAdY25 and ChAdOx1 are adenoviruses and no gene transfer to the host genome will occur; there is, therefore, there is a strong rationale for considering the risk to the health of any vulnerable population e.g. immunocompromised persons, will be of the same level of clinical risk from exposure to this viral vaccine vector.

It is expected that the survivability of ChAdOx1-HPV in the environment will be typical of adenoviruses in general. Adenoviruses can survive for up to 8 weeks on environmental surfaces at ambient temperatures. Adenoviruses are resistant to lipid disinfectants because they are non-enveloped but are inactivated by common chemical agents (e.g. 70% IPA, 1% sodium hypochlorite, ethyl alcohol, 2% glutaraldehyde and 0. 25% sodium dodecyl sulphate). The virus is also susceptible to inactivation by heat and autoclaving at 121°C for 15 minutes. The cleaning agent 70% IPA will be used by the study sites and non-disposable Personal Protective Equipment

(PPE) that is used for any handling of the vaccine will be cleaned using this agent or by autoclaving.

MVA-HPV:

The host range of MVA is significantly restricted (to replication in avian cells) and is non-pathogenic. The MVA vaccine vector has ~30kb of gene sequence deleted from the CVA parental virus. This renders the MVA replication-deficient and highly host restricted. CVA is not used in the process of viral vector manufacture. Although the CVA parental vector was used historically as a vaccine during the campaign to eradicate human Smallpox, the vector did cause some unwanted side effects and clinical symptoms in some immunocompromised individuals. The MVA vaccine was developed in response to this and is significantly better tolerated that the CVA parental vector. The MVA vector has been used in other vaccines in human trials of immunocompromised individuals e.g. HIV patients, in which good tolerability and no safety concerns were reported (Overton, 2015).

The MVA viral vaccine vector has been used in hundreds of human trials to date and has demonstrated a good safety profile. The MVA-HPV vaccine encodes a small portion of synthetic antigen sequence, originally identified from consensus regions from thousands of strains of HPV; however, the vaccine does not cause any of the harmful effects of the HPV. Both CVA and MVA are Orthopoxviruses and no gene transfer to the host genome will occur; there is, therefore, no reason to believe that any vulnerable population e.g. immunocompromised person will be of any higher clinical risk from exposure to this (MVA-HPV) viral vaccine vector.

The MVA viral vector has high environmental stability with high resistance to drying up to 39 weeks at 6.7% moisture at 4 ^{o}C and increased temperature tolerance compared to other viruses (Goossens, 2013). The MVA virus 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.

2.17. Potential for recombination with the parental virus *in vivo* and description of potential recombinants.

Discuss the potential for homologous recombination in vivo and describe all recombinants that might be generated by homologous recombination with e.g. the parental virus. Discuss the potential biological (including pathogenic) effects of any possible recombination for the population (including for vulnerable groups). Indicate whether the recombinants described have been monitored and detected in previous experiments or after administration to humans.

Confidential information should be provided in an annex, together with the reasons why it is considered confidential. The Section of the application form to which the information refers should be clearly identified. When confidentially is claimed, a summary that can be made public should be provided in this section.

ChAdOx1-HPV:

The probability of ChAdOx1 reversion is negligible for the following reasons:

(i) For homologous recombination to occur it requires co-localisation with a wild-type adenovirus, but ChAdOx1 is a chimpanzee adenovirus; therefore, homologues only circulate in chimpanzees. The circulation of wild-type chimpanzee adenoviruses is by typically by aerosol or the faecal-oral route; whereas ChAdOx1 will be administered intramuscularly in a controlled environment.

(ii) 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.

(iii) ChAdOx1 is a non-integrative virus, which means that viral DNA does not integrate into the host cell genome following infection.

(iv) The transgene segment is unable to reverse the replication-deficient genotype of the recipient ChAdOx1 vector; the vector is, therefore, incapable of completing a reproductive cycle and so cannot survive in the natural ecosystem.

(v) The immunogen proteins that are produced by the transduced cells are processed by the normal cellular machinery.

There is, therefore, no reason that any population, including potentially vulnerable populations e.g. immunocompromised persons, will be at risk from ChAdOx1 reversion following exposure to the ChAdOx1-HPV viral vaccine vector.

MVA-HPV:

The probability of MVA reversion is negligible for the reasons outlined in Section 2. 7. The recombinant MVA vector, with a variety of different transgenes, has been used extensively in clinical trials for vaccination and gene therapy applications, generating a significant safety database with no apparent major safety concerns raised (Verheust, 2012; Goossens, 2013).

2.18. Biodistribution and shedding.

Detailed data on vector shedding (including information on the administered dose, the route of administration, and –where available- immune status of the treated subjects) from previous clinical trials with the clinical vector should be provided. Where available and if relevant for the environmental risk assessment, biodistribution data should be provided.

If there is no prior clinical experience with the same clinical vector, the potential for shedding should be discussed based on non-clinical data and/or clinical experience from related clinical vectors. If the applicant relies on data from related clinical vectors, the relevance of the data to the product that is the object of this application should be explained considering, in particular, the dose and route of administration.

When shedding occurs, the estimated duration should be specified.

The methods used for detection of viral shedding including information on the specificity (including ability to detect revertants) and sensitivity thereof should be provided.

Confidential information should be provided in an annex, together with the reasons why it is considered confidential. The Section of the application form to which the information refers should be clearly identified. When confidentially is claimed, a summary that can be made public should be provided in this section.

ChAdOx1-HPV:

This is a First-in-Human (FIH) study being conducted on ChAdOx1-HPV. It will be administered in a prime-boost regime with MVA-HPV in women with persistent hrHPV infection.

The vector ChAdOx1 has been administered in other clinical studies of vaccines including Middle East Respiratory Syndrome (NCT 03399578; NCT 04170829), malaria (EudraCT: 2017-001049-28), influenza (NCT 01818362; NCT 01623518), tuberculosis (NCT 03681860; NCT 01829490), chikungunya disease (NCT 03590392) and prostate cancer (2017-001992-22) and the safety databases for these vaccines have not generated any safety signals to date.

ChAdOx1-HPV will be administered *via* IM injection. With this route of administration, studies show there is limited virus release and limited spread to other tissues, 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 ChAdOx1-HPV.

Adenoviruses of subgroup E use the CAR to enter cells and thus the biodistribution of ChAdOx1 would be equivalent to that of other subgroup E adenoviruses if it were not replication-incompetent. Therefore, although no biodistribution studies have been conducted with a ChAdOx1-vectored vaccine, results are expected to align with data from reports of other subgroup E replication-incompetent chimpanzee adenovirus vectors-. These include studies of three recombinant viral vectored vaccines based on E1, E3-deleted simian adenovirus C63 and C3 (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 only limited spread to other tissues, with no evidence

of viral shedding, as the virus vector does not replicate and remains mainly localised to the site of injection.

Biodistribution Studies for adenovirus vectored vaccines for malaria

1. A single intradermal injection of 3.3 x 10^9 vp AdCh63-ME-TRAP was made into each pinna. At Week 1, AdCh63 ME-TRAP was only detected at the vaccination site, at significantly reduced levels. No infectious AdCh63 ME-TRAP virus particles were detected in any internal organ (reproductive organs, spleen, liver, cervical lymph nodes). There was no evidence of replication of the virus or presence of a disseminated infection.
2. AdCh63 MSP-1 was administered by IM injection and virus was detected at the vaccination site immediately after injection, but not in organs or vaccination site at Week 1.

Biodistribution Studies for adenovirus vectored vaccines for Hepatitis C

1. AdCh3NSmut was administered by IM injection at a dose of 6.08 x 10^9 vp into each quadriceps. At Day 1 and Week 1, the presence of infectious particles was detected in draining lymph nodes. One hour post-vaccination, infectious AdCh3NSmut particles were found in the vaccination site and in regional lymph nodes, and not in any other organs (liver, spleen, reproductive organs). At Week 1, AdCh3NSmut was barely detectable in regional lymph nodes.
2. In the second study, at 1 hour post-vaccination. infectious Ad6NSmut particles could be found in the vaccination site and in regional lymph nodes, and not in any other organs. At Week 1, Ad6NSmut was barely detected only in the regional lymph nodes.

Results of these studies are consistent with the injection of a replication-incompetent virus.

Many other replication-incompetent simian adenovirus vectored vaccines (e.g. ChAdV63, HIVconsv, AdCh63 AMA1, ChAdOx1-85A, ChAdOx1-MERS and ChAdOx1-5T4) have been used in approved clinical studies without prior biodistribution studies as the information on replication-deficient E1 and E3 deleted ChAd63 was considered both relevant and sufficient; further studies would involve use of additional animals in experiments without providing any beneficial information; which would run counter to European Directive 2010/63/EU (Paragraph 42), which states that: *It is necessary to…eliminate unnecessary duplication of regulatory testing. For that purpose Member States should recognise the validity of test data produced using test methods provided for under the legislation of the Union*. Furthermore, the ChAdOx1 vector has been used in other vaccine programmes involving over 200 participants to date with no vector-related adverse effects reported.

MVA-HPV:

This is a First-in-Human (FIH) study being conducted on MVA-HPV. MVA-vectored vaccines have, however, been used in numerous other clinical studies using inserts from various infectious pathogens. Specifically, Vaccitech has an ongoing clinical programme with an MVA-vectored vaccine for influenza expressing the internal influenza proteins nucleoprotein and matrix 1 (MVA-NP+M1). In two of the completed studies (FLU004 and FLU005), MVA-NP+M1 was used to boost following priming with a ChAdOx1 vectored influenza vaccine (ChAdOx-NP+M1). A total of 144 participants have received MVA-NP+M1 manufactured in CEF cells and approximately 1521 participants have received MVA-NP+M1 manufactured in AGE1. CR. pIX®-cells with no vaccine-related serious adverse effects or study withdrawals due to vaccination.

In this FIH study, MVA-HPV will be administered by IM injection. No biodistribution studies have been conducted on MVA-HPV. MVA is replication-deficient in human cells due to a block in late-stage virion assembly. After the initial infection of the cells around the site of injection by the

viral vaccine vector, there is no further infection and no spread of infectious viral material within the body.

Given all of the data on biodistribution of recombinant MVA vaccines, it is not considered appropriate to repeat an in vivo study with a new recombinant MVA vaccine in which the insert does not affect viral replication in mammalian cells; the HPV transgene is not expected to alter the ability of the MVA to replicate in mammalian cells.

Biodistribution studies have been performed with other MVA-vectored vaccines (MVA85A [tuberculosis] and MVA-ME-TRAP and MVA-MSP1 [malaria]) by the University of Oxford, 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.

In these studies, the presence of infectious virus was assessed by an infectivity assay at the vaccination site immediately post-vaccination, and at the vaccination site, draining lymph nodes, spleen, liver and gonads at Week 1.

Results from 1 x 10^8 pfu IM injection of MVA-MSP1 in BALB/c mice are summarised as follows (Crook, 2009):

Location	**% of mice with tissue type positive for MVA-MSP1**		
	Immediately post-vaccination	**24 hours**	**Week 1**
Vaccination site	100	100	100
Draining lymph nodes	Not tested	66.6	0
Organs	Not tested	0	0

Virus was detected in all six mice at the vaccination site immediately post-vaccination, at 24 hours and Week 1. MVA was detected in draining lymph nodes of 4/6 mice at 24 hours but was absent from this tissue in all mice at Week 1. No MVA was detectable in spleen, gonads or liver after 24 hours at either post-vaccination timepoint.

Similar results were demonstrated in a study of 1 x IM injection of 10^7 or 10^8 plaque-forming units of MVA-MERS [Middle East Respiratory Virus] in C57BL/6N mice. PCR analysis of >240 tissue types detected MVA-DNA predominantly at the injection site and in draining lymph nodes at Days 1, 3 and 7 post-inoculation; in 3/27 animals MVA-DNA was detected in the lung and liver, considered to be due to marginal quantities of the vector being dislodged from the injection site and unlikely to represent viable virions. Necroscopies were conducted at 16 hours, 24 hours and 2, 3, 4, 6, 7 and 21 days; no lesions were detectable peripheral to the injection site and the draining lymph nodes (Langenmayer, 2018[1, 2]).

Importantly in these studies, viral DNA was not detected in excretory organs (kidneys, rectum and bladder). These results are overall as expected with the use of a replication-deficient viral-vectored vaccine; In vaccination site samples taken immediately post-vaccination, some virus will be present as it has not yet infected any cells. Once a cell has been infected with MVA, the virus unpackages, but it will not be able to assemble new viral particles, and so will not infect any other cell. Transfection of cells (even if permissive for MVA replication) with MVA DNA does not result in viral infection. The assay therefore only detects viral particles that have not yet infected cells. Initially, concentrated virus is present in a small volume of liquid and only a portion of the virus is able to infect cells immediately. Over the course of a week more viruses infect cells, either through influx of antigen presenting cells to the area or a gradual wider

distribution of the virus. There may also be some vascular leakage from the vaccination site. MVA infects a wide range of cell types and so any virus leaking into the blood will infect the first suitable cell it comes into contact with. It is not possible to assess the destination of all the injected viral particles using the study design described above, which does however confirm that the virus is not replicating after injection.

SECTION 3 –INFORMATION RELATING TO THE CLINICAL TRIAL

3.1. General information about the clinical trial.

EudraCT-number (where available):	2019-001890-98
Deliberate release reference number (where available and applicable):	B/BE/20/BVW2
Title of the clinical trial:	A Phase 1b/2, Randomised, Placebo-controlled, Dose-ranging Study to Evaluate Safety, Tolerability and Immunogenicity of a 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
Name of principal investigator:	This information is provided in Annex 2 with confidential information.
Objective of the study:	Primary Objective: To determine the safety and tolerability of Chimpanzee Adenovirus Oxford 1-human papillomavirus (ChAdOx1-HPV) plus modified vaccinia virus Ankara (MVA-HPV) vaccines
Intended start and end date:	June 2020 LPV: November 2021
Number of trial subjects that will take part in the study:	105: 9 in lead-in phase (UK only) 96 in main phase (UK and Belgium; approx. 32 in BE)
Indicate if an application related to the same investigational medicinal product has been submitted -or is planned to be submitted- to other EEA Member States. In the affirmative, identify the countries concerned:	This study will also be conducted in the UK and a Clinical Trial application to the MHRA will be submitted.

3.2. Intended location(s) of the study.

The applicant should provide information about the clinical sites located in the country of submission of the application.

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

In some jurisdictions, the following additional information should be provided:

- *the location(s) of laboratories (in the country of submission) in which activities with the GMO are carried out under the framework of the clinical trial application should be stated.* [10]

Not applicable – no laboratories are located in Belgium.

- *information about the location where the investigational medicinal product is stored (to the extent that the location is in the country of submission but outside the clinical site).* [11]

Not applicable – the Investigational Medicinal product is stored in the clinical sites in Belgium.

- *information about the location where patient's samples that contain GMO's are stored (to the extent that the location is in the country of submission but outside the*

[10] Information about the location of laboratories is required for applications submitted to Austria, Belgium, Croatia, Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland, and Spain. In case of submissions to these jurisdictions, fill in the relevant table for laboratories that conduct specialised analysis referred in the protocol of the clinical trial only; laboratories that perform standard laboratory diagnostics analysis need not be listed.
[11] This information should be provided for applications submitted to Croatia, Germany, Ireland and Spain. This information should be provided for applications submitted to Belgium, Czech Republic and Finland, unless there is a contained use notification covering the storage of the product.

clinical site). [12]

Not applicable – all samples are sent outside Belgium to be stored.

(Applicant should complete as many tables as necessary)

3.3. Storage of the clinical vector at the clinical site.

The applicant should provide information about the storage location, conditions of storage (including restrictions of access), and the maximal storage duration. [13]

Throughout the conduct of the study, the cartons of vials are stored at the study site in GMO -labelled secondary containers in a continuously monitored and locked fridge at 2-8°C, in a Pharmacy area with restricted access. Specific storage information, including lot number, concentration, volume and storage temperature is detailed on the vaccine product labels.

At least daily temperature logs of the fridge will be maintained. Any temperature excursions will be reported to the study Sponsor and the affected vials held in quarantine at the site; the affected vials will not be dispensed to study participants.

During the study, full accountability will be maintained of all vials of ChAdOx1-HPV and MVA-HPV including a record of all dispensing for individual study participants and the return of used cartons. The used vials are placed in relevant biohazard sharps containers which are maintained in the restricted access area of the Pharmacy with other packaging, needles, syringes and used dilution kits, until destroyed as GMO waste in accordance with the study site Standard Operating Procedures (SOPs).

Any vials that expire whilst they are being stored at the site will be removed from the fridge and the cartons marked clearly as "EXPIRED" with black ink; they will be returned to the manufacturer at the end of the study.

Unused vaccine vials will be returned to the manufacturer at the end of the study.

The overall duration of the study in Belgium is anticipated to be 17 months:

- Lead-in phase (not being conducted in Belgian sites) – overall duration for each participant will be up to 4.5 months (up to 1.5 months for screening and 3 months on the study with two doses of study vaccine given on Days 0 and 28).
- Main phase - overall duration for each participant will be up to 13.5 months (up to 1.5 months for screening and 12 months on the study with two doses of study vaccine given on Days 0 and 28).

<u>Preparation of vaccines</u>

In all sites in Belgium except Site 202, the vaccines will be prepared in Pharmacies.

[12] This information should be provided for applications submitted to Croatia, Ireland and Germany.
For applications submitted to Belgium, Czech Republic and Finland, this information should be provided if the patient samples contain replicative and infective viruses (unless there is a contained use notification covering the storage).

[13] In case of applications submitted to Austria, Belgium, Croatia, Czech Republic, Finland, France, Ireland, Italy, the Netherlands and Spain, the applicant should specify if the dose is being prepared in the hospital pharmacy. If the clinical dose is prepared at a location other than the hospital pharmacy, this should be explained.

At Site 202, the vaccine will be prepared in a Containment Level 2 laboratory.

3.4. Logistics for on-site transportation of the clinical vector.

The applicant should provide information about the logistics for in-house transportation (i. e. transfer of the clinical vector from storage to the administration site and –where applicable- site where dose is prepared). The applicant should provide information about the characteristics of the containers used addressing also disinfection procedures applied and labelling of the containers.

Before the 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; this is 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 as "GMO transport box" and remains in this until at 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.

3.5. Information about reconstitution, finished medicinal product and administration to patients.

Reconstitution (where applicable, summarise reconstitution steps):	The ChAdOx1-HPV and MVA-HPV injections will be prepared in designated rooms according to the instructions in the study-specific Pharmacy Manual provided by the study Sponsor, and also according to the study site SOPs relevant to the handling of GMOs. These designated rooms are within the Hospital Pharmacies at all sites except 202 (UZ Gent) at which it is within a Containment Level 2 Laboratory in which the Biosafety Cabinet is located. All staff will have received training by the Sponsor specific to the handling of the vaccines and training by the study site relevant to handling of GMOs; this will include training in the use of the required PPE which is used as standard clinical practice. To prepare, the vaccine vial is removed from the refrigerator. Following this, the vial may be stored at room temperature (15-25 ^{o}C), for up to 2 hours before being used; after this time has elapsed, the vial must be disposed of. Each vial is mixed thoroughly prior to administration according to the instructions in the study Pharmacy Manual. The ChAdOx1-HPV vaccine will be provided at the required concentration for the highest dose to be administered. The lower two doses will be prepared by dilution of individual vials with sterile 0.9% saline solution. The MVA-HPV vaccine will be provided at the required concentration for the highest dose to be administered. The lower dose will be prepared by dilution of individual vials with

	sterile 0.9% saline solution. Dilution kits of commercially available equipment will be provided by the Sponsor. Doses will be prepared on a per participant basis by unblinded study staff according to the documented procedures in the study Pharmacy Manual. This includes 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 before preparing the dilution. All preparation will take place within a biosafety cabinet.
Pharmaceutical form and strength:	ChAdOx1-HPV solution for injection 8×10^{10} vp/mL MVA-HPV solution for injection 2×10^{8} pfu/mL
Mode of administration:	Intramuscular injection into the deltoid muscle
Information on dosing and administration schedule (in case of repeated dosing):	Participants will be randomised to receive: 1 x ChAdOx1-HPV (2×10^{8} vp, 2×10^{9} vp or 2×10^{10} vp) or placebo at Day 1 and 1 x MVA-HPV vaccine (1×10^{7} pfu or 1×10^{8} pfu), or placebo at Day 28
Information on concomitant medication that may affect the shedding of the clinical vector/ environmental risks (*e.g.* administration of laxatives, administration of a medicinal product that could enhance the replication activity of the clinical vector, administration of a plasmid-based medicinal product):	Exclusion criteria include: • Receipt of any investigational drug, or vaccine within 3 months prior to Day 0 of the study, or prior participation in a clinical study of an HPV vaccine • History of receipt of any adenoviral or MVA-based vaccine • Receipt of any live vaccines within 30 days of Day 0 or inactivated vaccine within 14 days of Day 0, or planned within 2 months of Day 0.

3.6. Measures to prevent dissemination into the environment.

a) Control measures during reconstitution (if applicable), handling and administration.

Injections are prepared (including dilution of lower doses) and administered by intramuscular injection by a suitably qualified unblinded health professional who will utilise PPE consisting of gloves, eye protection and an apron or laboratory coat/gown during the preparation and administration. Preparation is conducted within a biosafety cabinet. All injections are administered in a single designated room within the clinic.

Following injection, the vaccination site will be cleaned with a standard alcohol wipe and then covered with a sterile, occlusive dressing to absorb any virus that may leak out through the needle track and 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 dry. The dressing will be discarded as GMO waste in accordance with the study site SOPs.

b) Personal protective equipment.

Personal Protective Equipment (PPE) is used as standard good clinical practice and will consist of gloves, eye protection and an apron or laboratory coat/gown which will be changed between participants. 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.

c) Decontamination/cleaning measures after administration or in the case of accidental spilling (i. e. decontamination /cleaning measures of potentially contaminated materials, surfaces and areas). In addition, the disinfection procedures applied should be justified by providing evidence that the chosen method is sufficiently active against the clinical vector.

Adenoviruses are resistant to lipid disinfectants (because they are non-enveloped), but they are inactivated by other common disinfectants including: 70% IPA, 1% sodium hypochlorite, ethyl alcohol, 2% glutaraldehyde, 0.25% sodium dodecyl sulphate. Poxviruses are susceptible to a variety of chemical disinfectants including formaldehyde, glutaraldehyde, ethanol and isopropanol. Such chemicals would, therefore, effectively remove any virus released by accidental spills during the study. The cleaning agent 70% IPA will be used by the study sites.

d) Elimination or inactivation of left-overs of the finished product at the end of the clinical trial.

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 waste.

e) Waste treatment (including also –where applicable- decontamination and disposal of potentially contaminated waste that accumulates outside the clinical trial site). Where applicable, identify also the company responsible for waste management.

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:

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 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 will be included as an Annex of the Pharmacy Manual. Waste management documentation will also be maintained.

f) Are there exclusion criteria applied to the enrolment of patients in the clinical trial to address environmental risks? Are the treated patients subject to restrictions after administration of the product?

No. The ChAdOx1-HPV is a replication-incompetent vaccine and MVA-HPV has an extremely limited host range and does not replicate in humans. Both vaccines express a synthetic

antigen lacking functionality beyond the induction of a t-cell immune response to HPV and it cannot, therefore, cause illness in humans, or spread in the general human population or the environment.

g) Recommendations given to clinical trial subjects to prevent dissemination.

- There are no recommendations to clinical trial subjects to prevent dissemination as no viral shedding is anticipated. However, to prevent any potential exposure during pregnancy, participants are informed:

  "If you take part in the trial, you must use (an) authorised method(s) of contraception, 4 weeks prior to administration of the first dose of trial vaccine and up to 8 weeks after the last dose of the trial vaccine."

  "You will be required to have a pregnancy test (urine) at trial start, before the administration of ChAdOx1-HPV and prior to administration of MVA-HPV. A repeated pregnancy test must be done if you miss any periods or your menstrual cycle becomes irregular."

  "Nevertheless, if you become pregnant during the trial (or during the 6 months after the last vaccination if you withdraw from the trial early), you should inform immediately the investigator and your treating physician."

- Participants are informed that "It is important that you report any new or worsened health problems immediately to the investigator, regardless of whether or not you think it has to do with the trial."

h) Recommendations on donation of blood/cells/tissues/organs by the clinical trial subject.

Participants are informed that "It is also not allowed to do egg/ovum donation during and after your participation in the trial for up to 8 weeks after administration of the second dose."

i) Other measures.

Following vaccination, the dressing will be removed after approximately 10 minutes and the participant will only leave the clinic after 30 minutes observation, by which time the injection site will be dry.

3.7. Sampling and further analyses of samples from study subjects

This Section should be filled in where samples that may contain GMOs are being taken from patients in the context of the clinical trial and the application is submitted to the following jurisdictions: Croatia, Czech Republic, Denmark, Germany, Ireland, Italy, Hungary, the Netherlands and Spain.

Not Applicable for Belgium.

a) Describe how samples will be handled/stored/transported.

To the extent that handling/ storage and transport of samples are treated under same procedures as the clinical vector, cross-reference can be made as appropriate.

b) Indicate whether and at which time points samples that may contain the administered clinical vector are taken from study subjects.

c) If samples are stored at the clinical site, describe storage location and storage conditions.

d) Explain if there is any non-routine[14] testing of the samples and indicate whether the clinical vector is generated *de novo* during the testing.

3.8 Emergency response plans. [15]

Emergency response plans for accidental self-administration during handling or administering the clinical vector:	Accidental exposure of study site personnel will be minimised by relevant staff undergoing training to ensure correct procedures and SOPs are followed. Staff will also utilise PPE according to the study site SOPs. Any accidental exposure will be reported accordingly and dealt with as specified in the study Pharmacy Manual and site SOPs: • Needle-stick injury: the area will be thoroughly cleaned with a suitable disinfectant and the incident reported to the Sponsor and relevant study site personnel • Ingestion: the mouth will be washed thoroughly with clean water and the incident reported to the relevant physician at the study site; the Poisons Centre and Sponsor will be contacted • Inhalation: the person will be moved outside into the fresh air and their breathing monitored; a physician at the study site will be contacted if any symptoms occur • Skin/eye exposure: the exposed area will be washed/flushed for at least 15 minutes; a physician at the study site will be contacted if any symptoms occur • Spills: the affected vicinity will be cleared of all people and cleaned by suitably trained site personnel wearing PPE and using a viricidal disinfectant; all waste will be disposed of in relevant GMO biohazard containers.
Emergency response plans for accidental release into the environment of the clinical vector:	1. Methods and procedures for controlling the GMOs 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 decontamination of the areas affected, for example eradication of the GMOs,

[14] Standard clinical care tests as well as tests required to fulfil long-term follow-up of clinical trial subjects need not be mentioned.

[15] In the case of applications submitted in Austria, Finland, France and Ireland, information on emergency response plans is only required if the clinical vector has been classified as BSL 3 or 4. In the case of submissions to Italy, the emergency response plan does not need to be provided; an emergency response plan may, however, be requested by the authorities if appropriate.

	MVA and ChAdOx1 are susceptible to common disinfectants as listed in Section 2. 9. Any surfaces and areas that are affected by an accidental spillage will be cleaned up using an appropriate disinfectant according to the study site SOPs. 3. Methods for disposal or sanitation of plants, animals, soils, etc., that were exposed during or after the spread, No contact with plants, animals or soil with ChAdOx1 or MVA 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 2. 9. 4. Methods for the isolation of the area affected by the spread, Absorbent tissue will be used to contain the spillage, and this will be discarded as GMO waste in accordance with the study site SOPs. The contaminated surface and area will be cleaned using an appropriate disinfectant and the area will only be re-used when all surfaces are completely dry, and the required time period stated in the study site SOPs has elapsed. Staff will have to undertake a change of protective clothing and cleaning procedure prior to treating another participant. 5. Plans for protecting human health and the environment in case of the occurrence of an undesirable effect. The proposed clinical trial is a FIH for ChAdOx1-HPV and MVA-HPV. The target recruitment is 105 participants, of whom 73 will receive one of three doses of ChAdOx1-HPV: 2 x 10^8 vp, 2 x 10^9 vp and 2 x 10^{10} vp (prime), followed by one of two doses of MVA-HPV: 1 x 10^7 pfu or 1 x 10^8 pfu (boost); it is expected that 45 participants will be recruited in Belgium. No adverse effects to the ChAdOx1 or MVA vector 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. Both ChAdOx1-HPV and MVA-HPV express a benign antigen string-containing polypeptide and are unable to replicate in humans, animals, or plants. The vaccines present effectively zero risk to human health or the environment.

SECTION 4 – OTHER DATA REQUIREMENTS

4.1 Plan of the site(s) concerned

Applicants should provide a copy of the plan of the site where the clinical trial takes place if the application is submitted to the following jurisdictions: Austria, Belgium, Croatia, Czech Republic, Finland, France, Hungary, Ireland and Italy.

This information will be included in the corresponding Contained Use Applications relevant to each of the four sites.

4.2 Other information

Submissions to Austria:

In addition to the plan of the site, a description of the location of the autoclave should be provided –as appropriate- as part of the description of the measures to prevent dissemination into the environment referred in Section 3. 6 (d) and (e).

Submissions to Belgium:

In addition to the plan of the site, a description of the location of the autoclave and the biosafety cabinet should be provided –as appropriate- as part of the description of the measures to prevent dissemination into the environment referred in Section 3. 6 (d) and (e).

This information will be included in the corresponding Contained Use Applications relevant to each of the four sites.

The applicant is also asked to provide an overview (table) of the rooms involved in the CT activity by indicating for each of those the number of the room, the type of handling carried out (e.g. storage, administration of the IMP, reconstitution of the IMP) and the containment level.

This information is included in the corresponding Contained Use Applications relevant to each of the the four sites.

SECTION 5 – ENVIRONMENTAL RISK ASSESSMENT

This Section should be filled in for submissions under Directive 2001/18/EC. 17

A. Risk Analysis

In filling this Section, applicants may refer to relevant literature data and results from earlier preclinical and/or clinical studies.

ChadOx1-HPV:

A.1. Risks to healthcare professionals and/or close contacts of the clinical trial subject (including vulnerable groups)

5.1 Hazard identification: *Provide a list of the potential adverse effects (e.g. immune reaction, integration in the genome of the exposed cells, adverse effects linked to the expression of the therapeutic gene, etc.) if transmission of the clinical vector or potential revertants to thirds-including vulnerable groups- occurs through shedding (as described in Section 2. 18).*

5.2 Hazard characterisation: *Provide an estimate of the magnitude of each of the identified potential adverse effects (it should be assumed that each of the hazards will occur). Where a quantitative estimation is not possible, a category ("high", "moderate", "low" or "negligible") should be assigned.*

5.3 Exposure characterisation: *Provide an estimate of the likelihood (probability) that each of the identified hazards will occur. Where a quantitative estimation is not possible, a category ("high", "moderate", "low" or "negligible") should be assigned*.

5.4 Risk characterisation: *Considering the magnitude of each of the effects identified and the likelihood of their occurrence, characterise the risk. Where a quantitative estimation is not possible, a category ("high", "moderate", "low" or "negligible") should be assigned.*

5.5 Risk management strategies: *The applicant should explain measures implemented to reduce the potential risks to thirds and/or the environment associated with the clinical use of the clinical vector. This includes -but is not limited to- the measures implemented to prevent the risks of accidental transfer during reconstitution, handling, administration of the product, or during manipulation of patient's samples (after administration of the clinical vector). The applicant should also explain the recommendations that will be provided to the clinical trial subject and/or close contacts to prevent dissemination/accidental contamination. Finally, the applicant should consider if clinical trial subjects should be prevented from donating blood/cells/ tissues/ organs after being administered the clinical vector. This information should be listed in Section 3. 6.*

Hazard identification	Hazard Characterisation	Exposure Characterisation	Risk Characterisation	Risk Management Strategies
Presence of replication-competent ChAdOx1 and infection of third-parties.	Potential consequence: Negligible. Antibodies are formed to wild chimp adenovirus in humans but no clinical symptoms have been reported (Xiang, 2006).	Likelihood of occurrence: Negligible. ChAdOx1, not ChAdY25 (wild type) was used in the manufacture of ChAdOx1-HPV: ChAdOx1 is replication incompetent and has been studied extensively and there is no evidence supporting the presence of wild type ChAdY25 virus by the molecular techniques used; PCR and DNA sequencing.	Combination of consequence and likelihood: Negligible.	None.
Lack of homogeneity of the parental ChAdOx1 strain, including the presence of replication competent virus, which could replicate in various mammalian (including human) cells.	Potential consequence: Negligible. A low dose of replication competent virus would, if capable of infecting humans, be unlikely to produce any clinical symptoms.	Likelihood of occurrence: Negligible. A homogeneous strain of ChAdOx1 has been used to manufacture the ChAdOx1-HPV viral vaccine vector: The ChAdOx1 parental material has been well characterised and widely used to generate many other viral vaccine vectors utilised in various clinical trials to date e.g. https://clinicaltrials.gov/ct2/show/NCT03203421 Both the parental ChAdOx1 viral vector and the ChAdOx1-HPV viral vector vaccine have	Combination of consequence and likelihood: Negligible.	The ChAdOx1-HPV seed material (MVS and DS) is tested for the presence of RCA using a well-established *in vitro* assay (specification: <1 RCA / 3×10^{10} viral vector particles; therefore the maximum dose to be used in this trial is 2×10^{10} vp; indicating that, 1 RCA per dose is confirmable). The confirmation of absence of RCA is a DS release test and has been demonstrated in all batches of ChAdOx1-HPV manufactured to date.

Hazard identification	Hazard Characterisation	Exposure Characterisation	Risk Characterisation	Risk Management Strategies
		both been subject to several rounds of single virion isolation, under controlled laboratory conditions, to maximise the purity/homogeneity of the product. Manufacture of the material is performed in a controlled laboratory environment, using stringent GMP protocols and validated reagents. This ensures a high specification and quality product. The final drug product (ChAdOx1-HPV) and intermediates have undergone suitable and sufficient molecular characterisation (quality) checks including PCR and DNA sequencing. These data confirm the presence of the ChAdOx1 backbone and the HPV antigen expression cassette. There is no evidence of heterogeneity in the final drug product (ChAdOx1-HPV) or any of the intermediates. The lack of homology between the ChAdOx1-HPV vaccine and the human Ad5 genome		

Hazard identification	Hazard Characterisation	Exposure Characterisation	Risk Characterisation	Risk Management Strategies
		sequences in the 293 complementing cell line used to manufacture the vaccine prevents the generation of replication-competent adenoviruses during vaccine manufacture.		
Biological activity of the transgene(s): Adverse effects associated with the HPV transgene or a recombinant event e.g. altered immune response, inflammatory reaction, auto-immune disease.	Potential consequence: Low The antigen expression cassette of ChAdOx1-HPV consists of a CMV promoter (modified to contain a tetracycline repressive element), a synthetic antigen sequence derived from consensus regions, not related to pathogenicity, from thousands of strains of HPV and a polyadenylation sequence. The transgene is not toxic and does not confer any advantage to the clinical viral vector in terms of viral vector survival, recombination or replication. The HPV sequence does not alter the transmission route or host range of the ChAdOx1 viral vector and a recombination	Likelihood of occurrence: Low. The therapeutic potential of ChAdOx1-HPV is conferred through expression of the HPV antigen and is designed to stimulate/prime a protective immune response in the recipient towards the HPV virus. The origin of the sequence for the HPV synthetic antigen is derived using consensus regions of genomes of HPV strains, and contains no naturally-occurring HPV genes. A recombination event with wild-type HPV is extremely unlikely due to the lack of sequence homology.	Combination of consequence and likelihood: Low.	Vaccines will be handled and administered by trained study site personnel.

Hazard identification	Hazard Characterisation	Exposure Characterisation	Risk Characterisation	Risk Management Strategies
	event with wild-type HPV is extremely unlikely due to the lack of sequence homology. There have been no serious, vaccine-related adverse events in any of the trials conducted to date. The ChAdOx1-HPV vector has been well tolerated and there have been no significant findings during pre-clinical testing.			
Unintentional exposure to the ChAdOx1-HPV viral vaccine vector. Including those in high risk groups e.g. immunocompromised individuals.	Potential consequence: Low. The ChAdOx1-HPV viral vector vaccine is a replication-incompetent chimpanzee adenovirus and therefore not expected to produce any clinical symptoms of that could be associated with some wild-type human adenoviral serotypes e.g. conjunctivitis. Based upon the long history of safety using ChAdOX1 viral vaccine vectors, there is no reason to believe that the ChAdOx1-HPV vaccine will pose any additional clinical risk to high risk groups e.g. immunocompromised	Likelihood of occurrence: Negligible. Due to the small injected volume, the viral titre administered and the replication-incompetence of the viral vector, it is highly unlikely that sufficient material could be released to be of any significance. Mammalian cells can be infected but the virus is devoid of elements required to replicate. The route of administration in clinical trial is IM; there have been no reports of biodistribution following this route of administration, therefore, no shedding is	Combination of consequence and likelihood: Low.	Although risk assessment of the replication incompetent vector and the non-toxic characteristics of the recombinant antigen carried by the vector indicate that ChAdOx1-HPV poses no risk to human health or the environment, accidental exposure to the vaccine will be controlled by a number of measures: The ChAdOx1-HPV viral vector vaccine will be supplied in sealed vials; each trial participant will receive a single IM injection into the deltoid muscle of 0.25 mL by trained personnel. To prevent shedding from the injection site after

Hazard identification	Hazard Characterisation	Exposure Characterisation	Risk Characterisation	Risk Management Strategies
	individuals. The viral vaccine vector is not expected to adversely affect immunocompromised individuals, pregnant individuals, or that of an unborn child.	expected.		needle withdrawal, the site will be wiped clean with an a standard alcohol wipe and then covered with a dressing to absorb any virus that may leak out through the needle track and 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 dry. The clinical trial will be conducted in 32 participants in Belgium; trial is placebo-controlled, so the expected number that will receive ChAdOx1 vaccine is *circa*. 21. Study site personnel will be trained and utilise PPE-as standard good clinical practice-(e.g. gloves) when handling the medicinal product and follow the study-specific Pharmacy Manual and study site SOPs for handling of GMOs from receipt to final disposal of the materials; full decontamination

Hazard identification	Hazard Characterisation	Exposure Characterisation	Risk Characterisation	Risk Management Strategies
				procedures will be conducted in the event of accidental exposure. Trial participants are required to use reliable contraceptive methods, as defined in the Clinical Trial Protocol; this must be used for 4 weeks prior to administration of the first dose of trial vaccine and up to 8 weeks after the last dose of trial vaccine. Female participants are required to have a pregnancy test before the administration of each vaccine dose. Study site personnel are fully trained in all handling of GMOs and decontamination procedure following accidental exposure.
Lack of genetic stability and integrity of the transgene:	Potential consequence: Low. Genetic instability is most likely to cause the antigen to be non-immunogenic e.g. lack of transgene expression.	Likelihood of occurrence: Negligible. Adenoviral based vaccine vectors are genetically stable. Furthermore, the genetic stability of the ChAdOx1-HPV viral vaccine vector has been tested to passages beyond those required for general	Combination of consequence and likelihood: Negligible.	None.

Hazard identification	Hazard Characterisation	Exposure Characterisation	Risk Characterisation	Risk Management Strategies
		manufacture purposes and no genetic instability has been indicated: As demonstrated by standard molecular biology techniques e.g. PCR, DNA sequencing.		
Recombination between ChAdOx1 and naturally occurring homologues	Potential consequence: Low. If a recombination event did occur, then it would be unlikely to produce a viable chimeric virus or one capable of producing clinical symptoms.	Likelihood of occurrence: Negligible. The ChAdOx1-HPV is very unlikely to recombine with wild-type adenovirus (especially human adenovirus) due to the significant lack of homology in the viral Chimpanzee and Human adenoviral DNA sequences. Adenovirus genome packaging limitations would mean that a replication-competent virus would have the HPV transgene deleted to allow incorporation of the E1 gene required for virus replication. ChAdOx1 homologues only circulate in chimpanzees and study participants in this trial are unlikely to encounter these; the wild-type virus will be unable to enter the healing needle track temporarily	Combination of consequence and likelihood: Negligible.	Study site personnel are fully trained in handling, decontamination and disposal procedures for genetically modified organisms (in this case ChAdOX1-HPV). The likelihood of sufficient material being released that could result in a recombination event is therefore low.

Hazard identification	Hazard Characterisation	Exposure Characterisation	Risk Characterisation	Risk Management Strategies
		created by the vaccination, or reach the draining lymph nodes of the vaccination site within twenty-four hours after vaccination – the locations shown to contain on infectious replication-incompetent chimpanzee adenoviral vector DNA in biodistribution studies.		
Dispersion of the ChAdOx1 from the administration site (shedding)	Potential consequence: Negligible: The ChAdOx1-HPV viral vector vaccine is a Chimpanzee adenovirus and therefore not expected to produce any clinical symptoms Based upon the long history of safety using ChAdOX1 viral vaccine vectors, there is no reason to believe that the ChAdOx1-HPV vaccine will pose any additional clinical risk to high risk groups e.g. immunocompromised individuals. The viral vaccine vector is not expected to adversely affect immunocompromised individuals, pregnant individuals, or that of an	Likelihood of occurrence: Low.	Combination of consequence and likelihood: Negligible.	The ChAdOx1-HPV vaccine is being administered by IM injection. Any potential shedding *via* the needle track is reduced by wiping the wound clean with a standard alcohol wipe after needle withdrawal and then covered with a dressing to absorb any virus that may leak out through the needle track. 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 dry.

Hazard identification	Hazard Characterisation	Exposure Characterisation	Risk Characterisation	Risk Management Strategies
	unborn child.			
Integration of vector sequences into the genome of the participant Insertional mutagenesis and/or inadvertent regulation (activation/silencing) of neighbouring genes which may lead to oncogenic and other adverse effects. Adverse effects associated with transmission to germline.	Potential consequence: Moderate.	Likelihood of occurrence: Negligible. ChAdOX1-HPV is an inherently non-integrative viral vector; it remains transiently episomally in the transduced host cell and does not contain elements which facilitate the integration of the viral vector its genome into the host. There is no evidence of germline transmission.	Combination of consequence and likelihood: Low.	Participants are required to use reliable contraceptive methods, as defined in the Clinical Trial Protocol; this must be used for 4 weeks prior to administration of the first dose of trial vaccine and up to 8 weeks after the last dose of trial vaccine. Female participants are required to have a pregnancy test before the administration of each vaccine dose. Female participants are not allowed to perform egg/ovum donation during and after their participation in the trial for up to 8 weeks after administration of the last dose of trial vaccine.
Dispersion of the ChAdOx1 from the administration site (shedding).	Potential consequence: Negligible: The ChAdOx1-HPV viral vector vaccine is a Chimpanzee adenovirus and therefore not expected to produce any clinical symptoms Based upon the long history of safety using ChAdOX1 viral	Likelihood of occurrence: Low. Biodistribution studies on ChAdOx-vectored vaccines administered by IM injection have shown that the vaccine remains localised largely at the site of injection; non-infectious viral DNA was detected only in draining lymph nodes at the	Combination of consequence and likelihood: Negligible.	The ChAdOx1-HPV vaccine is being administered by IM injection. Any potential shedding via the needle track is reduced by wiping the site clean with a standard alcohol wipe after needle withdrawal. Participants will remain at the study site for at least 30 minutes following

Hazard identification	Hazard Characterisation	Exposure Characterisation	Risk Characterisation	Risk Management Strategies
	vaccine vectors, there is no reason to believe that the ChAdOx1-HPV vaccine will pose any additional clinical risk to high risk groups e.g. immunocompromised individuals. The viral vaccine vector is not expected to adversely affect immunocompromised individuals, pregnant individuals, or that of an unborn child.	24 hour timepoint only. There is no evidence that viral elements detectable after injection are infectious (the vaccine virus is replication-incompetent), the vaccine is broken down and processed naturally by the immune system and therefore unlikely to disperse.		vaccination, by which time the injection site will be completely dry.
Viral vector transmission to other people by organ/blood donation.	Potential consequence: Negligible: The ChAdOx1-HPV viral vector vaccine is a Chimpanzee adenovirus and therefore not expected to produce any clinical symptoms Based upon the long history of safety using ChAdOX1 viral vaccine vectors, there is no reason to believe that the ChAdOx1-HPV vaccine will pose any additional clinical risk to high risk groups e.g. immunocompromised individuals. The viral vaccine vector is not	Likelihood of occurrence: Negligible. The ChAdOx1-HPV viral vector is a non-replicating. Therefore, the risk of transmission of ChAdOx1 through organ/tissue donation is considered very low.	Combination of consequence and likelihood: Negligible.	As per current national organ donation guidelines, the participant will be precluded from blood or organ donation for period of 1 month. Female participants are not allowed to perform egg/ovum donation during and after their participation in the trial for up to 8 weeks after administration of the last dose of trial vaccine.

Hazard identification	Hazard Characterisation	Exposure Characterisation	Risk Characterisation	Risk Management Strategies
	expected to adversely affect immunocompromised individuals, pregnant individuals, or that of an unborn child.			
Inadvertent contamination of laboratory personnel, care keepers or close relatives of the participant with ChAdOx1 injected person	Potential consequence: Negligible: The ChAdOx1-HPV viral vector vaccine is a Chimpanzee adenovirus and therefore not expected to produce any clinical symptoms Based upon the long history of safety using ChAdOX1 viral vaccine vectors, there is no reason to believe that the ChAdOx1-HPV vaccine will pose any additional clinical risk to high risk groups e.g. immunocompromised individuals. The viral vaccine vector is not expected to adversely affect immunocompromised individuals, pregnant individuals, or that of an unborn child.	Likelihood of occurrence: Low.	Combination of consequence and likelihood: Negligible.	Only designated and trained study site personnel will be involved in handling of the GMO, which will be kept in a locked fridge in a restricted Pharmacy area. All supplies will be labelled as GMOs and handled according to the Pharmacy Manual and study site SOPs from receipt of study supplies to final disposal of materials; full decontamination procedures will be conducted in the event of accidental exposure. Participants will remain at the study site until the injection site is completely dry. Infection control procedures will be followed when any samples are taken from trial participants. All participants will be monitored for adverse effects as per the study protocol and

Hazard identification	Hazard Characterisation	Exposure Characterisation	Risk Characterisation	Risk Management Strategies
				provided with a contact number for the clinic to report any potential adverse effects that occur outside of assessments at the study site.

A.2. Risks to the environment

5.6 Hazard identification: *Provide a list of the potential adverse effects. As appropriate, consider specific environmental conditions that may affect the survival, replication or ability to colonise (wind, water, soil, temperatures, pH, etc).*

5.7 Hazard characterisation: *Provide an estimate of the magnitude of each of the identified potential adverse effects (it should be assumed that each of the hazards will occur). Where a quantitative estimation is not possible, a category ("high", "moderate", "low" or "negligible") should be assigned.*

5.8 Exposure characterisation: *Provide an estimate of the likelihood (probability) that each of the identified hazards will occur. Where a quantitative estimation is not possible, a category ("high", "moderate", "low" or "negligible") should be assigned.*

5.9 Risk characterisation: *Considering the magnitude of each of the effects identified and the likelihood of their occurrence, characterise the risk. Where a quantitative estimation is not possible, a category ("high", "moderate", "low" or "negligible") should be assigned.*

5.10 Risk management strategies: *The applicant should implement adequate measures to prevent dissemination into the environment. These should be listed in Section 3. 6.*

Hazard identification	Risk Characterisation	Hazard Characterisation	Exposure Characterisation	Risk Management Strategies
Environmental release and persistence of ChAdOx1 vector in the environment. Adenoviruses have a worldwide distribution. Wild-type adenoviruses have been detected in waters around the	Potential consequence: Low.	Likelihood of occurrence: Negligible.	Combination of consequence and likelihood: Negligible.	Adenoviruses are inactivated by common chemical agents (e.g. 70% IPA, 1% sodium hypochlorite, ethyl alcohol, 2% glutaraldehyde and 0. 25% sodium dodecyl sulphate). The virus is also susceptible to

Hazard identification	Risk Characterisation	Hazard Characterisation	Exposure Characterisation	Risk Management Strategies
world, including waste water, river water, drinking water, oceans and swimming pools. Humans and animals are the natural reservoirs for wild-type adenoviruses. Adenoviruses can survive for up to 8 weeks on environmental surfaces at ambient temperatures and are resistant to lipid disinfectants.				inactivation by heat and autoclaving at 121°C for 15 minutes. The release of the ChAdOx1-HPV viral vector into the environment is controlled by the following measures: ChAdOx1-HPV cannot replicate outside of the laboratory. The medicinal product will be stored securely under strict pharmacy regulations. Only trained and authorised personnel have access to the viral vaccine vector. Any spillages will be dealt with promptly and decontaminated using established and effective methods. Any leakage from the injection site of the participant following IM injection, will be wiped clean with a standard alcohol wipe, which will be disposed of through an approved clinical waste route and the study subject will be retained until the injection site is completely dry. Study site personnel will be

Hazard identification	Risk Characterisation	Hazard Characterisation	Exposure Characterisation	Risk Management Strategies
				fully trained in the conduct required to decontaminate and dispose of equipment and consumables used in the study; all GMO waste will be disposed of from the study sites by certified vendors. These control methods are deemed effective at minimising any possibility of release and persistence in the environment.
Ability to replicate in mammalian cells.	Potential consequence: Negligible. A replication-competent virus is unlikely to produce clinical symptoms (there is evidence that human exposure to wildtype chimp adenovirus, produces antibodies, but no clinical symptoms).	Likelihood of occurrence: Negligible. The likelihood of replication is low. ChAdOx1 has been engineered to be replication-incompetent. ChAdOx1-HPV can only replicate in laboratory cells which supplement the missing viral replication elements transiently. The packaging limitation on the virus genome and insertion of the transgene into the E1 gene locus preclude the creation of a replication-competent virus containing the HPV antigen gene cassette.	Combination of consequence and likelihood: Low.	Testing for replication competent adenovirus using established approved methods is always performed and to date, no replication competent virus has been detected in any of the ChAdOx1-HPV viral vector vaccines.

Hazard identification	Risk Characterisation	Hazard Characterisation	Exposure Characterisation	Risk Management Strategies
Recombination between ChAdOx1 and naturally occurring homologues	Potential consequence: Low. Any product of such a recombination is not likely to display any increased pathogenicity compared to the wild-type adenovirus. Moreover, the product of such a recombination would not be able to contain both the replication-inducing E1 gene and the transgene due both to the colocalization these genes in the respective parental virus genomes and the packaging constraints on the virus genome size.	Likelihood of occurrence: Negligible. ChAdOx1 is unlikely to recombine with human adenovirus, due to the lack of sequence homology. It is possible, but highly unlikely (given the relatively low abundancy and likelihood of contact with Chimpanzee adenovirus in the environment), that the ChAdOx1-HPV could recombine with wild-type Chimpanzee adenovirus. However, the process would be highly inefficient and would cause the deletion of the transgene.	Combination of consequence and likelihood: Low.	Study site personnel are fully trained in all handling of GMOs and decontamination thereby reducing the possibility of contact and therefore recombination with other naturally occurring variants of the virus.
Integration of vector sequences into the genome of mammalian/plant species. Insertional mutagenesis and/or inadvertent regulation (activation/silencing) of neighbouring genes which could be transferred.	High: Insertional mutagenesis. Negligible. The HPV antigen is not toxic and does not confer any advantage to the clinical viral vector in terms of viral vector survival, recombination or replication. The HPV sequence does not alter the transmission route or host range of the ChAdOx1 viral vector.	Negligible. Adenovirus is a non-integrating virus and the intact virus remains transiently episomally in the host cell; it does not possess the inherent characteristics to be able to actively integrate into the genome of the host cell. It is unable to infect plant cells.	Negligible.	Study site personnel will be fully trained in the decontamination and disposal of equipment and materials used in the study; all GMO waste will be disposed of from the study sites by certified vendors.

A. 3 Overall risk evaluation and conclusions

5.11 *Evaluate the overall risk of the clinical vector for humans (healthcare professionals and close contacts of the patient) and the environment considering, as applicable, the risk management strategies described in Section 3. 6.*

The overall risk of exposure to the ChAOx1-HPV viral vector is considered **low**. This is based upon the non-replicating properties of the viral vector, the benign nature of the HPV antigen polypeptide encoded by the transgene, and the control measures put in place during the trial to prevent any significant exposure or any transfer outside the clinical trial site.

The overall risk to the environment and human health is also considered **low**. This is based upon the non-replicating, non-integrating nature of the viral vector and the low likelihood of recombination events occurring. It is also supported by the good clinical safety profile and history of use for the current ChAdOx1 viral vectors.

MVA-HPV:

A.1. Risks to healthcare professionals and/or close contacts of the clinical trial subject (including vulnerable groups)

5.1 Hazard identification: *Provide a list of the potential adverse effects (e.g. immune reaction, integration in the genome of the exposed cells, adverse effects linked to the expression of the therapeutic gene, etc.) if transmission of the clinical vector or potential revertants to thirds-including vulnerable groups- occurs through shedding (as described in Section 2. 18).*

5.2 Hazard characterisation: *Provide an estimate of the magnitude of each of the identified potential adverse effects (it should be assumed that each of the hazards will occur). Where a quantitative estimation is not possible, a category ("high", "moderate", "low" or "negligible") should be assigned.*

5.3 Exposure characterisation: *Provide an estimate of the likelihood (probability) that each of the identified hazards will occur. Where a quantitative estimation is not possible, a category ("high", "moderate", "low" or "negligible") should be assigned*.

5.4 Risk characterisation: *Considering the magnitude of each of the effects identified and the likelihood of their occurrence, characterise the risk. Where a quantitative estimation is not possible, a category ("high", "moderate", "low" or "negligible") should be assigned.*

5.5 Risk management strategies: *The applicant should explain measures implemented to reduce the potential risks to thirds and/or the environment associated with the clinical use of the clinical vector. This includes -but is not limited to- the measures implemented to prevent the risks of accidental transfer during reconstitution, handling, administration of the product, or during manipulation of patient's samples (after administration of the clinical vector). The applicant should also explain the recommendations that will be provided to the clinical trial subject and/or close contacts to prevent dissemination/accidental contamination. Finally, the applicant should consider if clinical trial subjects should be prevented from donating blood/cells/ tissues/ organs after being administered the clinical vector. This information should be listed in Section 3. 6.*

Hazard identification	Hazard Characterisation	Exposure Characterisation	Risk Characterisation	Risk Management Strategies
Lack of homogeneity of the parental MVA strain, including the presence of replication competent virus, which could replicate in various mammalian (including human) cells.	Potential consequence: Low. A low dose of replication competent virus would, if capable of infecting humans, would be unlikely to produce any significant clinical symptoms.	Likelihood of occurrence: Negligible. The viral vaccine vector and the MVA vector vaccine used to create it, have been through several serial plaque picks to maximise the purity and	Combination of consequence and likelihood: Negligible.	The MVA-HPV seed material (MVS) and drug substance is tested for the presence of replication-competent virus. The release criteria for this particular viral vector is the confirmation of sequence specificity of the vector and the

Hazard identification	Hazard Characterisation	Exposure Characterisation	Risk Characterisation	Risk Management Strategies
		homogeneity of the drug product. MVA-HPV was shown to be homogeneous by next-generation sequencing, proving that the MVA strain is not polyclonal and does not contain any variants that may differ in their attenuation profile. Manufacture is performed in a controlled laboratory environment, by certified contract manufacturing organisations. Stringent GMP protocols and validated cell lines and reagents are used throughout. These processes ensure a high specification and quality product. The final drug product (MVA-HPV) and intermediates have undergone suitable and sufficient characterisation (quality) checks including PCR and DNA sequencing: These data confirm the presence of the MVA backbone and the HPV antigen expression cassette.		absence of any other contaminating vectors; there is no evidence for heterogeneity in the final drug product (MVA-HPV) and no evidence of replication-competent virus.
Biological activity of the transgene(s): Adverse effects	Potential consequence:	Likelihood of occurrence:	Combination of consequence	The vaccine will be handled and administered by trained

Hazard identification	Hazard Characterisation	Exposure Characterisation	Risk Characterisation	Risk Management Strategies
associated with the HPV transgene or a recombinant event e.g. altered immune response, inflammatory reaction, auto-immune disease.	Moderate. The antigen expression cassette of MVA-HPV consists of the F11 promoter, a synthetic antigen sequence derived from consensus regions, not related to pathogenicity, from thousands of strains of HPV and a polyadenylation sequence. The transgene is not toxic and does not confer any advantage to the clinical viral vector in terms of viral vector survival, recombination or replication. The HPV sequence does not alter the transmission route or host range of the MVA viral vector and a recombination event with wild type HPV is extremely unlikely due to the lack of sequence homology. There have been no serious, vaccine-related adverse events in any of the trials conducted to date. The MVA-HPV vector has been well tolerated and there have been no significant findings during pre-clinical testing.	Negligible. The therapeutic potential of MVA-HPV is conferred through expression of the HPV antigen and it is designed to stimulate/prime a protective immune response in the recipient towards the HPV virus. The origin of the sequence for the HPV synthetic antigen is derived using consensus regions of genomes of HPV strains and contains no naturally-occurring HPV genes. A recombination event with wild-type HPV is extremely unlikely due to the lack of sequence homology.	and likelihood: Low.	study site personnel.

Hazard identification	Hazard Characterisation	Exposure Characterisation	Risk Characterisation	Risk Management Strategies
Unintentional exposure to the MVA-HPV viral vaccine vector. Including those in high risk groups e.g. immunocompromised individuals.	Potential consequence: Low. Based upon the long history of safety using MVA viral vaccine vectors, including MVA-BN®, which is approved as a smallpox vaccine in Canada and the EU (under the trade names IMVAMUNE® and IMVANEX® respectively, there is no reason to believe that the MVA-HPV viral vector will pose any additional clinical risk to high risk groups e.g. immunocompromised individuals. The viral vaccine vector is not expected to adversely affect pregnant individuals, or that of an unborn child.	Likelihood of occurrence: Negligible. Due to the small injected volume, the viral titre administered and the replication-deficiency of the viral vector, it is highly unlikely that sufficient material could be released to be of any significance. Mammalian cells can be infected, but the virus is devoid of elements required to replicate. The route of administration in clinical trial is IM; there have been no reports of biodistribution following this route of administration, therefore, no shedding is expected.	Combination of consequence and likelihood: Low.	Although risk assessment of the replication incompetent vector and the non-toxic characteristics of the recombinant antigen carried by the vector indicate that MVA-HPV poses no risk to human health or the environment, accidental exposure to the vaccine will be controlled by a number of measures: The MVA-HPV viral vector vaccine will be supplied in sealed vials; each trial participant will receive a single intramuscular injection into the deltoid muscle of 0.5 mL by trained personnel. To prevent shedding from the injection site after needle withdrawal, the site will be wiped clean with a standard alcohol wipe and then covered with a sterile, occlusive dressing to absorb any virus that may leak out through the needle track and minimise dissemination into the environment. The dressing will be removed approximately 10 minutes after vaccination and the participant will remain

Hazard identification	Hazard Characterisation	Exposure Characterisation	Risk Characterisation	Risk Management Strategies
				in the clinic for at least a further 20 minutes, by which time the injection site will be dry. The clinical trial is being conducted in approximately 32 participants in Belgium; trial is placebo-controlled, so the expected number that will receive MVA vaccine is *circa*. 21. Study site personnel will be trained and utilise PPE-as standard good clinical practice-(e.g. gloves) when handling the medicinal product and follow the study-specific Pharmacy Manual and study site SOPs for handling of GMOs from receipt to final disposal of the materials; full decontamination procedures will be conducted in the event of accidental exposure. Trial participants are required to use reliable contraceptive methods, as defined in the Clinical Trial Protocol; this must be used for 4 weeks prior to administration of the first dose of trial vaccine and up to 8

Hazard identification	Hazard Characterisation	Exposure Characterisation	Risk Characterisation	Risk Management Strategies
				weeks after the last dose of trial vaccine. Female participants are required to have a pregnancy test before the administration of each vaccine dose and if their menstruation becomes irregular, or if a period is missed. Study site personnel are fully trained in all handling of GMOs and decontamination procedure following accidental exposure.
Lack of genetic stability and integrity of the transgene	Potential consequence: Low. Genetic instability is most likely to cause an impaired immune response e.g. lack of transgene expression.	Likelihood of occurrence: Negligible. MVA based vaccine vectors are genetically stable. Furthermore, the genetic stability of the MVA-HPV viral vaccine vector has been tested to passages beyond those required for general manufacture purposes and no genetic instability has been indicated: As demonstrated by standard molecular biology methods e.g. PCR, DNA sequencing.	Combination of consequence and likelihood: Negligible.	None.

Hazard identification	Hazard Characterisation	Exposure Characterisation	Risk Characterisation	Risk Management Strategies
Recombination between MVA-HPV and naturally occurring homologues	Potential consequence: Low. If recombination did occur, the possibility of producing a pathogenic strain of virus is remote. MVA is thought to have been derived from horsepox, which is thought to now be extinct. Cowpox is more distantly related but is now rare in cattle and this causes self-limiting infections in humans.	Likelihood of occurrence: Low. The MVA-HPV is unlikely to recombine with wild-type pox virus due to the significant lack of homology in the modified viral vector and the wild type virus.	Combination of consequence and likelihood: Low.	Study site personnel are fully trained in handling, decontamination and disposal procedures for GMOs. The likelihood of sufficient material being released that could result in a recombination event is therefore low.
Integration of vector sequences into the genome of the participant Insertional mutagenesis and/or inadvertent regulation (activation/silencing) of neighbouring genes which may lead to oncogenic and other adverse effects. Adverse effects associated with transmission to germline.	Potential consequence: Moderate.	Likelihood of occurrence: Negligible. MVA-HPV is an inherently non-integrative viral vector and remains localised in the cytoplasm of the infected host cell; it does not enter the nucleus or contain elements which facilitate the integration of the viral vector genome into the host. There is no evidence of germline transmission.	Combination of consequence and likelihood: Low.	Participants are required to use reliable contraceptive methods, as defined in the Clinical Trial Protocol; this must be used for 4 weeks prior to administration of the first dose of trial vaccine and up to 8 weeks after the last dose of trial vaccine. Female participants are required to have a pregnancy test before the administration of each vaccine dose and if their menstruation becomes irregular, or if a period is missed. Female participants are not

Hazard identification	Hazard Characterisation	Exposure Characterisation	Risk Characterisation	Risk Management Strategies
				allowed to perform egg/ovum donation during and after their participation in the trial for up to 8 weeks after administration of the last dose of trial vaccine.
Dispersion of the MVA from the administration site to other tissues of the treated study participant.	Potential consequence: Negligible. Based upon the long history of safety using MVA viral vaccine vectors, there is no reason to believe that the MVA-HPV vaccine will pose any additional clinical risk to high risk groups e.g. immunocompromised individuals. The viral vaccine vector is not expected to adversely affect immunocompromised individuals, pregnant individuals, or that of an unborn child.	Likelihood of occurrence: Low.	Combination of consequence and likelihood: Negligible.	The MVA-HPV vaccine is being administered by IM injection. Any potential shedding *via* the needle track via the needle track is reduced by wiping the site clean with a standard alcohol wipe after needle withdrawal. Participants will be retained for at least 30 minutes after vaccination by which time the injection site will be dry.
Viral vector transmission to other people by organ/blood donation	Potential consequence: Low. Pre-clinical toxicity studies of the MVA vector have shown no significant adverse effects in any organs or tissues sampled. Clinical studies conducted on the MVA vector with inserts	Likelihood of occurrence: Negligible. The MVA-HPV vaccine is non-replicating. Therefore, the risk of transmission of MVA through organ/tissue donation is considered low. Biodistribution studies on	Combination of consequence and likelihood: Low.	The MVA-HPV viral vector is non-replicating. Therefore, the risk of transmission of MVA through organ/tissue donation is considered low. However, as per current national organ donation guidelines, the participant will be precluded from blood or organ donation

Hazard identification	Hazard Characterisation	Exposure Characterisation	Risk Characterisation	Risk Management Strategies
	from other infectious pathogens have been conducted with no serious adverse effects reported to date.	MVA-vectored vaccines administered by IM injection have shown that the vaccine remains localised largely at the site of injection; viral DNA was detected only in draining lymph nodes at the 24 hour timepoint only.		for period of 1 month. Female participants are not allowed to perform egg/ovum donation during and after their participation in the trial for up to 8 weeks after administration of the last dose of trial vaccine.
Inadvertent contamination of laboratory personnel, care keepers or close relatives of the participant with MVA injected person	Potential consequence: Negligible. Based upon the long history of safety using MVA viral vaccine vectors, there is no reason to believe that the MVA-HPV vaccine will pose any additional clinical risk to high risk groups e.g. immunocompromised individuals. The viral vaccine vector is not expected to adversely affect immunocompromised individuals, pregnant individuals, or that of an unborn child.	Likelihood of occurrence: low.	Combination of consequence and likelihood: Negligible.	Only designated and trained study site personnel will be involved in handling of the GMO, which will be kept in a locked fridge in a restricted Pharmacy area. All supplies will be labelled as GMOs and handled according to the Pharmacy Manual and study site SOPs from receipt of study supplies to final disposal of materials; full decontamination procedures will be conducted in the event of accidental exposure. Participants will be retained at the study site for at least 30 minutes after vaccination by which time the injection site will be dry. Infection control procedures will be followed when any samples are taken from trial

Hazard identification	Hazard Characterisation	Exposure Characterisation	Risk Characterisation	Risk Management Strategies
				participants. All participants will be monitored for adverse effects as per the study protocol and provided with a contact number for the clinic to report any potential adverse effects that occur outside of assessments at the study site.

A.2. Risks to the environment

5.6 Hazard identification: *Provide a list of the potential adverse effects. As appropriate, consider specific environmental conditions that may affect the survival, replication or ability to colonise (wind, water, soil, temperatures, pH, etc).*

5.7 Hazard characterisation: *Provide an estimate of the magnitude of each of the identified potential adverse effects (it should be assumed that each of the hazards will occur). Where a quantitative estimation is not possible, a category ("high", "moderate", "low" or "negligible") should be assigned.*

5.8 Exposure characterisation: *Provide an estimate of the likelihood (probability) that each of the identified hazards will occur. Where a quantitative estimation is not possible, a category ("high", "moderate", "low" or "negligible") should be assigned.*

5.9 Risk characterisation: *Considering the magnitude of each of the effects identified and the likelihood of their occurrence, characterise the risk. Where a quantitative estimation is not possible, a category ("high", "moderate", "low" or "negligible") should be assigned.*

5.10 Risk management strategies: *The applicant should implement adequate measures to prevent dissemination into the environment. These should be listed in Section 3. 6.*

Hazard identification	Hazard Characterisation	Exposure Characterisation	Risk Characterisation	Risk Management Strategies
Environmental release and persistence of MVA-HPV vector into the environment.	Potential consequence: Low.	Likelihood of occurrence: Negligible.	Combination of consequence and likelihood: Negligible.	Poxviruses are inactivated by common chemical agents (e.g. 70% IPA, 1% sodium

Hazard identification	Hazard Characterisation	Exposure Characterisation	Risk Characterisation	Risk Management Strategies
Pox virus have a worldwide distribution. Humans and animals are the natural reservoirs for wild-type pox viruses, and some have zoonotic potential. However, highly virulent strains of pox virus e.g. small pox have been successfully eradicated through vaccination with replication competent versions of a related viral vector. MVA has a high environmental stability. Infectious virions could survive up to 39 weeks at 6.7% moisture at 4°C environmental surfaces at ambient temperatures and are resistant to lipid disinfectants.				hypochlorite, ethyl alcohol, 2% glutaraldehyde and 0.25% sodium dodecyl sulphate). The virus is also susceptible to inactivation by heat and autoclaving at 121°C for 15 minutes. The general release of the MVA-HPV viral vector into the environment is controlled by the following measures: MVA-HPV cannot replicate outside the laboratory. The medicinal product will be stored securely under strict pharmacy regulations. Only trained and authorised personnel have access to the viral vaccine vector. The MVA vector has been utilised tested as a vaccine platform for various diseases and there are marketed and approved vaccines utilising this vector are in clinical use: MVA-BN® is approved as a smallpox vaccine in Canada and the EU (under the trade names IMVAMUNE® and IMVANEX® respectively) for use in healthy individuals and those

Hazard identification	Hazard Characterisation	Exposure Characterisation	Risk Characterisation	Risk Management Strategies
				with immunodeficiency. Any spillages that occur will be dealt with promptly and decontaminated using established and effective methods. Any leakage from the injection site of the participant following intramuscular injection, will be wiped away with a standard alcohol wipe which will be disposed of through an approved clinical waste route and the study participant will be retained at the site for at least 30 minutes, by which time the injection site will be completely dry. Study site personnel will be fully trained in the conduct required to decontaminate and dispose of equipment and consumables used in the study; all GMO waste will be disposed of from the study sites by certified vendors. These control methods are deemed effective at minimising any possibility of release and persistence in the

Hazard identification	Hazard Characterisation	Exposure Characterisation	Risk Characterisation	Risk Management Strategies
				environment.
Ability to replicate in non-human cells.	Potential consequence: Low. Since MVA-HPV cannot replicate, the potential consequence would depend on the extent in a change in the ability to replicate in non-human cells. The consequence would fall between negligible and that of an infection with vaccinia virus; which is attenuated in immunocompetent animals and was used as the basis of the oral rabies vaccine used to eliminate the disease in Europe.	Likelihood of occurrence: Negligible. MVA-HPV cannot replicate, and testing has confirmed that the drug product is devoid of replication competent virus. There is a history of safe use of these type of vectors with no reports of replication competent virus being generated.	Combination of consequence and likelihood: Low.	Study site personnel will be fully trained in the conduct of the required decontamination and disposal procedures; all GMO waste will be disposed of from the study sites by certified vendors.
Recombination between MVA and naturally occurring homologues.	Potential consequence: Low.	Likelihood of occurrence: Negligible. MVA-HPV cannot replicate, and testing has confirmed that the drug product is devoid of replication-competent virus. Route of administration in the clinical trial is intramuscular; there have been no reports of biodistribution following this route of administration.	Combination of consequence and likelihood: Low.	Study site personnel are fully trained in all handling of GMOs and decontamination thereby reducing the possibility of contact and therefore recombination with other naturally occurring variants of the virus.

Hazard identification	Hazard Characterisation	Exposure Characterisation	Risk Characterisation	Risk Management Strategies
		Lack of sequence homology between wild type virus and MVA-HPV make recombination events very unlikely. MVA is thought to have been derived from horsepox, which is thought to now be extinct. Cowpox is more distantly related but is now rare in cattle.		
Integration of vector sequences into the genome of mammalian/plant species. Insertional mutagenesis and/or inadvertent regulation (activation/silencing) of neighbouring genes which could be transferred.	Potential consequence: High: Insertional mutagenesis Negligible: The HPV antigen does not confer any advantage to the clinical viral vector in terms of viral vector survival, recombination or replication. The HPV sequence does not alter the transmission route or host range of the MVA-HPV vector.	Likelihood of occurrence: Negligible. MVA-HPV is non integrative and cannot infect germline cells. MVA-HPV is unable to infect plant cells.	Combination of consequence and likelihood: Negligible.	Study site personnel will be fully trained in the decontamination and disposal of equipment and materials used in the study; all GMO waste will be disposed of from the study sites by certified vendors.

A. 3 Overall risk evaluation and conclusions

5.11 *Evaluate the overall risk of the clinical vector for humans (healthcare professionals and close contacts of the patient) and the environment considering, as applicable, the risk management strategies described in Section 3. 6.*

The overall risk of exposure to the MVA-HPV viral vector is considered **low**. This is based upon the non-replicating properties of the viral vector and the control measures put in place during the trial to prevent any significant release.

The overall risk to the environment and human health is also considered **low**. This is based upon the non-replicating, non-integrating nature of the viral vector and the low likelihood of recombination events occurring. It is also supported by the good clinical safety profile and history of use for the current MVA viral vectors.

Submissions to Czech Republic:

In addition to the plan of the site, a description of the location of the autoclave should be provided –as appropriate- as part of the description of the measures to prevent dissemination into the environment referred in Section 3. 6 (d) and (e).

Submissions to France:

The plan of the site should indicate clearly the location of a PSMII or an equivalent device.

Submissions to Germany:

- *The applicant is not required to provide further information in Section 3(6)(c) if he/she confirms that the disinfectant and decontamination procedure are included in the list of the Robert Koch Institute of currently approved disinfectants and disinfectant procedures or the VAH (Verbund für Angewandte Hygiene e. V) list of disinfectants.*
- *The applicant should explain if left-overs are stored at the clinical site and, if in the affirmative, for how long as part of the information submitted in Section 3(6)(d).*
- *The applicant should provide the following information on waste treatment in Section 3(6)(e):*
  - *Whether and for how long the waste will be stored (or frequency of waste disposal),*
  - *Storage location,*
  - *Logistics for on-site transportation of the waste (similar as asked for the clinical vector in Section 3. 4), and*
  - *In case of chemical decontamination whether the chosen disinfectant and method is sufficiently active against the clinical vector (similar as in Section 3. 6. c)*

- *If samples are stored at the clinical site, the maximum duration of the storage should be stated in Section 3. 7 (c).*

Submissions to Ireland:

- *In addition to the plan of the site, a description of the location of the autoclave should be provided –as appropriate- as part of the description of the measures to prevent dissemination into the environment referred in Section 3. 6 (d) and (e).*
- *If samples are stored at the clinical site, the maximum duration of the storage should be stated in Section 3. 7 (c).*

Submissions to Italy:

- *In addition to the plan of the site, a description of the location of the autoclave should be provided –as appropriate- as part of the description of the measures to prevent dissemination into the environment referred in Section 3. 6 (d) and (e).*
  If the manufacturer of the clinical vector is located in Italy, the authorisation issued to the premises should be declared in Section 1. 3.

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