Janssen Vaccines & Prevention B.V.

PART 1 (COUNCIL DECISION 2002/813/EC)

Summary Notification Information Format for the Release of Genetically Modified Organisms Other Than Higher Plants in Accordance with Article 11 of Directive 2001/18/EC

Ad26COVS1

NETHERLANDS

Status: Approved
Date: 6 July 2020
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TABLE OF CONTENTS

Abbreviation list

Ad	Adenovirus
Ad26	Adenovirus type 26
BRMAC	Biological Response Modifiers Advisory Committee
COVID	Coronavirus disease
DNA	Deoxyribonucleic acid
E	Adenovirus early genes
EBV	Epstein Barr virus
EMA	European Medicines Agency
ENVA	Envelop A
FDA	Food and Drug Administration
GMO	Genetically modified organism
HIV	Human immunodeficiency virus
HPV	Human papillomavirus
IM	Intramuscularly
IMP	Investigational Medicinal Product
PCR	Polymerase chain reaction
Ph. Eur.	European pharmacopeia
polyA	Polyadenylation
RCA	Replication-competent adenovirus
RSV	Respiratory syncytial virus
S protein	Spike protein
SARS-CoV-2	Severe acute respiratory syndrome coronavirus type 2
SNIF	summary notification information format
TetO	Tetracycline operator
TetR	Tetracycline repressor
Vp	Virus particle
ZEBOV	Zaire Ebola virus

1 GENERAL INFORMATION

1.1 Details of notification

Member State of notification	Netherlands
Notification number	B/NL/20/008
Date of acknowledgement of notification	08/07/2020
Title of the project	Clinical trials with Ad26COVS1
Proposed period of release	Recruiting as of July 2020
Period of release	The study duration will depend on the outcome of the immunizations

1.2 Notifier

Name of institution or company:

UMC Utrecht

Heidelberglaan 100

3584 CX Utrecht, the Netherlands

1.3 GMO characterisation

The Genetically Modified Organism (GMO) is denoted as Ad26COVS1 throughout this document. Ad26COVS1 is a replication-incompetent adenovirus (human adenovirus type 26) based vector encoding the spike (S) protein of the severe respiratory syndrome coronavirus type 2 (SARS-CoV-2).

Indicate whether the GMO is a:

- viroid (.)
- RNA virus (.)
- DNA virus (X) Ad26 vector, recombinant replication-incompetent vector
- bacterium (.)
- fungus (.)
- animal
    - mammals (.)
    - insect (.)
    - fish (.)
    - other animal (.)

specify phylum, class …

Identity of the GMO (genus and species)

The identity of the GMO is Ad26COVS1 and is a replication incompetent adenovirus vector containing the sequence for the spike (S) protein of SARS-CoV-2. The Ad26COVS1 vector is derived from the human adenovirus group D type 26 (genus *Mastadenoviridae*).

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

After administration of Ad26COVS1 to the subjects of the clinical trial, it will remain epichromosomal in the host cells, thus avoiding the risk of integration of viral DNA into the host genome. In addition, as Ad26COVS1 is replication incompetent, it is not able to replicate its genome and can therefore be considered to be genetically stable and alterations in the genome are not expected.

During the production process candidate vaccine seed lots are extensively tested and characterized, which includes sequence analysis. Also, candidate vaccine lots are subjected to sequence analysis.

The Ad26COVS1 vector has been made replication-incompetent by removing the E1 region of the Ad26 genome, which is required for replication. A large portion of the E3 region, which promotes persistence within the host cell, has also been removed to create sufficient space in the viral genome for insertion of foreign antigens. For productive infection and replication during manufacturing, the E1 defect is supplemented by engineered E1- (from Ad5) complementing cell lines (Fallaux et al., 1998). Due to the absence of any DNA sequence overlap between the Ad26 adenoviral vector and the cell line, the formation of replication-competent adenovirus (RCA) is prevented which is confirmed by specific safety testing (RCA test). The RCA test is reported as “no RCA detected” if it complies with the acceptance criterion of <1 RCA/3 x 10^{10} VP. This acceptance criterion is based on the FDA guidance document and the FDA Biological Response Modifiers Advisory Committee (BRMAC) meeting number 30 (Adenovirus Titer Measurements and RCA levels; April 5, 2001).

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

Yes (X) No (.)

If yes, insert the country code(s) [1]: ES, DE

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

Yes (.) No (X)

If yes:

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

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

Yes (.) No (X)

If yes:

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

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

In this Phase 2 VAC31518COV2001clinical study Ad26COVS1 will be administered intramuscularly in subjects. The aim of the clinical trial is to demonstrate the safety and immunogenicity of Ad26COVS1 when given as a single-dose or multi-dose vaccination regimen, as compared to placebo, in SARS-CoV-2 seronegative adults. The investigational COVID-19 vaccine includes Ad26COVS1, a component containing organisms with modified genetic material. The administration is taking place under conditions similar to contained use and during as well as following the administration of the vaccine no release into the environment is expected.

Shedding

The biodistribution profile of the Ad26 vector has been evaluated in **non-clinical studies** using several Ad26-based vaccines. These platform data showed that the Ad26 vector did not widely distribute following intramuscular (IM) administration in rabbits. Ad26 vector DNA was primarily detected at the site of injection, draining lymph nodes and (to a lesser extent) the spleen. Clearance of the Ad26 vector from the tissues was observed. The data indicate that the Ad26 vector does not

[1] **Please use the following country codes:**
Austria AT; Belgium BE; Germany DE; Denmark DK; Spain ES; Finland FI; France FR; United Kingdom GB; Greece GR; Ireland IE; Iceland IS; Italy IT; Luxembourg LU; Netherlands NL; Norway NO; Portugal PT; Sweden SE

replicate and/or persist in the tissues following IM injection. Despite differences in the encoded antigen transgenes, both Ad26 vectors showed a similar pattern of biodistribution and clearance when delivered via the IM route in the rabbit. These non-clinical biodistribution results are considered sufficient to inform on the biodistribution profile of other Ad26-based vaccines, including Ad26COVS1, for which the same Ad26 vector backbone is used, when administered via the same (IM) route.

Shedding of the Ad26 vector has also been evaluated in **clinical studies** for several Ad26-based vaccines. These shedding studies show that Ad26 clinical vector DNA is very rarely found in secreted body fluids after vaccination, further indicating that the Ad26 clinical vector does not persist and/or replicate in the tissues following vaccination.

It is concluded that the chance that the Ad26COVS1 clinical vector is shed to the environment in relevant quantities upon administration, beyond its initial potential presence at the injection site (injection site leakage) is negligible, taking into account:

- information from other Ad26 vector vaccines confirms that vector DNA is only infrequently detected, and if so, is only found at very low levels. Furthermore, the presence of vector DNA does not necessarily mean the presence of infectious virus particles.
- the potential for shedding is considered to be independent of the transgene insert, as the transgene insert does not have an impact on the vector particle, and thus does not change cell tropism.
- the same routes (i.e. intramuscular injection) and dose (1× 10^{11} VP or below) of inoculation will be used for vaccination as have been used for previous shedding studies.

Pathogenicity

The modified Ad26 virus is replication incompetent and therefore is not pathogenic and has a minimal likelihood to colonize in natural ecosystems. If it is exposed to the environment, it is unlikely to survive for extended periods.

In regard to humans, the infection risk with Ad26COVS1 is negligibly since no release from the injected subjects is expected (see above). In the case of transmission to unintended persons working with, coming into contact with, or in the vicinity of Ad26COVS1 administration, the consequences for the individual are expected to be minimal.

Persistence and/or invasion

The likelihood of Ad26COVS1 becoming persistent and invasive in natural habitats is minimal for the following reasons. Ad26COVS1 has been tested negative for RCA. The probability that the missing E1 function is complemented in humans is extremely low. In the theoretical situation that Ad26COVS1 re-acquired the ability to replicate reliably, the consequences would likely be minimal.

Horizontal gene transfer

Horizontal gene transfer is unlikely and due to Ad26COVS1 sequence characteristics there is no possibility to confer a selective advantage to bacteria or other microorganisms because it does not contain any prokaryotic promoters, any antibiotic or other types of resistance genes which would enhance or constrain their growth.

Unintended release during transport or disposal

Ad26COVS1 will be shipped in qualified, insulated shippers to clinical sites. The vaccines will be supplied in sealed single-dose vials, which will be stored in a secured location with no access for unauthorized personnel. Subjects of the trials will be vaccinated with Ad26COVS1 at the clinical sites under controlled conditions. Considerable care will be taken that personnel and the areas are not exposed to Ad26COVS1.

All wastes resulting from Ad26COVS1 handling are to be treated according to regular site procedure for infectious wastes. In case of accidental spillage, specific recommendation for decontamination and destruction are to be followed to avoid any risk of dispersion to the environment.

Taking the above information into consideration, dispersion of Ad26COVS1 to the environment is unlikely and its potential environmental impact is considered negligible. The negligible environmental

impact of Ad26-based vectors is further highlighted by the positive CHMP opinion that was received on the 28th of May 2020 for an Ad26.ZEBOV environmental risk assessment (https://www.ema.europa.eu/en/medicines/human/summaries-opinion/zabdeno).

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

2.1 Recipient or parental organism characterisation

Indicate whether the recipient or parental organism is a:

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

specify phylum, class …

- other, specify …

Name

• order and/or higher taxon (for animals)	*Rowavirales, Adenoviridae*
• genus	Mastadenovirus
• species	Human adenovirus group D
• subspecies	N.a.
• strain	Type 26
• pathovar (biotype, ecotype, race, etc.)	N.a.
• common name	Human adenovirus type 26 (Ad26)

Human adenovirus type 26, group D, has presumably been isolated from an anal specimen from a 9-month-old male, in Washington, DC, in 1956.

Geographical distribution of the organism

Indigenous to, or otherwise established in, the country where the notification is made:

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

Indigenous to, or otherwise established in, other EC countries:

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

If yes, indicate the type of ecosystem in which it is found: (see description)

Atlantic ..
Mediteranean ..
Boreal ..
Alpine ..
Continental ..
Macaronesian ..

Adenovirus is prevalent worldwide, and is ubiquitous throughout the year, especially during late winter and early spring. Low to moderate titers of baseline Ad26 specific neutralizing antibodies have been observed in populations in Belgium, sub-Saharan Africa, Thailand, and Brazil, amongst other regions (Mast et al., 2010; Barouch et al., 2018). A seroprevalence study showed that several Ad serotypes from subgroup B and D were rare in a West European population (Vogels et al., 2003; Abbink et al., 2007). Epidemiological studies indicate that the parental Ad26 is endemic in the EU (Vogels et al., 2007; Mast et al., 2010).

Is it frequently used in the country where the notification is made?

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

In the country of notification, a low seroprevalence of Ad26 virus in humans is expected.

Is it frequently kept in the country where the notification is made?

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

Natural habitat of the organism

If the organism is a microorganism

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

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

Not applicable

Detection and identification techniques

Detection of wild type replication competent Ad26 adenoviruses can be performed by adenoviral cultures in MRC5 and A549 cells and detection using an anti-hexon antibody that shows reactivity against the Ad26 adenovirus hexon protein. Alternatively, Ad26 viruses can be detected with PCR using adenovirus general or Ad26 virus-specific sequences.

Wild type Ad26 viruses are identified using PCR with sequences specific for Ad26 virus. DNA sequencing can also be used for the identification of adenoviruses.

Is the recipient organism classified under existing Community rules relating to the protection of human health and/or the environment?

Yes	(X)	No	(.)

If yes, specify

Human adenovirus is classified as a group 2 biological agent as per the European Economic Community classification for the protection of workers with biological agents (Directive 2000/54/EC). The group 2 designation applies to agents (1) that can cause human disease and might be a hazard to workers, (2) that are unlikely to spread to the community and (3) for which there is usually effective prophylaxis or treatment available.

Based on the mild disease of human adenovirus 26 in healthy humans and results of toxicity studies that demonstrated safety and tolerability of Ad26-based clinical vectors, the GMO is not considered to pose a risk to human health.

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

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

If yes:

to which of the following organisms:

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

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

Adenoviruses can be transmitted through close contact, aerosol droplets or via the oral/fecal route. Upon exposure, they generally cause an asymptomatic infection despite virologic and serological proof of infection, or are responsible for self-limiting and mild respiratory, gastrointestinal, and/or ocular disease in immunocompetent hosts. They are a common pediatric pathogen. Among patients with compromised immunity, adenovirus may cause severe disseminated disease associated with increased morbidity and mortality.

Group D adenoviruses are considered less pathogenic than e.g. B or C group adenoviruses, although only a limited number of studies on the clinical manifestations of Ad26 have been published.

Information concerning reproduction

Generation time in natural ecosystems:

The adenoviral life cycle starts with binding of the viral fiber knob to cell surface receptors. After other specific binding and recognition events including the viral penton protein and the host's integrins, the virus is taken up into the cell by endocytosis. The virus particle escapes from the endosome and the viral DNA is released into the host cell nucleus. There, early genes and late genes are transcribed. Early gene products are regulatory proteins that enable efficient viral DNA replication, activate other viral proteins and ensure escape from the host's immune response. After DNA replication, late genes are transcribed that code for structural proteins. These proteins together with replicated DNA molecules form new viral particles that leave the cell by cell lysis. Wild type adenovirus replication is an efficient process and progeny virus can be produced in less than 2 days.

Generation time in the ecosystem where the release will take place:

See section above

Way of reproduction:

Sexual (.) Asexual (X)

Factors affecting reproduction:

The outcome of an adenovirus infection depends on the animal species and cell type involved. Adenovirus type 26 is restricted to humans.

Survivability

Ability to form structures enhancing survival or dormancy

Adenovirus do not form structures enhancing survival or dormancy.

- endospores (.)
- cysts (.)
- sclerotia (.)
- asexual spores (fungi) (.)
- sexual spores (funghi) (.)
- eggs (.)

- pupae (.)
- larvae (.)
- other, specify …

Relevant factors affecting survivability:

Wild type Ad26 virus is able to persist in aerosols and water. Stability decreases significantly as temperature is increased. Under normal environmental conditions, Ad26 is expected to lose viability within days to weeks. Adenoviruses are resistant to lipid disinfectants but are inactivated by e.g. formaldehyde, bleach and chlorine (https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/adenovirus-types-1-2-3-4-5-7-pathogen-safety-data-sheet.html).

Ways of dissemination

Adenoviruses can be transmitted through close contact, aerosol droplets or via the oral/fecal route.

Factors affecting adenovirus dissemination, in general, are exposure dose, formation of aerosols, and closeness of contacts.

Previous genetic modifications of the recipient or parental organism already notified for release in the country where the notification is made (give notification numbers)

Not applicable

3 Information relating to the genetic modification

3.1 Type of the genetic modification

- insertion of genetic material (X)
- deletion of genetic material (X)
- base substitution (.)
- cell fusion (.)
- others, specify …

3.2 Intended outcome of the genetic modification

This clinical vector is intended to be used for prophylactic immunization against coronavirus disease 2019 (COVID-19). To this aim, a synthetic sequence is designed based on antigen(s) of SARS-CoV-2 and inserted in the replication-incompetent Ad26 vector.

3.3 Vector information

Has a vector been used in the process of modification?

Yes (X) No (.)

If no, go straight to section 0.

If yes, is the vector wholly or partially present in the modified organism?

Yes (X) No (.)

If no, go straight to section 0. If the answer to 3(b) is yes, supply the following information:

Type of vector

- plasmid (X)

Single genome plasmid in which the whole genetic sequence of the clinical vector is encoded, including the transgene

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

Identity of the vector

To generate the replication incompetent Ad26COVS1 vector, a single genome plasmid in which the whole genetic sequence of the clinical vector is encoded, including the transgene, is linearized and transfected into complementing cells (PERC6® TetR). The Ad26COVS1 vector is produced after transfection of the cells, after which plaque purification steps are performed to ensure clonality of the vector. The vector is further amplified and a small-scale batch is purified which leads to the vaccine candidate seed.

Host range of the vector

E. coli laboratory strains

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

Yes (X) No (.)

- Antibiotic resistance (X)
- other, specify …

Indication of which antibiotic resistance gene is inserted: ampicillin resistance gene

Note that the antibiotic resistance genes are only part of the plasmid backbone. Upon recombination in the cells and generation of the final vaccine vector Ad26COVS1, no antibiotic resistance gene is present.

Constituent fragments of the vector

The vector contains a human viral promotor, the transgene and a viral polyA.

Method for introducing the vector into the recipient organism

- transformation (.)
- electroporation (.)
- macroinjection (.)
- microinjection (.)
- infection (.)
- other, specify Transfection of linearized plasmid

What was the method used in the process of modification?

- transformation (.)
- microinjection (.)
- microencapsulation (.)
- macroinjection (.)
- other, specify …

Composition of the insert

Composition of the insert

Ad26COVS1 harbours a transgene expression cassette in place of the E1 deletion at the left end of the Ad26 vector genome. The transgene expression cassette consists of a human viral promoter, a

tetracycline resistance operon (TetO), and a viral polyadenylation signal and encodes a synthetic SARS-CoV-2 S protein sequence. The synthetic sequence for the transgene was cloned into the plasmid by standard molecular cloning techniques. The transgene encodes a structural protein of SARS-CoV-2. No effects on the vector sequences due to the insertion of the transgenes into the vector were found.

Source of each constituent part of the insert

The synthetic sequence encodes a spike protein (S) derived from SARS-CoV-2. Expression of the transgene is controlled by a strong ubiquitous human viral promoter. The polyadenylation signal is also derived from a virus. The promoter and polyadenylation signal are commonly used genetic control elements.

Intended function of each constituent part of the insert in the GMO

A synthetic transgene, encoding the SARS-CoV-2 S protein is inserted into the adenoviral vector genome. This transgene is expressed in the vaccinated individual and elicits the vaccinee's immune response against SARS-CoV-2.

Location of the insert in the host organism

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

Integrated in the double-stranded DNA genome in the adenovirus ds DNA replacing the E1 region. No integration of the insert into the genome of vaccinated individuals.

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

Yes (.) No (X)

If yes, specify …

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

4.1 Donor organism characterisation

Indicate whether the recipient or parental organism is a:

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

  specify phylum, class …
- other, specify …

Name

- order and/or higher taxon (for animals) *Coronaviridae*
- genus Betacoronavirus
- species Severe acute respiratory syndrome coronavirus

- subspecies SARS-CoV-2
- strain Wuhan-Hu-1
- pathovar (biotype, ecotype, race, etc.) N.a.
- common name SARS-CoV-2

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

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

If yes:

to which of the following organisms:

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

Are the donated sequences involved in any way to the pathogenic or harmful properties of the organism

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

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

In the context of wild type SARS-CoV-2, the S protein gene encodes a structural virus protein. During viral infection, the S protein fuses the viral and host cell membranes. The S protein was selected as a transgene because it is highly conserved and forms an important target of neutralizing antibodies. The encoded transgene is a synthetic sequence. The transgene does not have an influence on the transduction capacity of Ad26, and does not change the host spectrum, cell tropism or environmental stability of the Ad26 vector.

Is the donor organism classified under existing Community rules relating to the protection of human health and/or the environment?

Yes (X) No (.)

If yes, specify

SARS-CoV-2 is classified as a group 3 biological agent as per the European Economic Community classification for the protection of workers with biological agents (Directive 2000/54/EC). The group 3 designation applies to agents (1) that cause severe human disease and present a serious hazard, (2) that may present a risk of spreading to the community, but (3) there is usually effective prophylaxis or treatment available.

Do the donor and recipient organism exchange genetic material naturally?

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

5 Information relating to the genetically modified organism

5.1 Genetic traits and phenotypic characteristics of the recipient or parental organism which have been changed as a result of the genetic modification

Is the GMO different from the recipient as far as survivability is concerned?

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

Specify: Survivability or stability of the GMO Ad26COVS1 is expected to be similar to the wild type Ad26 virus. Ad26COVS1 is however replication-incompetent.

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

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

Specify: Ad26COVS1 is replication-incompetent.

Is the GMO in any way different from the recipient as far as dissemination is concerned?

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

Specify: Wild type adenovirus replication is an efficient process and progeny virus can be produced in less than 2 days, whereas Ad26COVS1 cannot replicate in cells which do not express the adenoviral E1 region and dissemination is limited, unlike the wild type virus would be present. Dissemination is restricted to the subjects receiving the GMO in the clinical study.

Is the GMO in any way different from the recipient as far as pathogenicity is concerned?

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

Specify: Ad26.COVS1 cannot replicate in cells which do not express the adenoviral E1 region and is therefore not considered to be pathogenic.

5.2 Genetic stability of the genetically modified organism

Ad26COVS1 is considered genetically stable. Genetic stability is tested during different steps of the production process. In brief, plaque purified Ad26COVS1 vector was used to produce virus seed lots. Virus seed lots are extensively tested and characterised, which includes sequence analysis (compared to reference sequence). Virus seed lots containing the correct sequence serve as starting material for the production of each virus lot. Also, virus lots are subjected to sequence analysis. During manufacturing, the GMO identity is confirmed to ensure that the vector with correct type and insert is present in the drug product. The adenovirus vector identity test is according to Ph. Eur. 5.14, EMA/CHMP/VWP/141697/2009, and ICH Q6B.

5.3 Is the GMO significantly pathogenic or harmful in any way (including its extracellular products), either living or dead?

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

To which of the following organisms?

- humans (.)
- animals (.)
- plants (.)
- other …

Give the relevant information specified under Annex III A, point II(A)(11)(d) and II(C)(2)(i)

Safety

No safety data are available yet for the Ad26COVS1 vaccine. However, as of 30 March 2020, more than 67.000 subjects have been vaccinated with other Ad26 clinical vectors expressing different transgenes, including vaccines against human immunodeficiency virus (HIV) (Ad26.ENVA.01 (Baden et al., 2013, 2016; Barouch et al., 2018; Colby et al., 2020)), malaria (Ad26.CS.01 (Radosevic et al., 2010; Talley et al., 2012), Ebola virus (Ad26.ZEBOV (Anywaine et al., 2019; Milligan et al., 2016; Mutua et al., 2019)) and respiratory syncytial virus (RSV) (Ad26.RSV.PreF (Williams et al., 2020) without any safety concerns.

Biodistribution and shedding

As outlined above, the Ad26 vector does not replicate and/or persist in the tissues following IM injection as evidenced by a variety of non-clinical and clinical studies with other Ad26 clinical vectors. The

chance that the Ad26COVS1 clinical vector is shed to the environment in relevant quantities upon administration, beyond its initial potential presence at the injection site (injection site leakage) is negligible.

Pathogenicity

The modified Ad26 virus is replication incompetent and therefore is not pathogenic and has a minimal likelihood to colonize in natural ecosystems. If it is exposed to the environment, it is unlikely to survive for extended periods.

In regard to humans, the infection risk with Ad26COVS1 is negligibly since no release from the injected subjects is expected (see above). In the case of transmission to unintended persons working with, coming into contact with, or in the vicinity of Ad26COVS1 administration, the consequences for the individual are expected to be minimal.

Persistence and/or invasion

The likelihood of Ad26COVS1 becoming persistent and invasive in natural habitats is minimal for the following reasons. Ad26COVS1 has been tested negative for RCA. The probability that the missing E1 function is complemented in humans is extremely low. In the theoretical situation that Ad26COVS1 re-acquired the ability to replicate reliably, the consequences would likely be minimal.

Horizontal gene transfer

Horizontal gene transfer is unlikely and due to Ad26COVS1 sequence characteristics there is no possibility to confer a selective advantage to bacteria or other microorganisms because it does not contain any prokaryotic promoters, any antibiotic or other types of resistance genes which would enhance or constrain their growth.

Recombination

Potential recombinants resulting from recombination with wildtype adenoviruses would not pose a higher risk than a wildtype infection that is already present and these recombinants would not be able to disseminate in the human body or to the environment. The unlikely event of trans-complementation of the E1 missing function by other viruses (e.g. HPV, EBV) cannot be excluded, but would be limited to transduced co-infected cells only, as Ad26COVS1 remains replication incompetent. Therefore, dissemination of the vector will not take place. Furthermore, the absence of any sequence homology between the vector and the cells during the manufacturing process prevents the formation of RCA. All produced clinical vector batches at Janssen Vaccines & Prevention B.V., including this GMO, have been tested negative for a control test for the detection of RCA.

Genome integration

Adenovirus vectors are known to be present as episomal structures in the transduced cells, and do not likely present a risk of integration in the host genome. According to EMA guideline on non-clinical testing for inadvertent germline transmission of gene transfer vectors (EMEA/273974/2005), adenoviral vectors are considered as non-integrating vectors (https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-non-clinical-testing-inadvertent-germline-transmission-gene-transfer-vectors_en.pdf).

Description of identification and detection methods

Techniques used to detect the GMO in the environment

Virus identity testing is performed to confirm the adenoviral subtype of the vector and transgene by polymerase chain reaction (PCR). First, the viral DNA is extracted and purified. Purified DNA from the test sample is used for PCR with primers that have been designed to specifically amplify the transgene, as well as the adenovirus specific regions. Upon amplification, the size of the PCR product obtained with the test article is compared to a control amplification performed in parallel on purified plasmid DNA. Concordance between the size of the test article and the control is indicative of the virus identity. In addition, identity is confirmed by sequencing the transgene and its flanking regions

Techniques used to identify the GMO

See above

6 Information relating to the release

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

Janssen Vaccines & Prevention B.V. is developing a prophylactic SARS-CoV-2 vaccine to prevent COVID19.

Ad26COVS1 will be administered by intramuscular injection (IM) to subjects participating in this international, multicenter, randomized, placebo-controlled, observer-blind Phase 2 clinical study to evaluate the safety, tolerability and immunogenicity of Ad26COVS1 in participants aged 18 to 55 years. Each participant in the clinical trial will receive a maximum of 10^{11} vp IM per dose, with a maximum of 3 doses and a maximum of a 84 day interval of the vaccine or a placebo. Study duration is 14 months and includes a 6 months follow up. It is anticipated that a maximum of 1000 subjects will be enrolled during this international multi-center study.

While the candidate vaccine addresses an urgent need for protecting human health, there is no expected benefit for the environment.

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

Yes (.) No (x)

If yes, specify …

Not applicable, the GMO is not found in a natural habitat besides, to a limited percentage, in the human population.

6.3 Information concerning the release and the surrounding area

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

N.a. (separate SNIF is submitted for each clinical site where Ad26COVS1 will be administered).

Size of the site (m2)

- actual release site (m2): … m2
- wider release site (m2): … m2

No specific size for the release, immunizations are going to be performed in separate examination rooms. No environmental sites outside the examination rooms are involved.

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

The primary release is the moment where the vaccine is administered to the participant. No release is anticipated in environmental sites outside the examination rooms. Containment measures during the administration of Ad26COVS1 to subjects will exclude the release of Ad26COVS1 into the environment. Personal protective equipment will be used to avoid exposure to Ad26COVS1 to the medical personnel involved in the administration of the product. Therefore, the likelihood that Ad26COVS1 will be released to the proximity of significant biotopes, protected areas or drinking water supplies as possible potential sites, which could be affected, is negligible.

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

Not relevant

6.4 Method and amount of release

Quantities of GMOs to be released

In the notified country, Ad26COVS1 will be administered to maximally 1000 subjects who will receive maximum 3 doses of 10^{11} vp. In total, it is estimated that maximally 3x 1000 (3000) vials of Ad26COVS1 will be administered during this trial.

Duration of the operation:

Immunization of the subjects via IM will take a few minutes. The entire study duration will depend on the outcome of the immunizations.

Methods and procedures to avoid and/or minimize the spread of the GMOs beyond the site of the release

At the clinical sites, considerable care will be taken to ensure that Ad26COVS1 is contained and that personnel and the areas are not exposed. All wastes resulting from handling of Ad26COVS1 are to be treated according to regular site procedure for infectious wastes. In case of accidental spillage, specific recommendations for decontamination and destruction are to be followed to avoid any risk of dispersion to the environment.

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

Not applicable: given that Ad26COVS1 is prepared for administration and given to subjects in a clinical environment, it is not anticipated that Ad26COVS1 will be released into the environment.

6.6 Relevant data regarding previous releases carried out with the same GMO, if any, specially related to the potential environmental and human health impacts from the release.

As described above, the Ad26COVS1 vector is not expected to replicate and/or persist in the tissues following IM injection. The chance that the Ad26COVS1 clinical vector is shed to the environment in relevant quantities upon administration, beyond its initial potential presence at the injection site (injection site leakage) is negligible.

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

7.1 Name of target organism (if applicable)

- order and/or higher taxon (for animals) …
- family name for plants …
- genus homo
- species sapiens
- subspecies …
- strain …
- cultivar/breeding line …
- pathovar …
- common name human

7.2 Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)

Ad26COVS1 will induce an immune response (humoral and cellular responses) in the vaccinated individuals but will not modify the characteristics of the human recipients. The induced immune response will eliminate the transduced cells and thereby also the presence of Ad26COVS1 in the human being.

7.3 Any other potentially significant interactions with other organisms in the environment

Ad26COVS1 will be administered in a clinical site setting and is replication incompetent, therefore it is highly unlikely that the GMO will come in contact with other organisms or the environment. As Ad26COVS1 cannot replicate there is no basis that the inserted genetic trait (SARS-CoV-2 S) could be transferred to the environment at large.

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

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

Give details:

Upon administration, Ad26COVS1 will induce an immune response (humoral and cellular responses) in the vaccinated individuals but the induced immune response will eliminate the transduced cells and thereby also the presence of Ad26COVS1 in the human being. As such, there is no induction of increased competitiveness, or increased invasiveness.

A theoretically event is that Ad26COVS1 transduces a cell which has been infected with a wildtype adenovirus, to form a new GMO that is recombination competent, which might spread further in the environment. Recombination events are conceivable but not likely. The resulting recombinants would however not pose a higher risk level than a wildtype infection that is already present and the recombinants would not be able to disseminate in the human body or to the environment.

Another theoretical event is the release into the environment by trans-complementation of E1 deleted functions by alternative viral proteins from other viruses. Trans-complementation events are conceivable but not likely. Produced Ad26COVS1 could potentially transduce neighbouring cells. No further dissemination will take place, as Ad26COVS1 produced particles remain replication incompetent in all other cells. This further supports that the risk for release into the environment by trans-complementation is negligible.

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

Ad26COVS1 will be administered to subjects in a separate examination room. Considerable care will be taken to ensure that Ad26COVS1 is contained and that personnel and the areas are not exposed to it. In the very unlikely event that Ad26 vectors are released into the environment, they are not able to generate infectious progeny (see above).

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

- order and/or higher taxon (for animals) …
- family name for plants …
- genus …
- species …
- subspecies …
- strain …
- cultivar/breeding line …
- pathovar …
- common name …

Not applicable.

7.7 Likelihood of genetic exchange in vivo

from the GMO to other organisms in the release ecosystem

Highly unlikely, see section 7.3

from other organisms to the GMO

Highly unlikely, see section 7.3

likely consequences of gene transfer

Highly unlikely, adenoviruses are considered non-integrating viruses because of the inability of the virus to integrate into the host chromosomes.

7.8 Give references to relevant results (if available) from studies of the behaviour and characteristics of the GMO and its ecological impact carried out in stimulated natural environments (e.g. microcosms, etc.)

Not available

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

Not applicable

8 Information relating to monitoring

8.1 Methods for monitoring the GMOs

The intended function of Ad26COVS1 is to induce a SARS-CoV-2 specific immune responses, which will be measured by assessment of the humoral and cellular responses against SARS-CoV-2. In addition, subjects participating in the clinical trial using Ad26COVS1 will be monitored for clinical assessment (e.g. physical examinations), and adverse event monitoring.

8.2 Methods for monitoring ecosystem effects

Ecosystems effects will not be monitored as Ad26COVS1 is not naturally present in any ecosystem.

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

It is highly unlikely that transfer of donor genetic material from Ad26COVS1 will be transferred to other organisms (see above).

8.4 Size of the monitoring area (m2)

Not applicable

8.5 Duration of the monitoring

Not applicable

8.6 Frequency of the monitoring

Not applicable

9 Information on post-release and waste treatment

9.1 Post-release treatment of the site

The rooms in the medical facility used to prepare and administer the vaccine will be cleaned before and after manipulation with a standard disinfectant active against adenoviruses. Surfaces shall be decontaminated and cleaned after use with standard disinfectant active against adenoviruses.

The injection site will be covered with a plaster of bandage.

9.2 Post-release treatment of the GMOs

Materials in contact with Ad26COVS1 should be considered as contaminated and all wastes (including vaccine vials, needles and syringes) should be placed in bins suitable for biohazardous waste directly following administration of the IMP.

Used study materials will be destroyed by the clinical site following institutional procedures for the disposal of biohazardous material.

9.3 Specifications of waste treatment

Type and amount of waste generated

Based on the assumption that maximally 1000 subjects will receive Ad26COVS1 in the notified country, a maximum total of 3x 1000 (3000) vials are planned to be used. The Ad26COVS1 is supplied in a glass vial, stoppered, and capped with seals. As such, the amount of biohazardous waste is estimated to be 3000 glass vials, stoppers, caps and anticipated needles syringes, paper towels, gloves and packaging.

Treatment of waste

All disposable waste that has been in contact with the GMO during preparation and administration will be disposed of as hazardous medical waste (UN3291) in line with national legislation.

10 Information on emergency response plans

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

Ad26COVS1 is an IMP intended for use in a controlled clinical trial to be conducted at qualified medical facilities and under controlled conditions and handling procedures.

In case of accidental spillage, specific recommendation for decontamination and destruction are to be followed to avoid any risk of dispersion to the environment, capturing all remaining liquid into spill absorbent material and place them in leak-proof container suitable for disposal in accordance with applicable waste disposal regulations.

In case of skin contact. Disinfect skin and wash off with plenty of water and soap. Consult a physician.

In case of eye contact. Flush eyes immediately 10-15 minutes under running water. Remove contact lenses. Consult a physician.

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

See section 10.1.

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

Not applicable

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

Subjects included in the clinical trial will be monitored for the occurrence of adverse events (AE) and serious adverse events (SAE) according to the clinical protocol. Each SAE will be recorded and assessed by the site staff and the study sponsor, and Health Authorities will be notified when applicable. Adverse events will be registered and reported according to detailed procedures in the clinical study protocol. In case of an undesirable effect, this medicine will be put on hold until the effects are fully assessed, and measures are taken to mitigate further risks. All areas and facilities that had been used to administer the product would be cleaned and decontaminated using agents effective against adenoviruses.

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