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

GMO N.1 - GAd20-209-FSP

A. General information (GAd20-209-FSP)

1. Details of notification

(a) Member State of notification Belgium
(b) Notification number
(c) Date of acknowledgement of notification
(d) Title of the project A Phase I/II, Multicenter, Open-Label Study of Nous-209 Genetic Vaccine for the Treatment of Microsatellite Unstable Solid Tumors

(e) Proposed period of release From October 2022-to December 2024

2. Notifier

Name of institution or company: **Nouscom Srl, Via di Castel Romano, 100, 00128 Roma RM, Italy**

3. GMO characterization (**GAd20-209-FSP**)

(a) Indicate whether the GMO is a:

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

specify phylum, class: **Phylum Preplasmaviricota; Class Tectiliviricetes, Order Rowavirales; Family Adenoviridae; Genus Adenovirus, Species GAd20 Gorilla adenovirus (similar to human subgroup C adenoviruses).**

(b) Identity of the GMO (genus and species): **Genus Adenovirus, Species GAd20 Gorilla adenovirus** (**genetically modified to encode human tumor neoantigens and to impair replication)**

(c) Genetic stability – according to Annex IIIa, II, A(10)
The genetic stability of the GMO, according to Annex IIIa, A(10) is verified by NGS and restriction analysis.

4. Is the same **GAd20-209-FSP** 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) **ES, IT, BE, GB**

5. Has the same GMO been notified for release elsewhere in the Community by the same notifier?
Yes (.) No (**X)**
If yes:
- Member State of notification …
- Notification number B/../../…

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

6. Has the same GMO been notified for release or placing on the market outside the Community by the same or other notifier?
Yes (.) No (**X**)
If yes:
- Member State of notification …
- Notification number B/../../…

7. Summary of the potential environmental impact of the release of the GMOs.
No specific environmental impact is expected from the GMO since GAd20-209-FSP GMO is unable to replicate due to introduced mutations (deletions of viral E1, E3 and E4 coding regions) and consequently is unable to spread.
Residual experimental material will be destroyed according to national and local biohazard procedures.

B. Information relating to the recipient or parental organism from which the GAd20-209-FSP GMO is derived

1. Recipient or parental organism characterisation:

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

(select one only)

viroid (.)
RNA virus (.)

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

other, specify …

2. Name
(i) order and/or higher taxon (for animals)
(ii) genus **Adenovirus**
(iii) species **Gorilla adenovirus** (natural host: Gorilla gorilla gorilla, Western lowland gorilla)
(iv) subspecies **N/A**
(v) strain **GAd20** (similar to human subgroup C adenoviruses)
(vi) pathovar (biotype, ecotype, race, etc.) **N/A**
(vii) common name **GAd20**

3. Geographical distribution of the organism

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

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

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

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

(ii) No (**X**)
(iii) Not known (.)

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

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

4. Natural habitat of the organism

(a) If the organism is a microorganism

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

(b) If the organism is an animal: natural habitat or usual agroecosystem:
Gorilla, as the natural host, in Africa

5. (a) Detection techniques
Restriction analysis, PCR (Polymerase Chain Reaction), NGS (Next Generation Sequencing)

(b) Identification techniques
PCR (Polymerase Chain Reaction)/ NGS (Next Generation Sequencing)

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

Yes (.) No (**X**)

If yes, specify
…

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

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

If yes:

(a) to which of the following organisms:

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

(b) give the relevant information specified under Annex III A, point II. (A)(11)(d) of Directive 2001/18/EC
Pathogenicity in the natural host: **no known pathology in the Gorilla.**

8. Information concerning reproduction

(a) Generation time in natural ecosystems: **Adenoviral DNA replication and assembly of progeny virions generally occurs in the host cell nucleus and takes 24–36 h.**

(b) Generation time in the ecosystem where the release will take place: **N/A; the organism will be released only as a GMO and it will be unable to replicate due to modifications of its genome.**

(c) Way of reproduction: Sexual .. Asexual **X**

(c) Factors affecting reproduction:
N/A

9. Survivability

(a) ability to form structures enhancing survival or dormancy: **Not Applicable**

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

(b) relevant factors affecting survivability: **The organism will be release only as a GMO. However, relevant factor affecting survivability are the same for both parental organism and the derived GMO: CO_2 gas and storage above -60°C outside natural host.**

10. (a)Ways of dissemination: **through the natural host (Gorilla). However, the organism will be released only as a GMO and it will be unable to replicate due to modifications of its genome.**

(b) Factors affecting dissemination: **availability of the natural host (Gorilla).**

11. Previous genetic modifications of the recipient or parental organism already notified for release in the country where the notification is made (give notification numbers):
Not applicable
…, B/../../…

C. Information relating to the genetic modification (GAd20-209-FSP)

1. Type of the genetic modification

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

2. Intended outcome of the genetic modification

Expression of inserted material (FSPs neoantigens) able to elicit human immune response after intramuscular administration/inability of the GMO to replicate, infect and spread

3. (a) Has a vector been used in the process of modification?
Yes **(X)** No (.)

If no, go straight to question 5.

(b) If yes, is the vector wholly or partially present in the modified organism?
Yes (.) No (X)

If no, go straight to question 5.

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

(a) Type of vector

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

(b) Identity of the vector

(c) Host range of the vector

(d) Presence in the vector of sequences giving a selectable or identifiable phenotype
Yes (.) No (.)

antibiotic resistance	(.)
other, specify	…

Indication of which antibiotic resistance gene is inserted

(e) Constituent fragments of the vector

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

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

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

(i) transformation (.)
(ii) microinjection (.)
(iii) microencapsulation (.)
(iv) macroinjection (.)
(v) other, specify **Homologous recombination (recombineering) in SW102 E. Coli cells containing GAd20 genome cloned into a BAC (Bacterial Artificial Chromosome) construct**

6. Composition of the insert

(a) Composition of the insert
GAd20-209-FSP is composed of 4 replication-incompetent non-human Great Ape Adenoviruses (gorilla-derived GAd20). Each of the four GAd20 vectors present in the vaccine encode a synthetic transgene, named FSP-A1, FSP-A2, FSP-A3 and FSP-A4 respectively. Each transgene encodes a string of approximately 50 selected FSPs (Frame-Shifted Peptides) neoantigens selected among the most common found in colorectal, gastric and endometrial MSI (Microsatellite Instable) cancer patients.

(b) Source of each constituent part of the insert
GAd20-209-FSP encodes for a set of 209 FSPs identified following an analysis of dMMR/MSI-H CRC, gastric, G-E junction and endometrial tumor sequences in the TCGA database (https://gdc-portal.nci.nih.gov/).

(c) Intended function of each constituent part of the insert in the GMO
FSP neoantigens are non-self-proteins generated by tumor specific frameshift mutations. These mutations are not present in the healthy human protein repertoire and are consequently expected to be potent and safe immunogens, since they are unlikely to induce cross-reactive responses against self-proteins. The incorporation of multiple shared FSPs into the vaccine is desirable to maximize the probability of inducing effective immune responses in a large and heterogeneous cohort of patients.

(d) Location of the insert in the host organism

- on a free plasmid (.)
- integrated in the chromosome (.)
- other, specify: **The insertion fragments are cloned in a vector derived from GAd20 in which the E1, E3 and E4 sequences involved in replication have been eliminated. The transgene expression cassette is inserted in place of E1 deletion. A fragment corresponding to Orf6 of E4 from human adenovirus 5 is inserted in place of E4 deletion.**

(e) Does the insert contain parts whose product or function are not known?
Yes (**X**) No (.)
If yes, specify **The small neoepitopes do not have a function per se when extrapolated from the context of the original protein of which they are part, besides that of eliciting immune responses.**

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

1. Indicate whether it is a:

viroid	(.)	
RNA virus	(.)	
DNA virus	(.)	
bacterium	(.)	
fungus	(.)	
animal		
- mammals	(**X**)	
- insect	(.)	
- fish	(.)	
- other animal	(.)	
(specify phylum, class)		**Phylum Chordata; Class Mammalia**
other, specify	…	

2. Complete name

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

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

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

If yes, specify the following:

(a) to which of the following organisms:

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

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

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

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

…

4. Is the donor organism classified under existing Community rules relating to the protection of human health and the environment, such as Directive 90/679/EEC on the protection of workers from risks to exposure to biological agents at work?

Yes (.) No (**X**)
If yes, specify …

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

E. Information relating to the genetically modified organism (GAd20-209-FSP)

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

(a) is the GMO different from the recipient as far as survivability is concerned?
Yes (**X**) No (.) Not known (.)
Specify **The GAd20-209-FSP GMO is unable to replicate outside permissive cells in culture, since E1, E3 and E4 regions needed for replication have been deleted.**

(b) is the GMO in any way different from the recipient as far as mode and/or rate of reproduction is concerned?
Yes (**X**) No (.) Unknown (.)
Specify **The GAd20-209-FSP GMO is unable to replicate due to the referred E1, E3 and E4 deletions.**

(c) is the GMO in any way different from the recipient as far as dissemination is concerned?
Yes (**X**) No (.) Not known (.)
Specify **The GAd20-209-FSP GMO is unable to replicate due to introduced mutations (deletions of viral E1, E3 and E4 coding regions) and consequently is unable to spread.**

(d) is the GMO in any way different from the recipient as far as pathogenicity is concerned?
Yes (.) No (**X**) Not known (.)
Specify **The GAd20-209-FSPP GMO is unable to replicate due to introduced deletions, hence it doesn't show pathogenicity.**

2. Genetic stability of the genetically modified organism
GMO stability is verified by NGS and restriction analysis.

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

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

plants (.)
other …

(b) give the relevant information specified under Annex III A, point II(A)(11)(d) and II(C)(2)(i)
The GMO GAd20-209-FSP is unable to replicate in any natural host and to spread in the environment, hence none of the risks listed in Annex III A, point II(A)(11)(d) and II(C)(2)(i) are applicable to this GMO.

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment
N/A

(b) Techniques used to identify the GMO
PCR (Polymerase Chain Reaction)/ NGS (Next Generation Sequencing)

F. Information relating to the release (GAd20-209-FSP)

1. Purpose of the release (including any significant potential environmental benefits that may be expected)
Use in clinical trials as Investigator Medicinal Product (cancer immunotherapy). The use will imply no deliberate release in the environment of residual GMO IMP and of contaminated waste that has not been activated/destroyed. However, a deliberate release cannot be excluded referring to potential shedding of the GMO through body fluids of the treated patients inside and outside of the clinical centres. For this reason, inspite of the fact that the trial participants are advised to avoid contacts with the GAd20 natural host in order to eliminate the risk of recombination between the GAd20 wild type and the GMO.

2. Is the site of the release different from the natural habitat or from the ecosystem in which the recipient or parental organism is regularly used, kept or found?
Yes (**X**) No (.)
If yes, specify **The parental organism (GAd20) is a natural virus infecting gorillas in their natural habitat (Africa). The specific isolate used as a recipient to produce the GMO derives from the stools of a captive Gorilla. The GMO is being used in clinical trials (in humans) in US and will be used in clinical trials (in humans) in Europe. No release will occur in the environment of the product that has not been inactivated/destroyed.**

3. Information concerning the release and the surrounding area
The administration of GAd20-209-FSP will be performed in 1 site in Belgium:

Cliniques Universitaires Saint-Luc - Centre du Cancer
Avenue Hippocrate 10
Brussels
1200

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

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

NA

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

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

4. Method and amount of release

(a) Quantities of GMOs to be released:
GAd20-209-FSP will be administered to each individual patient at the dose of 1.88 x10^{11} vp.

(b) Duration of the operation:
Vaccination will take a few seconds; following vaccination, patient must remain in the unit for 1 hour for observation.

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

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

Administration is conducted by suitably qualified health professionals, fully trained to the study-specific protocol and pharmacy manual.

Biosafety precautions (BSL-1): all personnel handling GAd20-FSP-209 should wear protective gowns, gloves and eye protection. Universal blood precautions should be observed. Contaminated sharps and no-sharps waste should be disposed of as per local biohazard destruction procedures. Procedures according to study Pharmacy manual. In summary, drain the area with paper tissues, treat the surface with 1:10 dilution of household bleach (the solution must not be older than one day- for at least 10 minutes, rinse and dry. Alternatively, wipe the area with ethanol 70% or Virkon S. Dispose residual contaminated material as per local biohazard destruction procedures.

To minimise dissemination of the recombinant vectored vaccine virus into the environment, the inoculation site must be fully covered with an appropriate dressing (band-aid type) immediately following immunisation. Inoculation site should remain covered for at least 30 minutes. The dressing will be removed from the injection site and disposed as GMO waster, as per local biohazard destruction procedures. It is recommended to rinse the are of the inoculation site, applying 70% ethanol. Not mandatory: a second bandage may also be

applied to better protect the injection site, removed when the patients at home and disposed of as ordinary waste.

Any residual amount of IMP has also to be destroyed on site after the administration, in agreement with local biohazard destruction procedures.

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

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.
N/A

G. Interactions of the GMO with the environment and potential impact on the environment, if significantly different from the recipient or parent organism (GAd20-209-FSP).

N/A.

1. Name of target organism (if applicable)

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

N/A

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)
N/A

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

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?
Yes (.) No (.) Not known (.)
Give details
N/A

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

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
N/A

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

7. Likelihood of genetic exchange in vivo
N/A

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

(b) from other organisms to the GMO:
…

(c) likely consequences of gene transfer:
…

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.):
N/A

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

H. Information relating to monitoring (GAd20-209-FSP):

1. Methods for monitoring the GMOs
According to the current state of knowledge, environmental monitoring is not expected to afford any meaningful results for the following reasons:
- **GMO is replication-incompetent, which is verified and confirmed again at the manufacturing process.**
- **In preclinical distribution studies in mammals only small quantities of the virus could be detected by PCR at the injection site, lymph nodes and spleen for a limited time, whereas PCR tests for distant organs, body fluids and time points > 30 d remained negative.**
- **Routine precautions for potentially infectious material are taken for the injection site and patient samples.**

In summary, the chances to detect even traces of GMOs in the environment are considered extremely low, let alone to establish a rudimentary distribution pattern.

2. Methods for monitoring ecosystem effects
Not applicable

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms
Not Applicable

4. Size of the monitoring area (m^2)
… m^2
Not applicable

5. Duration of the monitoring
GAd20-209-FSP GMO administration will take a few seconds; following vaccination, patients must remain in the unit for 1 hour for observation. GAd20-209-FSP GMO is administered 1 time (visit 1-week 1). Patients will be then follow up for safety and tolerability.

6. Frequency of the monitoring
After the administration of the GMO GAd20-209-FSP (visit 1, week 1), each patient will undergo an additional 29 visits in 83 weeks in Cohort C and week 59 for Cohort D.

I. Information on post-release and waste treatment (GAd20-209-FSP)

1. Post-release treatment of the site
After intramuscular injection of the GAd20-209-FSP GMO into the deltoid muscle, the injection point will be covered for 30 min with a bandage as indicated in the Pharmacy manual. The bandage will then be disposed of according to clinical site procedures for biohazard waste.

2. Post-release treatment of the GMOs
The GAd20-209-FSP GMO will be destroyed and disposed according to clinical site procedures for biohazard substances.

3. (a) Type and amount of waste generated
Up to 2 band-aid per patient / up to 2 disposable syringe/patient / 1.2 ml product per unused vial / lower volume per vial as residual volume after injection

3. (b) Treatment of waste
The waste will be destroyed and disposed of according to local biohazard destruction procedures.

J. Information on emergency response plans (GAd20-209-FSP)

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread
The laboratories and clinical sites where the GMO will be used will have SOPs where the instructions about the actions to be taken in case of spillage are described in detail and the related tools will be available. In synopsis, the area where the spillage occurred has to be washed with a 1:10 dilution of household bleach (the solution must not be

older than one day), starting at the perimeter and working toward the centre; allow sufficient contact time (10 min), rinse and dry. Alternatively, wipe the area with ethanol 70% or Virkon S. The materials used to clean the area have to be disposed of according to the local procedures for GMO waste disposal. Information is also available in the study Pharmacy manual.

2. Methods for removal of the GMO(s) of the areas potentially affected
The area where the spillage occurred has to be drained with absorbent paper and then sanitized by freshly prepared disinfectant (usually 1:10 dilution of bleach or equivalents) or alternatively with 70% Ethyl alcohol or Virkon (or equivalent). The materials used to clean the area have to be disposed of according to the local procedures for GMO waste disposal.

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

4. Plans for protecting human health and the environment in the event of an undesirable effect
Use of personal protective equipment, such as: eye protection, gloves and a lab coat for exposed personnel. Availability of spill kits and eye washers for accidental exposure.

GMO N.2 - MVA-209-FSP

A'. General information (MVA-209-FSP)

1. Details of notification

(a)	Member State of notification	Belgium
(b)	Notification number	
(c)	Date of acknowledgement of notification	
(d)	Title of the project	A Phase I/II, Multicenter, Open-Label Study of Nous-209 Genetic Vaccine for the Treatment of Microsatellite Unstable Solid Tumors
(e)	Proposed period of release	From October 2022-to December 2024

2. Notifier
Name of institution or company: **Nouscom Srl, Via di Castel Romano, 100, 00128 Roma RM, Italy**

3. GMO characterisation (**MVA-209-FSP**)

(a) Indicate whether the GMO is a:

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

specify phylum, class: **Phylum Nucleocytoviricota; Class Pokkesviricetes; Order Chitovirales; Family Poxviridae; Genus Orthopoxvirus; Species Vaccinia virus**

(b) Identity of the GMO (genus and species): **Orthopoxvirus, Vaccinia Virus (strain MVA genetically modified to encode human tumor neoantigens and to impair replication)**

(c) Genetic stability – according to Annex IIIa, II, A(10)
The genetic stability of the GMO, according to Annex IIIa, II A(10) is verified by NGS and restriction analysis.

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

   Yes (**X**) No (.)

   If yes, insert the country code(s) **ES, IT, BE**, **GB**

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

   Yes (.) No (**X**)

   If yes:
   - Member State of notification …
   - Notification number B/../../…

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

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

   Yes (.) No (**X**)

   If yes:
   - Member State of notification …
   - Notification number B/../../…

7. Summary of the potential environmental impact of the release of the GMOs.
   No specific environmental impact is expected from the GMO since it is unable to replicate and to spread after experimental injection in humans. Residual experimental material will be destroyed according to national and local procedures.

B'. Information relating to the recipient or parental organism from which the GMO is derived (MVA-209-FSP)

1. Recipient or parental organism characterisation:

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

   (select one only)

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

     (specify phylum, class) …

   other, specify …

2. Name

(i) order and/or higher taxon (for animals)
(ii) genus **Orthopoxvirus**
(iii) species **Vaccinia virus**
(iv) subspecies **N/A**
(v) strain **Vaccinia virus Ankara***
(vi) pathovar (biotype, ecotype, race, etc.) **N/A**
(vii) common name **MVA**

***The Modified Vaccinia Ankara (MVA) a derivative of the Chorioallantois vaccinia virus Ankara(CVA) strain of Vaccinia Virus. It is a highly attenuated strain derived by more than 570 passages in chicken embryo fibroblasts (CEF), that was developed towards the end of the campaign for the eradication of smallpox by Anton Mayr in Germany and used in that massive vaccination campaign. The attenuation resulted in the loss of 30 kb from the original genome including sequences determining host range. MVA is capable of replication in the cytoplasm of avian cultured cells but is replication-deficient in mammalian cells and mammalian hosts.**
Due to its high-level safety profile, MVA is widely used as a vector for vaccination against non-poxvirus diseases.

The parental MVA backbone isolate used for the generation of the GMO MVA-209-FSP is the MVA pre-vaccine 476 MG/14/78 that was manufactured by former Bayrische Landesimpfanstalt in 1978 by using the then approved virus seed batch MVA 460 MG (passage 271). Previous MVA-based vaccines against infectious diseases were based on the same isolate.

3. Geographical distribution of the organism (**MVA**)

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

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

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

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

(ii) No (**X**) **The recipient is a laboratory strain, not a natural virus**
(iii) Not known (.)

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

Yes (**X**) No (**.**)
The recipient is frequently used as a vector in clinical investigations because of its high safety profile.

(d) Is it frequently kept in the country where the notification is made?
Yes **(X)** No ( )
The recipient is kept as a vector in clinical trials

4. Natural habitat of the organism

(a) If the organism is a microorganism
water (.)
soil, free-living (.)
soil in association with plant-root systems (.)
in association with plant leaf/stem systems (.)
other, specify **avian cell cultures**

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

5. (a) Detection techniques
PCR (Polymerase Chain Reaction), NGS (Next Generation Sequencing), restriction analyses

(b) Identification techniques
PCR (Polymerase Chain Reaction)/ NGS (Next Generation Sequencing)

6. Is the recipient organism classified under existing Community rules relating to the protection of human health and/or the environment?
Yes (**X**) No ()
If yes, specify
The human vaccinia virus is classified as a group 2 biological agent according to the standard community 2000/54 / EC. Although the recombinant MVA strain (recipient organism) is not classified, it is considered to belong to group 1 since it is a highly attenuated replication-defective vaccinia virus in human cells, showing a range limited number of hosts to infect. MVA is derived from the Chorioallantois Vaccine Ankara (CVA) strain. The attenuated strain was renamed MVA after the 516th passage of CVA strain on primary chicken embryo fibroblasts (CEF). Consequent genetic mutations occurring in MVA and described in several studies make the virus defective for replication in human cells and unable to cause infection in mammals (*Verheust, C., et al., Biosafety aspects of modified vaccinia virus Ankara (MVA)-based vectors used for gene therapy or vaccination. Vaccine, 2012. 30(16): p. 2623-32*.)

7. Is the recipient organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?
Yes () No (**X**) Not known (.)

If yes:
(a) to which of the following organisms:

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

(b) give the relevant information specified under Annex III A, point II. (A)(11)(d) of Directive 2001/18/EC
Pathogenicity in the natural host: **Not Applicable. MVA is not pathogenic**

8. Information concerning reproduction

(a) Generation time in natural ecosystems:
MVA does not have a known natural host. Virus replication is restricted to a few permissive host cell systems not generally found in natural ecosystems, such as: BHK-21 (baby hamster kidney cell line), CEF (fibroblasts chick embryo primaries), DF-1 (chick embryo fibroblast cell line).

(b) Generation time in the ecosystem where the release will take place: **N/A; the organism will be released only as a GMO.**

(c) Way of reproduction: Sexual .. Asexual **X**

(d) Factors affecting reproduction:
MVA is strictly restricted to host cells: it grows well in avian cells, but cannot multiply in human cells and in most other mammals tested due to six major deletions in its genome.

9. Survivability

(a) ability to form structures enhancing survival or dormancy: not applicable

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

relevant factors affecting survivability: **Survival of MVA is not expected as it is found exclusively in the cytoplasm of the cell and is unable to produce vector particles in human cells outside the site of inoculation.**

10. (a) Ways of dissemination:
MVA is unable to replicate in humans.

(b) Factors affecting dissemination:
Not applicable

11. Previous genetic modifications of the recipient or parental organism already notified for release in the country where the notification is made (give notification numbers):
Not applicable
…, B/../../…

C'. Information relating to the genetic modification (MVA-209-FSP)

1. Type of the genetic modification

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

2. Intended outcome of the genetic modification
Expression of inserted material (FSPs neoantigens) able to elicit human immune response after intramuscular administration/inability of the GMO to replicate, infect and spread.

3. (a) Has a vector been used in the process of modification?
Yes **(X)** No **(.)**

If no, go straight to question 5.

(b) If yes, is the vector wholly or partially present in the modified organism?
Yes (.) No (**X**)

If no, go straight to question 5.

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

(a) Type of vector

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

(b) Identity of the vector

(c) Host range of the vector

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

Yes (.) No (.)

antibiotic resistance (.)
other, specify …

Indication of which antibiotic resistance gene is inserted

(e) Constituent fragments of the vector

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

A. transformation (.)
B. electroporation (.)
C. macroinjection (.)
D. microinjection (.)
E. infection (.)
F. other, specify …

5. If the answer to question B.3(a) and (b) is no, what was the method used in the process of modification?
(i) transformation (.)
(ii) microinjection (.)
(iii) microencapsulation (.)
(iv) macroinjection (.)
(v) other, specify **insertion of foreign sequences (FSPs neoantigens) by homologous recombination in infected CEF cells**

6. Composition of the insert

(a) Composition of the insert
MVA-209-FSP is composed of 4 attenuated, replication-defective orthopoxvirus MVA. Each of the four MVA vectors present in the vaccine encode a synthetic transgene, named FSP-A1, FSP-A2, FSP-A3 and FSP-A4 respectively. Each transgene encodes a string of approximately 50 selected FSPs (Frame-Shifted Peptides) neoantigens selected among the most common found in colorectal, gastric and endometrial MSI (Microsatellite Instable) cancer patients.

(b) Source of each constituent part of the insert
MVA-209-FSP encodes for a set of 209 FSPs identified following an analysis of dMMR/MSI-H CRC, gastric, G-E junction and endometrial tumor sequences in the TCGA database (https://gdc-portal.nci.nih.gov/).

(c) Intended function of each constituent part of the insert in the GMO
FSP neoantigens are non-self-proteins generated by tumor specific frameshift mutations. These mutations are not present in the healthy human protein repertoire and are consequently expected to be potent and safe immunogens, since they are unlikely to induce cross-reactive responses against self-proteins. The incorporation of multiple shared FSPs into the vaccine is desirable to maximize the probability of inducing effective immune responses in a large and heterogeneous cohort of patients.

(d) Location of the insert in the host organism
- on a free plasmid (.)
- integrated in the chromosome (.)
- other, specify: **cloned in the viral vector MVA**

(e) Does the insert contain parts whose product or function are not known?
Yes (**X**) No (.)
If yes, specify **The small neoepitopes do not have a function per se when extrapolated from the context of the original protein of which they are part, besides that of eliciting immune responses.**

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

1. Indicate whether it is a:

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

(specify phylum, class) **Phylum Chordata; Class Mammalia**

other, specify …

2. Complete name

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

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

(a) to which of the following organisms:
humans (.)
animals (.)

plants (.)
other ..

(b) are the donated sequences involved in any way to the pathogenic or harmful properties of the organism
Yes (.) No (**X**) Not known (.)

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

4. Is the donor organism classified under existing Community rules relating to the protection of human health and the environment, such as Directive 90/679/EEC on the protection of workers from risks to exposure to biological agents at work?
Yes (.) No (**X**)
If yes, specify …

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

E'. Information relating to the genetically modified organism

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

(a) is the GMO different from the recipient as far as survivability is concerned?
Yes (**.**) No (**X**) Not known (.)
Specify

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

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

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

2. Genetic stability of the genetically modified organism
GMO stability is verified by NGS and restriction analysis

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

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

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

(b) give the relevant information specified under Annex III A, point II(A)(11)(d) and II(C)(2)(i)
The GMO MVA-209-FSP is unable to replicate in humans but only in avian cells, hence only negligible risks listed in Annex III A, point II(A)(11)(d) and II(C)(2)(i) are applicable to this GMO.

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment
N/A

(b) Techniques used to identify the GMO
PCR (Polymerase Chain Reaction) / NGS (Next Generation Sequencing)

F'. Information relating to the release (MVA-209-FSP)

1. Purpose of the release (including any significant potential environmental benefits that may be expected)
Use in clinical trials as Investigational Medicinal Product (cancer immunotherapy). The use will imply no deliberate release in the environment of the product that has not been inactivated/destroyed. However, a deliberate release cannot be excluded referring to potential shedding of the GMO through body fluids of the treated patients inside and outside the clinical centres

2. Is the site of the release different from the natural habitat or from the ecosystem in which the recipient or parental organism is regularly used, kept or found?
Yes (**X**) No (.)
If yes, specify
The parental organism (MVA) is a laboratory strain of Vaccinia virus. The GMO is being used in clinical trials (in humans) in US and will be used in clinical trials (in humans) in Europe.

3. Information concerning the release and the surrounding area

(a) Geographical location (administrative region and where appropriate grid reference):
The administration of MVA-209-FSP will be performed in site in Belgium:
Cliniques Universitaires Saint Luc
Medical Oncology Unit
Avenue Hippocrate, 10
1200 Brussels, Belgium

-

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

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

(c) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO: **NA**

…

4. Method and amount of release

(a) Quantities of GMOs to be released:
MVA-209-FSP will be administered to each individual patient at the dose of 1.65 x10^8 ifu.

(b) Duration of the operation:
Vaccination will take a few seconds: following vaccination, patients must remain in the unit for 1 hour for observation

(c) Methods and procedures to avoid and/or minimise the spread of the GMOs beyond the site of the release
The GMO is only used within the treatment centres. As no shedding is expected, release beyond the trial centres is deemed highly unlikely.
Administration is conducted by suitably qualified health professionals, fully trained to the study-specific protocol and pharmacy manual.
Biosafety precautions (BSL-1): all personnel handling MVA-209 should wear protective gowns, gloves and eye protection. Universal blood precautions should be observed. Contaminated sharps and no-sharps waste should be disposed of as per local biohazard destruction procedures. Procedures according to study Pharmacy manual. In summary, drain the area with paper tissues, treat the surface with 1:10 dilution of household bleach (the solution must not be older than one day- for at least 10 minutes, rinse and dry. Alternatively, wipe the area with ethanol 70% or Virkon S. Dispose residual contaminated material as per local biohazard destruction procedures.

To minimise dissemination of the recombinant vectored vaccine virus into the environment, the inoculation site must be fully covered with an appropriate dressing (band-aid type) immediately following immunisation. Inoculation site should remain covered for at least 30 minutes. The dressing will be removed from the injection site and disposed as GMO waste, as per local biohazard destruction procedures. It is recommended to rinse the are of the inoculation site, applying 70% ethanol. Not mandatory: a second bandage may also be applied to better protect the injection site, removed when the patients at home and disposed of as ordinary waste.

Any residual amount of IMP has also to be destroyed on site after the administration, in agreement with local biohazard destruction procedures.

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

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.
N/A

G'. Interactions of the GMO with the environment and potential impact on the environment, if significantly different from the recipient or parent organism
Not applicable

1. Name of target organism (if applicable)

(j)	order and/or higher taxon (for animals)	**Primate, Hominidae**
(ii)	family name for plants	…
(iii)	genus	**Homo**
(iv)	species	**Sapiens**
(v)	subspecies	**N/A**
(vi)	strain	**N/A**
(vii)	cultivar/breeding line	**N/A**
(viii)	pathovar	**N/A**
(ix)	common name	**human**

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)
N/A

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

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?
Yes (.) No (.) Not known (.)
Give details
N/A

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

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
N/A

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

7. Likelihood of genetic exchange in vivo
N/A

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

(b) from other organisms to the GMO:
…

(c) likely consequences of gene transfer:
…

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.):
N/A

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

H'. Information relating to monitoring (MVA-209-FSP):

1. Methods for monitoring the GMOs
According to the current state of knowledge, environmental monitoring is not expected to afford any meaningful results for the following reasons:
- GMO is replication incompetent, which is verified and confirmed again at the manufacturing process.
- In preclinical distribution studies in mammals only small quantities of the virus could be detected by PCR at the injection site, lymph nodes and spleen for a limited time, whereas PCR tests have distant organs, body fluids and time points > 8 days remained negative.
-Routine precautions for potentially infectious material are taken for the injection site and patient samples.

In summary, the chances to detect even traces of the GMOs in the environment are considered extremely low, let alone to establish a rudimentary distribution

2. Methods for monitoring ecosystem effects
Not applicable

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms
Not Applicable

4. Size of the monitoring area (m^2)
… m^2
Not applicable

5. Duration of the monitoring
MVA-209-FSP GMO administration will take a few seconds; following vaccination, patients must remain in the unit for 1 hour for observation. MVA-209-FSP GMO is administered 3 times (week 2, week 4 and week 7). Patients will be then follow up for safety and tolerability.

6. Frequency of the monitoring
After the first administration of the GMO MVA-209-FSP (week 2), each patient will undergo an additional 28 visits in 83 weeks in Cohort C and 59 weeks in Cohort D.

I'. Information on post-release and waste treatment (MVA-209-FSP)

1. Post-release treatment of the site
After intramuscular injection (three total in the study) of the MVA-209-FSP GMO into the deltoid muscle, the injection point will be covered for 30 min with a bandage as indicated in the study manual. The bandage will then be disposed of according to clinical site procedures for biohazard waste.

2. Post-release treatment of the GMOs
The MVA-209-FSP GMO will be destroyed and disposed according to clinical site procedures for biohazard material.

3. (a) Type and amount of waste generated
Up to 6 band-aid per patient / up to 6 disposable syringe/patient / 1.2 ml product per unused vial / lower volume per vial as residual volume after injection

3. (b) Treatment of waste
The waste will be destroyed and disposed of according to clinical site procedures for biohazard material.
…

J'. Information on emergency response plans

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread
In synthesis, the area where the spillage occurred has to be drained with absorbent paper and then sanitized by freshly prepared disinfectant (usually 1:10 dilution of

bleach or equivalents) or alternatively with 70% Ethyl alcohol or Virkon (or equivalent). The materials used to clean the area have to be disposed of according to the local procedures for GMO waste disposal.

2. Methods for removal of the GMO(s) of the areas potentially affected
The area where the spillage occurred has to be drained with absorbent paper and then sanitized by freshly prepared disinfectant (usually 1:10 dilution of bleach or equivalents) or alternatively with 70% Ethyl alcohol or Virkon (or equivalent). The materials used to clean the area have to be disposed of according to the local procedures for GMO waste disposal.

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

4. Plans for protecting human health and the environment in the event of an undesirable effect
Use of personal protective equipment, such as: eye protection, gloves and a lab coat for exposed personnel. Availability of spill kits and eye washers for accidental exposure.