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

A. General information

1. Details of notification

(a) Member State of notification **BE**
(b) Notification number
(c) Date of acknowledgement of notification ../../….
(d) Title of the project
An Open-Label, Multicenter, Non-Randomized, Dose-Confirmation and Cohort-Expansion Phase 1b Study to Evaluate the Safety, Tolerability, and Anti-Tumor Activity of Nous-PEV, with pembrolizumab, in Patients with Unresectable Stage III / IV Cutaneous Melanoma and with Stage IV NSCLC (PDL1≥ 50%)
(e) Proposed period of release **From Feb-2021 until Mar-2023**

2. Notifier
Name of institution or company: **Nouscom Srl, Rome, Italy**

3. GMO characterisation

(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 () No (X)

   If yes, insert the country code(s)

   GMO will be released in GB and ES under "Contained Use".

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

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

   If yes:

   - Member State of notification …
   - Notification number 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 as per local biohazard destruction procedures.

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

1. Recipient or parental organism characterisation:

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

   (select one only)

   viroid (.)
   RNA virus (.)
   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 replicate 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-PEV 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 (references are available in the Investigator Brochure which is part of the CTA package for the study NOUS-PEV-01).

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

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

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
Restriction analisis, PCR, NGS

(b) Identification techniques
PCR / NGS

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: **Not Applicable. MVA is not pathogenic**

8. Information concerning reproduction

(a) Generation time in natural ecosystems: **Not applicable. MVA is not a natural strain.**

(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**

(c) Factors affecting reproduction:
N/A

9. Survivability

(a) ability to form structures enhancing survival or dormancy:

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

(b) relevant factors affecting survivability: **storage above -60°C outside cell systems.**

10. (a) Ways of dissemination:

Not applicable. MVA is not a natural strain.

(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)
…, B/../../…

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

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 (cancer neoantigens) able to elicit human immune response after intramuscular administration

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 **insertion of foreign sequences (human cancer neoantigens) by classical cloning methods as described in the MVA-PEV CAF**

6. Composition of the insert

(a) Composition of the insert

Human neoantigens identified in NSCLC or melanoma cancers (prototype in pilot IMP lots, personalized sequences in lots manufactured for individual patients)

(b) Source of each constituent part of the insert

Human cancer patients

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

Neoantigen epitopes insert: epitopes of tumor neoantigens aimed at eliciting an immune response against the original tumor in patients (personalized vaccine)

(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

(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:

(b) 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
The GMO genetic 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-PEV 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 / NGS

F. Information relating to the release

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 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 centers.

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 will be used in clinical trials (in humans) in Europe (the application will be submitted to Spain, UK and Belgium Regulatory Authorities).**

3. Information concerning the release and the surrounding area
No release will occur in the environment of the product that has not been inactivated/destroyed.

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

The preparation and administration of MVA-PEV will be conducted in two hospitals in Belgium:

Site BE1:
Universitair Ziekenhuis Leuven – Campus Gasthuisberg
General Medical Oncology (Prof. Oliver Bechter)
Herestraat 49
3000 Leuven, Belgium

Site BE2:

Grand Hôpital de Charleroi
Service d'Oncologie et Hématologie (Dr. Javier Carrasco)
Grand Rue 3
6000 Charleroi, 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

Not applicable. For this study it is not relevant the surface of the center, rather the number of participants.
In this clinical trial, a planned number of 11 participants in Belgium will each receive 3 injections of MVA-PEV.

(c) Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs), which could be affected:
Not applicable – any effect on such areas is not considered possible due to this clinical trial.

(d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO
Not applicable – any effect on such areas is not considered possible due to this clinical trial.

4. Method and amount of release

(a) Quantities of GMOs to be released:

Study Treatment Name	**Dosage Formulation**	**Volume to administer**
MVA-PEV	Dose-Range (1×10^8 to 3×10^8 ifu)	From 0.5 up to 2 mL

For all patients, MVA-PEV 0.5 to 2mL, depending on the manufacturing process, will be administered 3 times during the boosting phase of the study. Planned number of patients to be enrolled in Belgium is 11.

(b) Duration of the operation:
Each 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 GAd-PEV should wear protective gowns, gloves, masks, and eye protection. Universal blood precautions should be observed. Contaminated sharps and non-sharp waste should be disposed of as per local biohazard destruction procedures. Spilled vector can be decontaminated as per Biosafety Protocols using a 1:10 dilution of household bleachand wiping the area with disinfectant 70% Ethanol or Virkon S.

To minimize 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 immunization. Inoculation site should remain covered for at least 30 minutes. This should absorb any virus that may leak out through the needle track. The dressing will be removed from the injection site and disposed as GMO waste, as per local biohazard destruction procedures.

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
N/A

1. Name of target organism (if applicable)
   (i) order and/or higher taxon (for animals) …
   (ii) family name for plants …
   (iii) genus …
   (iv) species …
   (v) subspecies …
   (vi) strain …
   (vii) cultivar/breeding line …
   (viii) pathovar …
   (ix) common name …
   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:

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:

- **Both GMOs are 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 the GMOs in the environment are considered extremely low, let alone to establish a rudimentary distribution pattern.

2. Methods for monitoring ecosystem effects

N/A

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

N/A

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

… m^2

N/A

5. Duration of the monitoring

N/A

6. Frequency of the monitoring

N/A

I. Information on post-release and waste treatment

1. Post-release treatment of the site

After each intramuscular injection (three total in the study) of the GMO into the deltoid muscle of the clinical trial NOUS-PEV-01 patients, 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 as per local biohazard destruction procedures.

2. Post-release treatment of the GMOs

The GMO will be destroyed and disposed as per local biohazard destruction procedures.

3. (a) Type and amount of waste generated

3 band-aid per patient / 3 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

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 70% Ethyl alcohol or Virkon S or equivalent. 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 70% Ethyl alcohol or Virkon S or equivalent. 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.

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 gloves, labcoats, white coats and goggles for exposed personnel. Availability of spill kits and eye washers for accidental exposure. Information is also available in the study Pharmacy manual.