COMMON APPLICATION FORM FOR VIRAL VECTORS CONTAINED IN INVESTIGATIONAL MEDICINAL PRODUCTS FOR HUMAN USE[1]

Note 1: The application form must be accompanied by the SNIF (summary of the dossier for the voluntary release into the environment of genetically modified organisms for purposes other than placing them on the market) in accordance with Directive 2001/18/EC.[2]

Document history	Publication date	Description of major changes
Version 1	28Jul2022	initial version
Version 2	27Sep2022	Updated based on comments Biosafety Advisory Council

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

[2] Council Decision 2002/813/EC establishing, in accordance with Directive 2001/18/EC of the European Parliament and of the Council, the summary notification information format for notifications concerning the voluntary release into the environment of genetically modified organisms for purposes other than placing on the market (OJ L 280, 18.10.2002, p. 62).

1. INTRODUCTION

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

Clinical trials with medicinal products that contain or consist of GMOs must also comply with applicable requirements under Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms5("deliberate release framework") and/or under Directive 2009/41/EC on the contained use of genetically modified micro-organisms ("contained use framework").6

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

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

The application form has been endorsed by Austria, Belgium, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, the Netherlands, Romania, Slovenia, Spain and Norway may be used for submissions to these countriesClinical trials conducted in the EU with investigational medicinal products containing or consisting of genetically modified organisms ("GMOs") must comply with the legislation governing the authorization of clinical trials.

2. Explanatory notes

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

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

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

[4]

Regulation (EU) No 536/2014 of the European Parliament and of the Council of 16 April 2014 on clinical trials on medicinal products for human use and repealing Directive 2001/20/EC, (OJ L158, 27.5.2014, p.1). Until the Regulation applies, Directive 2001/20/EC is applicable (Directive 2001/20/EC of the European Parliament and of the Council of 4 April 2001 on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use, OJ L121,1.5.2001, p.34).

[5] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC (OJ L 106, 17.4.2001, p. 1).

[6] Directive 2009/41/EC of the European Parliament and of the Council of 6 May 2009 on the contained use of genetically modified micro-organisms (OJ L 125, 21.5.2009, p. 75).

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

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

SECTION 1 – ADMINISTRATIVE INFORMATION

1.1 Identification of the applicant

Organisation Name:	ERGOMED d.o.o.
Address Details:	Sarajevo, Zmaja od Bosne 7-7a, Importanne Center, 5th floor, 71 000 Sarajevo, Bosnia and Herzegovina
Contact person:	Amila Djulovic
Telephone No:	+387 33 215 715
Email Address:	amila.djulovic@ergomedplc.com

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

Organisation Name:	Nouscom Srl
Address Details:	Via di Castel Romano, 100. 00128 Roma, Italy
Contact person:	Maria Arce-Tomas
Telephone No:	+390696036299
Email Address:	m.arce-tomas@nouscom.com / info@nouscom.com

1.3 Identification of the manufacturer of the clinical vectors

Organisation Name:	ReiThera Srl
Manufacturing location:	Castel Romano (Roma, Italy)

SECTION 2 – INFORMATION RELATING TO THE INVESTIGATIONAL MEDICINAL PRODUCT MVA-209-FSP

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

A.1 Characterization

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

Scientific name: Modified Vaccinia Ankara (MVA)

Strain and isolate: MVA

Other names (e.g. commercial name): Not applicable

Biosafety classification[8]: BSL-1

Parental virus attenuated: Yes ☐ No X

***2.2.* Phenotypic and Genetic Markers**

The parental MVA is ~170 kb long.

MVA can support a foreign insert of at least 24 kb. The longest size of inserts that have been inserted in MVA-209-FSP is about 5 kb.

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

The host of MVA are chick embryo fibroblasts (CEF) cells and cultured avian-derived cell lines. It does not infect mammals and cultured mammalian cells.

2.4. Zoonotic potential of the parental virus[9]

No previous experience or information about zoonosis due to MVA is known. MVA is not known to be able to infect and replicate in wild organisms; it only replicates in avian cell cultures.

2.5. Replication properties of the parental virus

The MVA parental virus is an enveloped double-stranded DNA highly attenuated virus which replicates in the cytoplasm of avian cells. It is unable to replicate in human and human cells.

[8] Explain if the classification varies between different territories in which the clinical trial will take place.
[9] This Section needs not be filled in case of replication incompetent clinical viral vectors.

A.2. Pathogenicity

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

Not applicable. Parental MVA is a laboratory strain, not replicating in animals and humans

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

Parental MVA was attenuated by more than 570 passages on CEF. The resulting selected strain lost more than 30kb genome corresponding to multiple genes, affecting escape immunity and host range restrictions

A.3. Ability to colonize

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

The MVA parental virus is a laboratory-selected strain. It does not replicate outside avian cultured cells.

2.9. Can the parental virus survive outside the host?

Such as with all poxviruses, MVA shows high environmental stability with high resistance to drying up to 39 weeks at 6.7% moisture at 4°C and increased temperature tolerance compared to other viruses. Nevertheless, there is only a limited environmental impact to be expected during unintended environmental spreading, due to the poor replicative and propagative characteristics of MVA. Moreover, vaccinia virus has no natural reservoir.

B. Genetic modification and manufacturing of the clinical vector.

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

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

2.12. Contaminating replication-competent virus.

Section B is Confidential. The whole section is included in the Confidential Annex to this document.

Summary that can be made public:

A) the recipient vector MVA was originally selected by more than 570 serial passages on CEF. This made the vector replication-restricted to avian cultured cells;

B) MVA was subsequently modified to obtain MVA-209 by insertion of antigen sequences corresponding to frame shift proteins. This modification is finalized at the expression of the specific neoantigens upon IMP administration to the patient and enhancing the natural immunological response against the tumour.

The cell bank used to produce the IMP and the product at all the subsequent manufacturing steps are subject to panels of assays aimed at ascertaining the quality of the product according to current regulations.

C. Clinical vector

MVA-209-FSP is a genetic vaccine that encodes a set of 209 Frame Shift Peptides (FSPs). FSPs are non-self proteins, neoantigens, generated by pathological frameshift mutations in solid tumors. These mutations arise in nucleotide repeats regions, in tumors characterized by defects in the DNA mismatch repair mechanism (MMR).
The FSPs are shared to a variable extent across individual *dMMR* or *MSI-H* solid tumors and, consequently, offer a compelling opportunity to develop an 'off-the-shelf' vaccine based on a shared portfolio of tumor neoantigens. dMMR/MSI-H solid tumors' FSPs are not present in the healthy human protein repertoire and are consequently expected to be potent and safe immunogens.

Paragraphs 2.13, 2.14 and 2.15 of Section C are CONFIDENTIAL. They are included in the Confidential Annex to this document.

Summary of paragraphs 2.13. 2.14 and 2.15 that can be made public:

Molecular characterization of the clinical vector(s)

Each MVA-209 based IMP to be used in the study NOUS-209-01 will express a different set of epitopes. The resulting final GMP product are unable to replicate (outside permissive cells in culture), due to the attenuation during the MVA parenteral vector selection, hence they are not able to productively infect animals and humans and to propagate in the environment. This is a very important statement that is at the basis of the risk assessment for this class of IMPs. The **inability to replicate** has of course a cascade impact on the possibility to spread in the environment and cause pathologies and adverse events.

The MVA-209 IMP class has been designed as an efficient vector to transduce neoepitope sequences into patients by intramuscular (IM) administration. The translated epitope sequences are aimed at eliciting an immune response against the tumour neoepitopes improving the natural immune response of the host during the therapy.

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

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

MVA (as MVA-209-FSP) is not an enveloped virus. Envelope sequences will not be provided *in trans*.

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

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

  Both the MVA vector and the GMO MVA-209-FSP only replicate in non-human permissive cell cultures (in labs).

- *Transmission route.*

  Not applicable

- *Pathogenic properties. Where relevant, consider potential effects in common population and in vulnerable groups such as immunosuppressed individuals, pregnant women, small children, or any other group with a higher risk.*

  Not applicable.

- *Ability to survive outside the host. If available, provide data on the loss of infectivity of the clinical vector on different materials or in liquids (e.g. waste water).*

  Data not available. Parental MVA is resistant to drying conditions and temperature but replication and spreading limitations make that resistance not risky. The GMO MVA-209-FSP is not changed with respect to the parental MVA.

2.17. Potential for recombination with the parental virus in vivo and description of potential recombinants.
Not applicable. Both the GMO and the parental virus do not replicate outside lab cultures.

2.18. Biodistribution and shedding.
There is no previous human pharmacology experience with MVA-209-FSP hence no shedding and biodistribution data are available. However, several clinical studies performed with very similar vectors as well as non-clinical PK results with the vectors of the study NOUS-209-01 show persistence of the IMP at the injection site or at the regional lymph nodes when the vector is administered intramuscularly. Bio-distribution studies revealed that MVA does not persist for more than 48h in the body (*Gomez, C.E., et al., The poxvirus vectors MVA and NYVAC as gene delivery systems for vaccination against infectious diseases and cancer. Curr Gene Ther, 2008. 8(2*): p. 97-120). In a Phase I study with an MVA that expressed human MUC1, urine samples collected 4 hours and 8 days after injection were negative for presence of vector sequences *(Rochlitz, C., et al., Phase I immunotherapy with a modified vaccinia virus (MVA) expressing human MUC1 as antigen-specific immunotherapy in patients with MUC1-positive advanced cancer. J Gene Med, 2003. 5(8): p. 690-9)*. In addition, the inoculation of MVA vaccine via the intramuscular route eliminates the development of skin pock lesions, reducing the shedding via those lesions *(Kennedy, J.S. and R.N. Greenberg, IMVAMUNE: modified vaccinia Ankara strain as an attenuated smallpox vaccine. Expert Rev Vaccines, 2009. 8(1): p. 13-24).* The shedding/spreading of vectors such as MVA which are unable to produce new viral progeny and to propagate in most mammalian cells is not expected to have environmental impact.

The target dose (1.65×10^8 ifu) has been selected based on safety and immunogenicity data obtained in previous human clinical trials with similar vectors at dosages equivalent to those proposed.
To date the patients treated with the target dose have not reported any DLT (Dose Limiting Toxicity).
References and updated data on the safety in the treated patients are included in the Investigator Brochure that is part of the CTA package.

SECTION 3 –INFORMATION RELATING TO THE CLINICAL TRIAL

3.1. General information about the clinical trial.

EudraCT-number (where available):	2021-002823-40
Deliberate release reference number (where available and applicable):	
Title of the clinical trial:	A Phase I/II, Multicenter, Open-Label Study of Nous-209 Genetic Vaccine for the Treatment of Microsatellite Unstable Solid Tumors
Name of principal investigator:	Marc Van den Eynde

Objective of the study:	Study Endpoints for Phase II (Cohort C and Cohort D) conducted in European Union **Primary Objective** **Cohort C** • To assess preliminary evidence of anti-tumor activity based on RECIST 1.1 of the vaccination regimen in combination with pembrolizumab in terms of ORR, based on Simon 2-stage design. **Cohort D** • To assess preliminary evidence of anti-tumor activity based on RECIST 1.1 of the vaccination regimen in combination with pembrolizumab in terms of ORR, based on Simon 2-stage design. **Secondary Objective** • To evaluate the overall safety and tolerability of the RP2D of GAd20-209-FSP and MVA-209-FSP vaccination regimen in combination with pembrolizumab. • Cohort C: To assess additional preliminary evidence of anti-tumor activity based on RECIST 1.1 of the vaccination regimen in combination with pembrolizumab in terms of Best Overall Response (BOR) anytime, Duration of Response (DoR) anytime, Progression-Free survival (PFS) at 6 m, 12 m and 18 m. • Cohort D: To assess additional preliminary evidence of anti-tumor activity based on RECIST 1.1 of the vaccination regimen in combination with pembrolizumab in terms of Best Overall Response (BOR) anytime, Duration of Response (DoR) anytime, Progression-Free survival (PFS).
Intended start and end date:	Start: October 2022 – End: December 2024
Number of trial subjects that will take part in the study:	Up to approximately 63 evaluable patients for Cohort C and 18 subjects in Cohort D
Indicate if an application related to the same investigational medicinal product has been submitted -or is planned to be submitted- to other EEA Member States. In the affirmative, identify the countries concerned:	The study was submitted in Belgium, Czech Republic, Italy and Spain and will be submitted in United Kingdom. The study is ongoing in USA and Spain. The study was withdrawn in Czech Republic. The application is still under review in Italy.

3.2 Intended location(s) of the study.

The applicant should provide information about the clinical sites located in the country of submission of the application.
In some jurisdictions, the following additional information should be provided:
•*the location(s) of laboratories (in the country of submission) in which activities with the GMO are carried out under the framework of the clinical trial application should be stated.* [10]
•*information about the location where the investigational medicinal product is stored (to the extent that the location is in the country of submission but outside the clinical site).*[11]
•*information about the location where patient's samples that contain GMO's are stored (to the extent that the location is in the country of submission but outside the clinical site).*[12]

Organisation Name:	Cliniques Universitaires Saint Luc
Address Details:	Medical Oncology Unit Avenue Hippocrate, 10 1200 Brussels, Belgium
Contact person:	Alexandre Colmant
Telephone No:	+32 2 764 11 11
Email Address:	alexandre.colmant@saintluc.uclouvain.be
Planned activities:	IMP handling, storage, preparation, administration and destruction of IMP
Containment level:	BSL1
Name and contact details of the responsible person[13]:	Alexandre Colmant biosecurite@saintluc.uclouvain.be

Site / Questions	Cliniques Universitaires Saint Luc
the location(s) of laboratories	Medical Oncology Unit Avenue Hippocrate, 10 1200 Brussels, Belgium
Location where the GMO will be administered	Unit 51, HR 501 to 515 (4301 → 4315)
location where the investigational medicinal product is stored	Pharmacy, level -3, room 4701 (2 Biological Safety Cabinets availables)
location where patient's samples that contain GMO's are stored	Unit 42, room 4505A

Name and contact details of the responsible person	Alexandre Colmant biosecurite@saintluc.uclouvain.be alexandre.colmant@saintluc.uclouvain.be +32 2 764 6734 (no personnal phone)

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

[11] This information should be provided for applications submitted to Croatia, Germany, Ireland and Spain. This information should be provided for applications submitted to Belgium, Czech Republic and Finland, unless there is a contained use notification covering the storage of the product.

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

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

The applicant should provide information about the storage location, conditions of storage (including restrictions of access), and the maximal storage duration.[14]

IMP will be stored in the freezer in the appropriate and certified area at the clinical site, with restricted and controlled access. Storage freezer will have appropriate temperature control system in place.
IMP must be stored at or below -60°C.
Each IMP shipment will be shipped to each site following individual subject screening/enrollment in the study. IMP will be stored at the site from the shipment receipt until the study end, or until the last IMP administration visit of the last patient at each site.

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

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

MVA-209-FSP viral vaccine is supplied within a 3 mL borosilicate glass vial closed with a rubber stopper and sealed with an aluminium flip-off cap. The vial is labelled with a technical label and a primary label. The vial is extremely resistant to shocks and accidental drops and protect against sample contamination thanks to their seal.

On the day of vaccination, the vial must be taken from the cardboard box and allowed to thaw putting it in an upright vertical position without inverting (to avoid producing foam within the vial content) in a rack or a similar support. Sealed containers are not necessary since the vials are already sealed thanks to the aluminium flip-off cap.
Since the dose to be injected is contained in the vial(s) and is ready-to-use (no dose preparation is required), the designee person who will administer the vaccine to the patient will only have to withdraw the appropriate volume from the vial.

If the syringe will be prepared at the administration site, the internal transportation of the vial(s) from the Pharmacy to the administration site must be done, as for the thawing, putting it in an upright vertical position. If the syringe will be prepared at the Pharmacy, the syringe will be moved to the administration site. For such a transport the syringe can be placed inside a large screw-capped plastic jar or alternatively in a sealed plastic bag inside a glass beaker. It is recommended to add bubble bags/pads inside the plastic bag to avoid shockproof.

Once administered the MVA-209-FSP vaccine by I.M. injection, the empty/used IMP packaging must be discarded per local biohazard materials disposal standards.

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

Reconstitution (where applicable, summarise reconstitution steps):	Not applicable
Pharmaceutical form and strength:	Suspension for injection Strength: ≥1 e11 to ≤4 e11 vp/mL
Mode of administration:	Intramuscular injection (IM)
Information on dosing and administration schedule (in case of repeated dosing):	MVA-209-FSP dose is 1.65 x 10^8 ifu. MVA-209-FSP will be administered three times as boost component of the candidate therapeutic agent.
Information on concomitant medication that may affect the shedding of the clinical vector/ environmental risks (*e.g.* administration of laxatives, administration of a medicinal product that could enhance the replication activity of the clinical vector, administration of a plasmid-based medicinal product):	MVA-209-FSP will be administered to cancer patients undergoing pembrolizumab Standard of Care therapy (commercial name Keytruda). Moreover, after the unique GAd20-209-FSP administration, 3 administrations of MVA-209-FSP will be performed as a boosting injection (see IMPDs, IB, Protocol of the NOUS-209-01 clinical study included in the CTA). Pembrolizumab concomitant medication cannot affect the spread of clinical vector.

3.6 Measures to prevent dissemination into the environment.

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

On the day of vaccination, the vial with the vaccine must be allowed to thaw putting it in an upright vertical position without inverting (to avoid producing foam within the vial content). During the thawing, sealed container is not necessary since the vial is already sealed thanks to the aluminum flip-off cap.
The dose to be injected is 1 mL and is ready-to-use (no dose preparation is required).
To further increase the probability of successful uptake of the vaccine, it will be administered half of the total volume (0.5 ml) in each arm (deltoid region) therefore the pharmacist or designee person, once the vial has been thawed, must prepare two syringes as following: to withdraw up 0.5 ml of vaccine from the vial in a sterile syringe and 0.5 mL of vaccine from the vial in another sterile syringe.

- If the syringe will be prepared at the administration site, the internal transportation of the vial from the Pharmacy (or the corresponding site of storage/thawing) to the administration site must be done keeping it in an upright vertical position. Sealed container to put in the vial is not necessary since the vial is already sealed thanks to the aluminum flip-off cap.
- If the syringe will be prepared at the Pharmacy, the syringe will be moved to the administration site. For such a transport the syringe can be placed inside a screw-capped plastic jar or alternatively in a sealed plastic bag inside a glass beaker. It is recommended to add bubble bags/pads inside the plastic bag to avoid shockproof.

Once administered the vaccine, the empty/used syringe must be discarded as per local biohazard materials disposal standards

b) Personal protective equipment.

Eye protection, gloves, masks and a lab coat

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

Procedures according to study Pharmacy manual.
In summary, 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, the solution must not be older than one day) for at least 30 minutes 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.

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

Dispose of according to local biohazard destruction procedures

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

Dispose of according to local biohazard procedures.

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

The treated patients will have the site of injection (deltoid region of the upper arm) covered with a bandage for 30 min, then the bandage will be disposed of as biohazard waste. The patients will be requested not to touch the injection site and, should this unwillingly occur, to wash their hands.

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

Not applicable. After 30 min bandage covering of the site no risk of dissemination is foreseen. However the patients will be requested not to touch the injection site and, if it occurs, to wash their hands.

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

Do not donate eggs or sperm for at least 180 days.

i) Other measures.

The use of effective contraceptive measures is included in the inclusion / exclusion criteria of the study NOUS-209-01 protocol, as well as egg and sperm donation for at least 180 days.

3.7 Sampling and further analyses of samples from study subjects

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

The biological samples will be handled and stored at site according to appropriate biohazard procedures and instructions provided by Nouscom in the Lab Manual(s) which will be sent to each site.
The biological samples will then be transported, managed, stored at and analyzed for the immediate objectives of the trial at the corresponding Central lab:

- Reithera Srl, Via di Castel Romano 100, 00128 Roma, Italy (for IMP specific antibodies isolated from blood)
- Guardant Health, Inc., 505 Penobscot Dr., Redwood City, CA 94063 (for liquid biopsy/cell free DNA)

Nouscom will organize and oversee transport from sites to the corresponding central labs according to GCP and IATA standards.

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

The time points at which the samples will be taken from study subjects during the trial NOUS-209-01 for cohort C and D are indicated in table below:

Visit	**Screening**	**V1**	**V2**	**V3**	**V4**	**V5**	**V6-10**	**V11**	**V12**	**V13**	**V14**	**V15**[2]	**V16-21/29 (cohrot C)**	**V22**[8]	**V30**
Week	-4 / -1	**1**	**4**	**7**	**10**	**11**	**13-25**	**28**	**31**	**34**	**37**	**38**	**40--79**	**59**	**83**
Day	-28 /-1	1	22	43	64	71	85-169	190	211	232	253	260	274-547	407	575
Visit window (days)[1]		+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3
Pembrolizumab[3]		**X**	**X**	**X**	**X**		**X**	**X**	**X**	**X**	**X**		**X**		
GAd20 -209-FSP (Prime)[4]		X						X[2]							
MVA -209-FSP (Boosts)[4]			X	X	X				X[2]	X[2]	X[2]				

Visit	**Screening**	**V1**	**V2**	**V3**	**V4**	**V5**	**V6-10**	**V11**	**V12**	**V13**	**V14**	**V15[2]**	**V16-21/29 (cohrot C)**	**V22[8]**	**V30**
Week	-4 / -1	**1**	**4**	**7**	**10**	**11**	**13-25**	**28**	**31**	**34**	**37**	**38**	**40--79**	**59**	**83**
Day	-28 /-1	1	22	43	64	71	85-169	190	211	232	253	260	274-547	407	575
Visit window (days)[1]		+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3	+/-3
Anti-GAd20 antibodies[5]		X		X				X		X[7]			Visit 21 only[7]		
Liquid/biopsy/ Cell free DNA[6]		X				X		X					Visit 19 (Cohort C and D)and 29 for Cohort C		

1.Scheduling window: plus/minus 3 days are always allowed. Whenever Pembrolizumab administration is withhold for IrAE; administration of the IP is also suspended and can restart as described in Section **Error! Reference source not found.**.

2. Re-treated subjects: Only subjects who are eligible for revaccination per Section **Error! Reference source not found.** will be retreated with GAd20-209-FSP and 3 MVA-209-FSP boosts s tarting at 6 months.

3. Pembrolizumab: Pembrolizumab to be administered by IV infusion Q3W per label. Pembrolizumab will be administered according to the country specific approved Keytruda Full Prescribing Information, until visit 4 (i.e. until the 3rd MVA boost). the 3rd MVA boost , this posology may be continued or changed to 400mg Q6W per Investigator's discretion.

4. GAd20 -209-FSP (Prime) and MVA-209-FSP (Boost): To be administered at least 1hr after the pembrolizumab on the days they are administered together (i.e. visits 1b and 11b for re-treated subjects), so long as there are no pembrolizumab infusion reactions.

5. Anti-GAd20 antibodies. Approximately 2.5-5 ml of blood will be collected.

6. Liquid biopsy/ Cell Free DNA: A sample of 20ml blood will be collected and will be used for plasma collection at indicated time points identified in the schedule of activities. The samples will be used for the extraction and testing of cell free DNA. Cell free DNA is also to be collected at the time of progression per RECIST1.1.

7. Duration for Cohort C: Study duration is up to 12 months for Cohort D, thus it is V16-21 (i.e. weeks 40-55 and days 274-379).

8. Duration for Cohort D: Study duration is up to 12 months for Cohort D, thus it is V22 (i.e. week 59 and day 407).

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

Currently proposed procedures for the various patients' samples storage are the following and will be included in lab manuals:

- Anti-GAd antibodies blood plasma samples can be stored at site's freezer under controlled conditions at -80C up to the shipment to central lab.
- Liquid biopsies can be stored at site's freezer under controlled conditions at -20C up to the shipment to central lab.

d) Explain if there is any non-routine[15] testing of the samples and indicate whether the clinical vector is generated *de novo* during the testing.

Not applicable

3.8 Emergency response plans.[16]

Emergency response plans for accidental self-administration during handling or administering the clinical vector:	The plan is described in the study Pharmacy Manual. The Medical monitor has to be informed immediately.
Emergency response plans for accidental release into the environment of the clinical vector:	The plan for spills is described at section 3.6 c)

SECTION 4 – OTHER DATA REQUIRMENTS

4.1. Plan of the site(s) concerned

Plan of the site together with a description of the location of the autoclave will be provided in contained use application per site.

4.2 Other information

SECTION 5.0 ENVIRONMENTAL RISK ASSESSMENT

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

A. Risk Analysis

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

5.1. Hazard identification*:*

MVA-209-FSP is unable to replicate outside of laboratory permissive cells, hence **it does not represent a risk for humans in any respect**. The intramuscular administration route has been demonstrated in studies with MVA-209-FSP prototypes not being associated with shedding and with tissue distribution in districts far from the injection site in rats. Previous clinical studies with MVA expressing different antigens also confirmed lack of shedding and tissue distribution results. In previous clinical studies with those IMPs no SAE related to the virus has ever been identified. Immune reactions against the expressed neoantigens are instead the basis of the mechanism of action of the IMP since the desired effect is to improve the pre-existing natural immune response against those sequences already specifically present in the tumor of the patients. No sequence homology of the MVA-209-FSP antigens with human sequences is expected. The cloned human epitopes are neoantigens only found in tumors and are specific for each patient. No homology between the vector and human sequences occurs. Illegitimate recombination is also not expected; the probability is not higher than for any other foreign or not foreign sequence introduced into an acceptor organism.

In the proposed clinical trial NOUS-209-01, MVA-209-FSP is used as a boosting vaccine, following a single administration of GAd20-209-FSP as a prime. Pharmacodynamic interactions other than those intrinsic to the fact of being prime-boost elements of the complete vaccine have not been observed in previous studies with similar vectors. Moreover, different types of interactions, such as recombination, have to be excluded considering the persistence timespan and localization of the first administered IMP (GAd20-209-FSP) and those of the second administered IMP (MVA-209-FSP). In addition to that, it has to be considered that the vectors in the IMPs do not present sequence homology and the outcome of a potential recombination event, that might only involve the inserts, would not be dangerous, since it might result only in a different arrangement of the FSP present in

the inserts of both the IMPs in different arrays. Even the production of a recombinant FSP would not be dangerous but only potentially not efficacious in enhancing the immune response against the tumor.

In the proposed trial, the anti-PD1 antibody pembrolizumab will be administered as an auxiliary product. Pharmacodynamics interactions between the auxiliary product and the IMPs are not expected.

At the clinical sites the GMOs IMPs shall be handled as a class-1 risk (in BSL-1 conditions) for all the operations including transportation, storage and handling for administration to patients, as it will be indicated in the Pharmacy and lab manuals. See also section 5.5.

At the clinical sites the GMO IMPs shall be handled as a class-I risk (in BSL-1 conditions) for all operations including transportation, storage and handling for administration to patients, as it will be indicated in Pharmacy and Lab manuals. See also Section 5.5

5.2. Hazard characterisation:

Negligible

5.3. Exposure characterisation:

Negligible

5.4. Risk characterisation:

Negligible

5.5. Risk management strategies:

The management of MVA-209-FSP to minimize the potential exposure of healthcare professionals and/or close contacts of the clinical trial subject is addressed through two primary efforts: 1) safe handling of the vector by health care professionals; 2) minimization of potential exposure of health care professionals and family, friends, and caregivers to the potential shedding of the virus.

<u>Biosafety Precautions (BSL-1)</u>

All personnel handling MVA-209-FSP 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 institutional standard operating procedures. Spilled vector can be decontaminated as per Biosafety Protocols using a 1:10 dilution of household bleach (the solution must not be older than one day) or 70% Ethanol or Virkon.

While the likelihood of exposure from shedding represents a negligible risk, management of the potential risk warrants attention.

The informed consent describes the potential risks of the study and advice for the patient has been added to address any potential GMO risks.
Since MVA-209-FSP is not considered a human pathogen, it is proposed to be classified as Class 1, to be handled in BSL-1 conditions. However, if a health care provider sustains a needle-stick injury while caring for a study patient, the Medical Monitor has to be notified.

Monitoring of Subjects in the Proposed Phase

Subjects enrolled in the study will undergo health controls as detailed in the study Protocol.

Pharmacy Manual

Instructions and precautions for its safe use and contained handling are provided in the Study Pharmacy Manual.

[17] In the case of applications submitted to Italy, this Section should always be filled-in.

A.2. Risks to the environment

5.6. Hazard identification

Not applicable.
The GMO vector is unable to replicate and does not survive outside specific cell culture systems in laboratory. Moreover, any residual GMO-contaminated material will be disposed of as biohazard waste.

The potential environmental hazards arising from MVA are as follows:

- Systemic toxicity of GMO (acute): **Not expected**
- Pathogenicity/tumorigenicity of GMO: **Not expected**
- Immunogenicity of GMO: **this is expected and is actually the desired mechanism of action, meaning immunogenicity of the insert**
- Systemic toxicity of expressed cytosine deaminase (chronic): **Not applicable**
- Immunogenicity: **Only expected referred to the insert as desired mechanism of action.**
- Recombination of GMO genome with genomes: **Not expected (lack of sequence homology between the GMO and the human genome)**
- Horizontal transmission: **Not applicable. The IMP is not infectious.**
- Release to environment (contamination of untreated sewage). **No shedding expected after IMP administration, based on previous observations in clinical trials with similar vectors and in non-clinical studies with the same vectors.**

5.7. Hazard characterisation:

Not applicable

5.8. Exposure characterisation:

Negligible

5.9. Risk characterisation:

Negligible

5.10. Risk management strategies:

The measures are listed in Section 3.6 and in the study Pharmacy manual.

A.3 Overall risk evaluation and conclusions

4.11. Overall risk evaluation and conclusions

The overall risk for of the GMO clinical vector MVA-209-FSP for both humans and environment is negligible or null, due to its inability to replicate outside permissive cultured cells, the extremely low probability of recombination with other vaccinia viruses and the lack of pathogenicity. MVA-209-FSP is proposed to be handled under BSL-1 conditions. However, the Sponsor cannot exclude the probability that indirect observations may take longer to be observed and that deferred effects on the human health or environment that are not observed during the period of release of the OMGs but that are expressed as direct or indirect effects on a later stage, after concluded the referred release.

References

- Barnes, E., et al., *Novel adenovirus-based vaccines induce broad and sustained T cell responses to HCV in man.* Sci Transl Med, 2012. **4**(115): p. 115ra1.
- *BE GSK SNIF ChAd155-RSV - 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*. 2018 https://www.biosafety.be/content/study-evaluate-safety-reactogenicity-and-immunogenicity-gsk-biologicals-rsv-investigational.
- Wold, W.S. and K. Toth, *Adenovirus vectors for gene therapy, vaccination and cancer gene therapy.* Curr Gene Ther, 2013. **13**(6): p. 421-33.
- Gomez, C.E., et al., *The poxvirus vectors MVA and NYVAC as gene delivery systems for vaccination against infectious diseases and cancer.* Curr Gene Ther, 2008. **8**(2): p. 97-120.
- Rochlitz, C., et al., *Phase I immunotherapy with a modified vaccinia virus (MVA) expressing human MUC1 as antigen-specific immunotherapy in patients with MUC1-positive advanced cancer.* J Gene Med, 2003. **5**(8): p. 690-9.
- Kennedy, J.S. and R.N. Greenberg, *IMVAMUNE: modified vaccinia Ankara strain as an attenuated smallpox vaccine.* Expert Rev Vaccines, 2009. **8**(1): p. 13-24.
- Verheust C et al. Biosafety aspects of Modified virus vaccinia virus Anakara (MVA)-based vectors used for gene therapy. Vaccina 2012; 30(16): 2623-2632
- EMA EPAR for MVAbea https://www.ema.europa.eu/en/medicines/human/EPAR/mvabea
- EMA EPAR for Imvanex https://www.ema.europa.eu/en/medicines/human/EPAR/imvanex

For MVA Biodistribution study:

- AdCh63 MSP-1 and MVA MSP-1 Tissue Distribution Study By Intra-Muscular Administration To Mice (attached)