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

In order to tick one or several possibilities, please use crosses (meaning x or X) into the space provided as (.)

A. General information

1. Details of notification

(a) Member State of notification:

Spain

(b) Notification number:

B/ES/18/25

(c) Date of acknowledgement of notification:

11/09/2018

(d) Title of the project:

Phase 1/2a First-in-Human Study of BMS-986277 Administered Alone and in Combination with Nivolumab in Advanced Epithelial Tumors

(e) Proposed period of release

First patient treated: 12/2018; Last patient treated: 09/2022

2. Notifier

Name of institution or company:

Bristol-Myers Squibb

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

other, specify (kingdom, phylum and class)

(b) Identity of the GMO (genus and species)

Genus: Mastadenovirus; Species: Human Chimeric Adenovirus

BMS-986277 is a replication-competent oncolytic adenovirus selective for human epithelial tumor cells. The genome has been modified to express 2 transgenes: a full-length human T-cell co-activating antigen, CD80, and a single chain variable fragment of the mouse anti-human CD3ε monoclonal antibody, OKT3 (anti-CD3-ScFv-TM), controlled under the virus endogenous major late promoter.

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

In general, as double-stranded DNA viruses with genome sizes of approximately 36 kb, adenoviruses are considered genetically stable. Adenovirus DNA polymerase has proofreading activity and removes mismatched nucleotides. However, the chance of co-infection enables the natural recombination among adenoviruses. Recombination plays an important role in shaping the phylogenetic relationships of adenovirus genomes (Lukashev et al 2008). Recombination occurs predominantly between strains of the same adenovirus species, in regions of homology, but not presumably between different adenovirus species. The seroprevalence of wild adenovirus type 5 (Ad5) is much higher as compared to adenovirus 11 (Ad11).

Genetic stability of the GMO is expected based on its design, manufacture and therapeutic use. The GMO is a double stranded DNA virus of 34,522 base pairs. This genome size is similar to wild-type adenovirus of 34,794 base pairs. Recombinant adenoviruses are generally considered genetically stable as long as the total base pairs do not exceed 105% of the parent genome size. Adenovirus DNA polymerase has proofreading activity and removes mismatched nucleotides. However, a chance of co-infection does enable natural recombination among adenoviruses. This recombination predominantly occurs between strains of the same adenovirus species in regions of homology. The GMO is a chimeric Ad11/Ad3 Group B adenovirus, with the majority of the genome derived from the Ad11 virus. Reports of clinical infection with wild type Ad11 virus are rare, and therefore, the likelihood of recombination with this chimeric vector is reduced as compared to a vector derived from the more common Ad5 serotype. Recombination with wildtype Ad3 is unlikely as the Ad3 chimeric portion of the GMO is limited to 197 non-homologous sequences in the E2b region. Manufacturing controls have also been instituted through use of a master viral seed stock, qualification of which includes genome sequencing, polymerase chain reaction (PCR) and restriction digest testing to confirm genetic integrity of the GMO. The production bioreactor is infected from this master seed stock, limiting the propagation of the virus during production. Each batch of product is additionally tested to ensure genetic integrity using PCR and restriction digest mapping. Lastly, the chimeric Ad11/Ad3 adenovirus was developed to ensure a high level of selectivity for malignant cells such that replication in healthy human cells is poor or non-existent.

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)

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

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 (X) No (.)

If yes:

- Member State of notification Canada
- Notification number N/A

Dossier ID: HC6-024-C209470, Control #: 209470

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

The GMO has been administered to humans in a Phase 1 clinical trial. Data are being collected concerning the GMO environment release. Release of the GMO has not been detected in urine, saliva, or stool samples that have been analyzed by quantitative PCR to date..

Data is also available on the release of the parental oncolytic adenovirus, enadenotucirev, the source of the GMO, which was administered by three intravenous infusions over 5 days. From day 17 of the virus administration, viral DNA was detected in rectal or mouth smears in fewer than 10% of patients (N= 31) and in only 1 patient 56 days after the final dose. The infectivity of samples for the parental oncolytic adenovirus, enadenotucirev, is not known since quantitative PCR does not discriminate between infectious viruses, non-infectious viruses, or virus assembly products. In the GMO clinical trial, urine, saliva, or stool samples with detectable GMO will be further tested in a plaque assay to assess the infectivity of the virus.

Additionally, humans are the natural adenovirus hosts. Adenoviral infections are primarily asymptomatic, self-limiting and restricted to few permissive tissues. Moreover, viral replication of the GMO is highly selective for replication in human cancer cells, which reduces its potential environmental impact.

Adenoviruses do not integrate their DNA into the host, so transmission along the recipient genome is not possible.

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

The information provided is based on the following definitions:
- GMO: BMS-986277
- Recipient: enadenotucirev
- Donor: human or mouse transgenes inserted to result in viral expression of a full length human T-cell co-activating antigen CD80 and a single chain variable fragment of the mouse anti-human CD3ε monoclonal antibody OKT3.

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) Adenoviridae

(ii) genus Mastadenovirus

(iii) species Human adenovirus

(iv) subspecies Group B adenovirus

(v) strain Human-specific chimeric Ad11p/Ad3 chimeric

(vi) pathovar (biotype, ecotype, race, etc.)

(vii) common name enadenotucirev

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, (X) specify	Specific to humans

(b) If the organism is an animal: natural habitat or usual agroecosystem: Not applicable

5. (a) Detection techniques

The parent adenovirus, enadenotucirev, may be detected by infection of a permissive cell line such as HEK293 or theoretically using PCR with a human adenovirus specific primer set [1] (J. Virols Methods. 2001 Apr;92(2):113-20)

(b) Identification techniques

The parent adenovirus, enadenotucirev, may be identified using techniques such as DNA sequencing, restriction digest mapping of isolated genomic DNA or PCR using primers diagnostic for identification. Restriction digest mapping and PCR tests that distinguish the parent adenovirus from the GMO are included in the panel of quality control release tests.

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

All human adenoviruses have been assigned to Risk Group 2 by the European Communities (Directive 2000/54/EC). Adenovirus 11 is a pathogen that can cause human disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment.

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

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

If yes:

(a) to which of the following organisms:

humans	(X)
animals	(.)
plants	()

other (.)

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

Enadenotucirev, the recipient organism, is a chimeric Ad11p/Ad3 adenovirus with the majority of the genome derived from the Ad11 virus. Ad11 and Ad3 are both Group B adenoviruses. The seroprevalence of wild type Ad11 has been examined by several groups and has been found to be relatively low compared to wild-type adenovirus type 5 (Ad5). In one study, only 8.3% of subjects, including those with cancer, have been shown to have neutralising Ad11 antibodies compared to 64.6% for Ad5. With regard to pathogenicity, infectivity and virulence of Ad11, reports of clinical infection with wild-type Ad11 are relatively rare. Ad11 infections have been reported in immunosuppressed patients due to bone marrow, stem cell and solid organ transplantation treatments, with the urogenital tract as the common site of infection. Enadenotucirev, the recipient organism, is not a known carrier of pathogens. The host range is limited to humans, as enadenotucirev does not replicate in cells of non-human species. Additionally, enadenotucirev was developed to ensure a high level of selectivity for malignant cells such that replication in healthy human cells is poor or non-existent. Enadenotucirev has been administered to more than 100 patients without evidence of latent virus activation, allergenicity or toxigenicity.

8. Information concerning reproduction

(a) Generation time in natural ecosystems:

The recipient organism is highly selective for replication in human tumor epithelial cells; therefore, there is no appreciable replication time in a natural ecosystem.

(b) Generation time in the ecosystem where the release will take place:

The recipient organism is highly selective for replication in human tumor epithelial cells; therefore, there is no appreciable replication time in a natural ecosystem.

(c) Way of reproduction: Sexual .. Asexual Not applicable

(d) Factors affecting reproduction: Not Applicable

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:

Adenoviruses quickly lose bioactivity at room temperature. Bioactivity losses are logarithmic and consequently the virus retains a certain degree of stability for durations of time but remaining levels are typically below the infectivity threshold. Adenoviruses are susceptible to commercially available viricidal disinfectant. Adenoviruses are also temperature-sensitive and inactivation may be accomplished by autoclaving at 121ºC for 30 minutes. Higher temperatures or longer autoclaving periods can also be used.

10. (a) Ways of dissemination

Ingestion, inhalation, mucous membrane contact and accidental injection (needle stick).

(b) Factors affecting dissemination

Dissemination is affected by the concentration of shed virus, the production of aerosols, the degree of contact, and individual factors such as prior exposure to related adenoviruses and overall immune status.

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

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

The 2 inserted transgenes, full-length human T-cell co-activating antigen, CD80, and a single chain variable fragment of the mouse anti-human CD3ε monoclonal antibody, OKT3 (anti-CD3-ScFv-TM), are controlled under the virus endogenous major late promoter. The inclusion of these transgenes is intended to result in activation of anti-tumor immunity in patients with epithelial derived tumors susceptible to the GMO infection.

(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 (X) No (.)

If no, go straight to question 5.

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

(a) Type of vector

plasmid	(X)
bacteriophage	(.)

virus (.)

cosmid (.)

transposable element (.)

other, specify

(b) Identity of the vector

- *E. coli* shuttle plasmid containing origin of replication, kanamycin resistance gene and GMO sequence (chimeric adenovirus and transgenes) is used as an intermediate step in the initial construction of the GMO. The viral genome of the GMO was excised from the shuttle plasmid and transfected to HEK293 cells to generate the initial isolate of the GMO product.

(c) Host range of the vector

- The shuttle plasmid only replicates in *E. coli.* The viral genome of the GMO that is present in the shuttle plasmid can replicate in permissible human tumor cell lines such as HEK293.

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

- In the shuttle plasmid

Yes (X) No ()

antibiotic resistance (X)

other, specify

Indication of which antibiotic resistance gene is inserted: kanamycin

- In the virus vector

Yes () No (X.)

antibiotic resistance)

other, specify

Indication of which antibiotic resistance gene is inserted:

(e) Constituent fragments of the vector

The *E. coli* shuttle plasmid contains a bacterial origin of replication, kanamycin resistance gene and the complete genome sequence of the GMO. The viral genome of the GMO contains the enadenotucirev parent virus sequence with the inserted transgene cassette composed of CD80 and anti-CD3-single chain variable fragment (ScFv)-transmembrane sequences, short splice acceptor sequence, high efficiency self-cleavable peptide (P2A) and Poly(A) sequence. The viral genome of the GMO does not contain an antibiotic resistance gene.

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

(i) transformation (.)

(ii) electroporation (X) shuttle plasmid

(iii) macroinjection(.)

(iv) microinjection (.)

(v) infection (.)

(vi) other, specify The linearized DNA fragment obtained from the shuttle plasmid, which contains the chimeric virus sequences and transgenes is introduced to HEK293 cells by transfection.

4. 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 (.)

5. Composition of the insert

(a) Composition of the insert

Transgene cassette composed of CD80 and anti-CD3-single chain variable fragment (ScFv)-transmembrane sequences, short splice acceptor sequence, high efficiency self-cleavable peptide (P2A) and Poly(A) sequence

(b) Source of each constituent part of the insert

- CD80 transmembrane sequence: human
- anti-CD3-ScFv transmembrane sequence: mouse
- short splice acceptor sequence: synthetic DNA
- high efficiency self-cleavable peptide: synthetic DNA
- Poly(A) sequence: synthetic DNA

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

(a) CD80 transmembrane sequence: encodes human CD80, a co-stimulatory molecule which binds to CD28 (signal 2) during T-cell activation

(b) anti-CD3-ScFv transmembrane sequence: encodes mouse anti-human CD3ε antibody, OKT3 which binds to the T-cell receptor (co-stimulatory signal 1) and activates T-cells

(c) short splice acceptor sequence: to permit efficient splicing of the transgene cassette mRNA and expression of the transgenes in mammalian cells

(d) high efficiency self-cleavable peptide: to inhibit the covalent bond formation between the nascent protein and amino acid on the tRNA in the ribosome

(e) Poly(A) sequence: to promote efficient post-transcriptional processing in mammalian cells

(d) Location of the insert in the host organism

- on a free plasmid (.)
- integrated in the chromosome (.)
- other, specify: encoded on viral genome under the Major Late Promoter (MLP)

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

Yes (.) No (X)

If yes, specify

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)

other, specify: synthetic DNA

2. Complete name

(i) order and/or higher taxon (for animals) …Transgene 1: Primates; Transgene 2: Rodentia

(ii) family name for plants …N/A

(iii) genus … Transgene 1:Homo; Transgene 2:Mus

(iv) species … Transgene 1:sapiens; Transgene 2: musculus

(v) subspecies …

(vi) strain …

(vii) cultivar/breeding line …

(viii) pathovar …

(ix) common name … Transgene 1: Human; Transgene 2: Mouse

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

Responses to the following are based on the recipient or parental organism as enadenotucirev

(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

Genetic stability of the GMO is expected based on its design, manufacture and therapeutic use. The GMO is a double-stranded DNA virus of approximately 34,522 base pairs which are generally considered genetically stable as the total base pairs do not exceed 105% of the parent genome size. Additionally, adenovirus DNA polymerase has proofreading activity and removes mismatched nucleotides. However, a chance of co-infection does enable natural recombination among adenoviruses. This recombination predominantly occurs between strains of the same adenovirus species in regions of homology. The GMO is a chimeric Ad11p/Ad3 Group B adenovirus, with the majority of the genome derived from the Ad11 virus. Reports of clinical infection with wild type Ad11 virus are rare, and therefore, the likelihood of recombination with this chimeric vector is reduced as compared to a vector derived from the more common Ad5 serotype. Recombination with wildtype Ad3 is unlikely as the Ad3 chimeric portion of the GMO is limited to 197 non-homologous sequences in the E2b region. Manufacturing controls have also been instituted through use of a master viral seed stock, qualification of which includes genome sequencing, polymerase chain reaction (PCR) and restriction digest testing to confirm genetic integrity of the GMO. The production bioreactor is infected from this master seed stock, limiting the propagation of the virus during production. Lastly, the chimeric Ad11p/Ad3 adenovirus was developed to ensure a high level of selectivity for malignant cells such that replication in healthy human cells is poor or non-existent.

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

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

(a) to which of the following organisms?

humans (X.)

animals (.)

plants (.)

other (.)

(b) give the relevant information specified under Annex III A, point II(A)(11)(d) and II(C)(2)(i) [**Give the details on pathogenicity, including infectivity, toxigenicity, virulence, allergenicity, carrier(vector) of pathogen, possible vectors, host range including non-target organisms and possible. Give details on toxic and allergic effects of the GMO]**

Enadenotucirev, the recipient organism, is a chimeric Ad11p/Ad3 adenovirus with the majority of the genome derived from the Ad11 virus. Ad11 and Ad3 are both Group B adenoviruses. The seroprevalence of wild type Ad11 has been examined by several groups and has been found to be relatively low compared to wild-type adenovirus type 5 (Ad5). In one study, only 8.3% of subjects, including those with cancer, have been shown to have neutralising Ad11 antibodies compared to 64.6% for Ad5. With regard to pathogenicity, infectivity and virulence of Ad11, reports of clinical infection with wild-type Ad11 are relatively rare. Ad11 infections have been reported in immunosuppressed patients due to bone marrow, stem cell and solid organ transplantation treatments, with the urogenital tract as the common site of infection. Enadenotucirev, the recipient organism, is not a known carrier of pathogens. The host range is limited to humans, as enadenotucirev does not replicate in cells of non-human species. Additionally, enadenotucirev was developed to ensure a high level of

selectivity for human malignant epithelial cells such that replication in healthy human cells is poor or non-existent. Enadenotucirev has been administered to more than 100 patients without evidence of latent virus activation, allergenicity or toxigenicity.

Based on the fact that enadenotucirev and the GMO have an identical outer capsid, the GMO is expected to have identical properties as the parent virus (enadenotucirev).

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment

In the ongoing Phase 1 clinical study, whole blood, urine, saliva, and stool samples will be collected. The urine, saliva, and stool samples will be analyzed to detect viral shedding. The whole blood samples will be used to characterize the pharmacokinetics of the administered GMO. The samples will be analyzed by quantitative polymerase chain reaction (qPCR) to quantify the viral DNA of the GMO in each sample type.

The viral shedding samples with quantifiable virus by qPCR will be tested in a plaque assay to assess infectivity of the detected virus. Since virus particles measured in circulation by qPCR could be inactive fragments or bound to antibodies rendering it inactive, it may be necessary to assess the percentage of infectious virus in circulation versus the quantified virus in circulation. To evaluate this, some whole blood samples will be tested in the plaque assay to compare the quantity of virus in circulation versus the quantity of infectious virus in circulation.

(b) Techniques used to identify the GMO

The DNA is extracted from each of the sample types and is analyzed in a qPCR assay. The qPCR assay utilizes a fluorogenic TaqMan probe which is complementary to the target sequence within the inserted hCD80 transgene of the GMO. In the presence of the primers, the probe, and Taq Polymerase, replication of the target sequence causes an increase in the fluorescent signal. This signal is used to interpolate the concentration of DNA based on known standards.

F. Information relating to the release

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

The purpose of the release is to administer the GMO in a Phase 1/2a clinical trial. The primary objective of the clinical trial is to characterize the safety and tolerability of the GMO when administered alone as monotherapy or in combination with the anti-PD-1 checkpoint, nivolumab, in human subjects with advanced epithelial tumors. The clinical trial is also designed to determine the recommended doses and dose schedules of the GMO as monotherapy and in combination with nivolumab. Secondary objectives include pharmacokinetics and shedding of the GMO, generation of anti-viral antibodies and preliminary anti-tumor activity of monotherapy and combination therapy in human subjects with advanced epithelial tumors. ..

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

Yes (.) No (X.)

If yes, specify

3. Information concerning the release and the surrounding area

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

3 to 4 academic hospitals specializing in Phase 1 clinical trials in Spain

Clínica Universitaria de Navarra. Avenida Pio XXI, 36. Pamplona 31008. Navarra.

Hospital Universitario Madrid Norte Sanchinarro. Calle Oña, 10. 28050 Madrid. Madrid.

Función Jiménez Díaz. Avda, Reyes Católicos, 2. Madrid 28040. Madrid.

(b) Size of the site (m^2):

(i) actual release site (m^2): Clinical administration area which will vary by institution

(ii) wider release site (m^2): Academic hospital where virus administration preparation will occur which varies by institution

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

Not applicable

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

None

4. Method and amount of release

(a) Quantities of GMOs to be released:

Study patients will receive a maximum of 7 doses. Approximately a total of 200 patients will be treated in the clinical trial with approximately 50 treated in Spain.

(b) Duration of the operation:

Treatment with the GMO will be administered over 5-15 minutes depending on the dose of viral particles assigned. Patients may receive up to 7 doses of the GMO over approximately 2 months. The trial is scheduled to take place approximately between December 2018 and September 2022.

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

Precautions must be followed and include wearing of personnel protective equipment such as a disposable gown, gloves, safety glasses, and a mask. Appropriate signage must be displayed in the preparation and treatment areas prior to handling the GMO. Signage must be a visible notice and include warnings about the treatment, the restrictions, and the possible risks. Disposal of all materials in contact with the GMO must follow appropriate disposal procedures in a container for Type III healthcare waste. Type III wastes are to be processed and disposed of by a specialized company for this type of waste. All preparation surfaces must be decontaminated using a disinfectant to which adenovirus is sensitive. All samples collected following GMO administration must be handled using personnel protective equipment. Transport of samples containing the GMO or specimens collected from patients administered the GMO must include a primary leak-proof receptacle, be wrapped in sufficient

absorbent material to absorb all fluid in case of breakage and a secondary leak-proof receptacle to enclose and protect the primary receptacle.

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

Mediterranean climate and controlled conditions in Hospital Area.

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.

Not Applicable, please see section B.7b.

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

1. Name of target organism (if applicable)

(i)	order and/or higher taxon (for animals)	Primates
(ii)	family name for plants	Not applicable
(iii)	genus	Homo
(iv)	species	homo sapiens
(v)	subspecies	homo sapiens
(vi)	strain	Not applicable
(vii)	cultivar/breeding line	Not applicable
(viii)	pathovar	Not applicable
(ix)	common name	Human

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

The GMO has been developed for selective oncolytic activity in human epithelial-derived tumor cells and is modified to express 2 transgenes: a full length human T-cell co-activating antigen CD80 and a single chain variable fragment of the mouse anti-human CD3ε monoclonal antibody OKT3. Therefore, the GMO is expected to have dual action of direct tumor cell killing via the oncolytic virus activity and promotion of anti-tumor immune responses through T-cell activation via the expression of the transgenes on the cell surface of the infected tumor cells. Expression of the transgenes is under the virus endogenous major late promoter (MLP) which restricts transgene expression to cells that are actively replicating virus. Off-target expression is limited because non-tumor cells are not permissive to virus infection.

The main objective of the trial is to investigate the safety and efficacy of administering the GMO to patients with advanced epithelial tumors.

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

The host range of the GMO is restricted to humans as the viral outer capsid is wild-type Ad11. Reports of clinical infection with wild-type Ad11 are relatively rare. Ad11 infections have been reported in immunosuppressed patients due to bone marrow, stem cell and solid organ transplantation treatments, with the urogenital tract as the common site of infection. Additionally, selection of the GMO viral parent has limited the ability of the GMO to replicate in human non-carcinoma cells.

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

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

Give details

Replication is highly selective for human epithelial tumor cells

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

Not applicable, highly species-specific (human) and will not replicate in cells of non-human origin. Additionally, the GMO is highly selective to human epithelial tumor cells.

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

Not 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 …

7. Likelihood of genetic exchange in vivo

(a) from the GMO to other organisms in the release ecosystem: Not applicable

(b) from other organisms to the GMO: Not applicable

(c) likely consequences of gene transfer: Not applicable

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

Not applicable

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

None

H. Information relating to monitoring

1. Methods for monitoring the GMOs

Study patients will be monitored for viral load, viral shedding, anti-virus antibodies, anti-tumor activity and adverse events following GMO administration. Viral shedding in blood, urine, saliva and stool samples will be assessed. Clinical evaluations of study patients will

include physical examinations, ECGs, vital signs, clinical laboratory assessments and adverse events including infusion site reactions and cytokine release syndrome. Additional assessments of tumor infiltrate and immune populations will be conducted.

2. Methods for monitoring ecosystem effects

Not applicable based on the low probability of transmission to non-treated subjects and the limited ability of the GMO to replicate in human non-carcinoma cells. Therefore, ecosystem effects are highly unlikely to occur.

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

Not applicable based on human epithelial tumor cell specificity of the GMO.

4. Size of the monitoring area (m2)

Clinical administration areas which vary by institution

5. Duration of the monitoring

Study patients will be monitored clinically for at least 60 days post-last study treatment and viral load/shedding will be assessed at least 60 days post-last study treatment.

6. Frequency of the monitoring

At least weekly while on study treatment with GMO and every 30 days during the first 2 months and then every 3 months during follow-up.

I. Information on post-release and waste treatment

1. Post-release treatment of the site

All waste generated during preparation and manipulation of the GMO must follow appropriate disposal procedures including disposal in a container for Type III healthcare waste. Type III wastes are to be processed and disposed of by a specialized company for this type of waste. All preparation surfaces must be decontaminated using a disinfectant to which adenovirus is sensitive.

2. Post-release treatment of the GMOs

All waste generated during preparation and manipulation of the GMO must follow appropriate disposal procedures including disposal in a container for group III healthcare waste. Type III wastes are to be processed and disposed of by a specialized company for this type of waste. . Patients will be instructed through the informed consent form on contact precautions following GMO administration such as avoiding close personal contact with vulnerable populations, not sharing eating utensils and reporting any infection symptoms.

3. (a) Type and amount of waste generated:

Waste will include all pharmacy preparation materials, personal protective equipment (PPE) worn by staff in contact with GMO and study drug administration materials.

(b) Treatment of waste

All waste generated during preparation and manipulation of the GMO must follow appropriate disposal procedures including disposal in a container for group III healthcare waste. Type III wastes are to be processed and disposed of by a specialized company for this type of waste.

J. Information on emergency response plans

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

All GMO preparation must be conducted in a class II biosafety cabinet by trained personnel. A detailed pharmacy manual will be provided to the sites and include all GMO preparation procedures and GMO administration instructions. In case of accidental spills, the spill must be contained with suitable, inert, absorbent material such as paper or cloth towels, commercial disinfect poured onto the spill area avoiding splashing or raising dust, and allowed to sit for at least 30 minutes with appropriate ventilation. After the 30 minute decontamination, all materials used during clean-up must be placed into plastic biohazard bags for disposal as Type III healthcare waste. The spill site should then be washed with cold water and all personnel protective equipment disposed of as Type III waste or decontaminated with commercial disinfectant. . Transfer of GMO containing material must occur following universal precautions. All samples collected following GMO administration must be handled using personnel protective equipment. Transport of samples containing the GMO or specimens collected from patients administered the GMO must include a primary leak-proof receptacle, be wrapped in sufficient absorbent material to absorb all fluid in case of breakage and a secondary leak-proof receptacle to enclose and protect the primary receptacle. All staff handling potentially infectious materials must be trained on safe handling practices and decontamination procedures. The parent virus of the GMO is sensitive to treatment with virostatic therapy such as cidofovir and ribavarin. As the viral characteristics of the parent virus are maintained by the GMO, the GMO is also expected to be sensitive to virostatic treatment.

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

All areas where the GMO is manipulated, stored or transportedmust be equipped with appropriate viricidal disinfectant and spill kits for disinfection of any potential splashes or spills including materials as described in J.1 above. All GMO preparation areas must be decontaminated using a disinfectant to which adenovirus is sensitive and all personnel protective equipment discarded as Type III healthcare waste or disinfected.

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

Not applicable. The GMO will only be administered at clinical sites.

4. Plans for protecting human health and the environment in the event of an undesirable effect:

Study patients will be monitored for the occurrence of serious adverse events (SAE) according to the clinical protocol. SAEs will be evaluated per protocol. Health Authorities will be notified per local legislation as required.

Due to the lack of infectivity in non-human cells and specificity to human tumor epithelial cells, the probability of the GMO disrupting population dynamics outside of individuals treated under the clinical protocol is negligible. However, the unintended infection of vulnerable individuals (e.g. immunocompromised, pregnant or breastfeeding woman, children < 1 year of age) cannot be fully excluded. Therefore, the informed consent documents for the GMO advise patients to avoid close contact with individuals at a high risk from viral infections until 30 days after the last dose of the GMO. As the transgenes are under the control of the virus endogenous MLP which restricts transgene expression to cells that are actively replicating virus, off-target expression and replication is limited. Additionally, adenoviruses including the parent virus of the GMO is sensitive to virostatic

treatment such as cidofovir and ribavarin. As the viral characteristics of the parent virus are maintained by the GMO, the GMO is also expected to be sensitive to virostatic treatment.

REFERENCES

[1] A. Avellon, P. Perez, J. Aguilar, R. Lejarazu, J. Echevarria, Journal of Virological Methods, 92 (2001), 113-120