

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/18/14

- (c) Date of acknowledgement of notification:

04/07/2018

- (d) Title of the project

"A Phase I Study to Evaluate the Safety, Tolerability, and Efficacy of VCN-01 in Combination With Durvalumab (MEDI4736) in Subjects With Recurrent/ Metastatic Squamous Cell Carcinoma of the Head and Neck "

- (e) Proposed period of release

September 2018 - December 2020

2. Notifier

Name of institution or company:

*Institut Català d'Oncologia (ICO)**Gran Via 199-203**08908 Hospitalet de Llobregat, Barcelona*

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)

Order: Adenoviriae

Genus: Mastadenovirus

Species: Adenovirus humano tipo 5 (HAd5)

The GMO (VCN-01) is an oncolytic adenovirus, which is a replication competent virus selective of tumoral cells. VCN-01 selectively expresses a matrix-degrading enzyme (hyaluronidase) and its genome contains 4 genetic modifications with respect to the wild-type virus (HAd5).

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

Generally, double stranded DNA viruses, such as adenovirus, are stable. Epidemiologic data demonstrate that, despite more than 45 years of circulation in the human population, the genome sequence of HAd5 remained remarkably conserved. Likewise, genome stability of the same strain has been observed despite circulating, co-infected with other serotypes and amplified in the research community for 39 years.

Genetically modified HAd5 can be genetically stable as long as the size does not exceed 105% of the normal HAd5 genome. Larger vectors grow poorly and undergo rapid rearrangement, resulting in loss of non-essential DNA sequences, usually the insert.

In immunocompromised patients, there is evidence of recombination between adenoviruses, which is thought to play a major role in the evolution of new strains with intermediate or unique immunogenic and tropic properties for these serotypes, but no recombinants containing group C adenoviruses have been isolated to date.

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 (X) No (.)

If yes:

- Member State of notification: *ES*

- Notification number: *B/ES/13/04, B/ES/13/05 and B/ES/15/15*

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

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

According to the facts summarized below, the likelihood of the GMO to become persistent and invasive in natural habitats under the conditions of the proposed release is very low.

- *VCN-01 has been designed to selectively target, replicate in and kill human cancer cells, therefore it is expected to be able to spread only in such human cells.*
- *The host-range of human wild-type adenovirus is restricted to humans, and the likelihood of horizontal transmission to other species is negligible due to its high species-specific nature.*
- *The genetic stability of VCN-01 has been confirmed at high selective pressure and its genome has demonstrated to be stable in such conditions; therefore, the likelihood of mutation or reversion to the wild-type negligible.*
- *The recombination/transcomplementation between the genomes of VCN-01 and wild-type adenoviruses by co-infection at cellular level is highly unlikely (since VCN-01 amplification in non-tumour cells is not possible), and in case of occurrence no alteration with respect to basal HAd5 pathology is expected. Additionally, the vast majority of primary infections with species C adenovirus occur early in life (within the first two years), infecting more than 80% of the human population. As a consequence, the human population is rapidly converted into sero-positive for adenovirus after systemic exposure, which implies that any adenoviral infection is easily neutralized. Moreover, such infections are mostly asymptomatic, auto-limiting and restricted to several permissive tissues.*
- *VCN-01 (as all adenoviruses) is a non-integrative virus, therefore the likelihood of integration in the host cell DNA is very low. Additionally, to date, no germline transmission of adenovirus genetic material to the offspring has been observed even with a route of administration that forces the entrance of virus into the gonads and that is able to infect non-germ cells.*
- *Based on the low probability of transmission to thirds and the inability of VCN-01 to amplify or replicate in normal population, and taking into consideration the controlled conditions for the GMO release, the probability of shedding from unintended population allowing further spreading of the virus is considered negligible. Additionally, from the data available with previous clinical trials with VCN-01 (two phase I clinical trials currently ongoing in Spain), it has never been reported any case of infection of health care professionals, or virus spread to family member/thirds in contact with these patients once they are discharged after having received VCN-01 in the clinical trial facilities.*
- *All the methods and procedures required will be applied for controlling the dissemination of the GMO.*

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

The following premises have been established to complete the information below.

- *GMO: VCN-01*
- *Receptor: HAd5*
- *Donor: synthetic and human genome fragments inserted in HAd5 to generate VCN-01 replication selective adenovirus.*

1. Recipient or parental organism characterization:

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

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

other, specify

2. Name

- (i) order and/or higher taxon (for animals): *Adenoviriae*
- (ii) genus: *Mastadenovirus*
- (iii) species: *Human wild-type adenovirus serotype 5 (HAd5)*
- (iv) subspecies: *Type-C adenovirus*
- (v) strain -
- (vi) pathovar (biotype, ecotype, race, etc.) -
- (vii) common name: *HAd5*

3. Geographical distribution of the organism

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

Yes	(X)	No	(.)	Not known	(.)
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(b) Indigenous to, or otherwise established in, other EC countries:

(i) Yes (X)

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

Most of the human population (up to 70%) is sero-positive for type 5 human adenovirus (HAd5).

Atlantic	X
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Mediterranean	X
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Boreal	X
Alpine	X
Continental	X
Macaronesian	X

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

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

(d) Is it frequently kept in the country where the notification is made?
Yes (X) 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

The host range of HAd5 is restricted to humans. Although it is also described that chimpanzees (Pan troglodytes), swine (Sus scrofa), cotton rats (Sigmodon hispidus) and some kind of hamsters (Mesocricetus auratus) are semi-permissive species for the replication of human adenovirus, no natural infections with HAd5 have been described in these species to date.

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

5. (a) Detection techniques

The detection of physical particles is done by real-time PCR (RT-PCR) directly from DNA obtained from the tissue/organ tested, and using oligonucleotides that amplify a non-coding region of the virus genome.

The detection of infectious particles is done by plaque assays (in which the in vitro generation of plaques in monolayers of human cells is examined), anti-hexon or TCID₅₀ (50% Tissue Culture Infective Dose).

(b) Identification techniques

The specific identification of adenovirus is performed at genomic viral DNA level by PCR using oligonucleotides with a defined complementary sequence and also by restriction enzyme analysis of the purified viral DNA.

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

In terms of biosafety, HAd5 are human adenoviruses classified as class 2 biological agents. Adenoviruses are pathogens that can cause human or animal disease but are unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventative measures are available and the risk of spread of infection is limited. Therefore HAd5 manipulation poses a moderate individual risk but low community risk.

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

In terms of biosafety, HAd5 are human adenoviruses classified as class 2 biological agents. Adenovirus can cause an array of clinical diseases, most of which occur in children younger than the age of 5 years. Adenoviral infections are generally self-limiting and asymptomatic despite virologic and serologic proof of infection, and only around 45% of infections are manifested by disease. Usual signs and symptoms of infection may include fever, nasal congestion, coryza, tonsillitis, pharyngitis, cervical adenopathy, cough with or without otitis media, and diarrhea. The incubation period is 2-14 days.

The vast majority of primary infections with species C adenovirus occur early in life (within the first two years), infecting more than 80% of the human population. By the age of 10, a majority of individuals have been infected with one or more serotypes. As a consequence, most of the human population is sero-positive for adenovirus, and since HAd-C is a widespread species, any adenoviral infection is easily neutralized.

Unlike retroviruses and lentiviruses, adenoviruses are non-integrative viruses. To date, no germline transmission of adenovirus genetic material to the offspring has been observed even with a route of administration that forces the entrance of virus into the gonads and that is able to infect non-germ cells.

8. Information concerning reproduction

- (a) Generation time in natural ecosystems:

Not applicable. HAd5 is not found in natural ecosystems, its only reservoir is human cells.

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

Not applicable

(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

None. Not applicable

(b) relevant factors affecting survivability:

Adenoviruses are very stable in the environment. But can be effectively inactivated by heat. Heating to 56 °C for 30 min; 60 °C for 2 min and autoclaving will destroy infectivity. HAd5 has been proven to be susceptible to sodium hypochlorite 1%, glutaraldehyde 2%, peracetic acid (1000 ppm), sodium dodecyl sulfate (SDS) 0.25% and alcohol-based hand gels (1-propanol 30% or ethanol ≥55%, v/v). Infectious titer is rapidly inactivated by exposure to any of the above biocides.

10. (a) Ways of dissemination

The mode of transmission of adenovirus is through the respiratory and oral-fecal routes. Infection can also spread through contaminated fomites, fingers, ophthalmic solutions, and airborne particulates. Children can shed non enteric adenovirus in throat and stool samples for 3 to 6 weeks following lower respiratory infection or generalized illness.

According to the public health agency of Canada, the inhalation of 5 infectious adenovirus particles (serotype 4) can cause disease in individuals free of serum antibodies against this serotype; and the infectious dose for adenovirus serotype 7 is above 150 plaque forming units as nasal drops. However, to the best of our knowledge, there has not been reported a clear infectious dose threshold for serotype 5.

Nonetheless adenoviral infections are usually self-limiting. Although other animal species have demonstrated to be semi-permissive for human adenoviruses replication (swine, cotton rats, Syrian hamster and chimpanzees), besides humans, there is no effective infection of other hosts.

(b) Factors affecting dissemination

Please refer to section 10.(a).

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

The following premises have been established to complete the information below.

- *GMO: VCN-01*
- *Receptor: HAd5*
- *Donor: synthetic and human genome fragments inserted in HAd5 to generate VCN-01 replication selective adenovirus.*

1. Type of the genetic modification

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

2. Intended outcome of the genetic modification

VCN-01 is an oncolytic adenovirus derived from HAd5 designed for the treatment of desmotic tumours. Its genome contains several modifications that confer tumour selectivity and anti-tumour activity. The 4 independent genetic modifications on the backbone of wild-type HAd5 adenovirus are: 1) the insertion of a tumour-specific synthetic promoter that inhibits the expression of viral proteins in normal cells; 2) the $\Delta 24$ mutation of the E1A gene that inhibits the viral replication in normal cells; 3) the RGD substitution of KKTK in the adenovirus fiber protein that results in improved tumour infectivity and lower liver infection; and 4) the inclusion of an expression cassette for the human sperm hyaluronidase (PH20) cDNA, that is a matrix-degrading enzyme that allows reducing interstitial fluid pressure, which facilitates the spread of therapeutic agents during treatment, thus enhancing the intratumour distribution of the oncolytic adenovirus and improves its therapeutic activity.

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 (X) No (.)

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	(X)
bacteriophage	(.)
virus	(.)
cosmid	(.)
transposable element	(.)
other, specify	

(b) Identity of the vector

To generate the replication selective virus, VCN-01, the plasmid pICOVIR-17KKTK was used.

(c) Host range of the vector

The vector genome was cloned in bacterial plasmids that also include genetic elements that allow its replication in yeasts.

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

Yes	(X)	No	(.)
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antibiotic resistance (.)

other, specify

Indication of which antibiotic resistance gene is inserted

(e) Constituent fragments of the vector

Plasmid pICOVIR-17KKTK contains the complete viral genome of VCN-01 flanked by sequences that allow its amplification and selection in bacteria and yeasts. Consequently, the plasmid contains the HAd5 genome with 4 genetic modifications (synthetic promoter E1A, Δ24 mutation, RDGK fiber protein modification and hyaluronidase expression cassette).

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

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

Transfection of a digested version of pICOVIR-17KKTK vector in A549 human lung adenocarcinoma cells to obtain the infectious VCN-01 adenovirus (containing the complete genome).

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	(.)

6. Composition of the insert
(a) Composition of the insert

HAd5 wild-type genome has been modified inserting four independent modifications.

<i>Composition of the insert</i>	<i>Source</i>	<i>Intended function</i>
<i>Tumour-specific synthetic promoter composed by 4 human E2F-1 transcription factor-binding boxes and 1 Sp-1-binding box</i>	<i>Synthetic</i>	<i>Controls the expression of E1A viral protein: inhibits the expression of viral proteins in normal cells and restricts E1A expression and activation of viral replication in tumour cells (which have high levels of active E2F-1).</i>
<i>Mutation Δ24 (deletion of 24 bp in E1A gene)</i>	<i>Not applicable</i>	<i>Inhibits that E1A viral protein binds to pRb and releases E2F transcription factor, thus, VCN-01 is unable to activate viral replication in normal cells.</i>
<i>⁹¹RGDK⁹⁴ substitution of ⁹¹KTK⁹⁴ in the adenovirus fiber protein</i>	<i>Synthetic</i>	<i>Such amino acids are involved in adenovirus interaction with heparin sulfate proteoglycans of the cell membrane. ⁹¹RGDK⁹⁴ substitution reduces the liver uptake after systemic administration, which improves the virus bioavailability, enhancing the tumour infectivity.</i>
<i>Insert of an expression cassette for the human sperm hyaluronidase (PH20) cDNA</i>	<i>Synthetic (promoter and signals) and human (cDNA)</i>	<i>Expresses a matrix-degrading enzyme that allows reducing interstitial fluid pressure, which facilitates the spread of therapeutic agents during treatment (such as chemotherapy), thus enhancing the intratumour distribution of the oncolytic adenovirus and improving its therapeutic activity.</i>

(b) Source of each constituent part of the insert

Please refer to section a).

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

Please refer to section a).

(e) Location of the insert in the host organism

- on a free plasmid (.)
- integrated in the chromosome (.)
- other, specify: *Integrated in the HAd5 genome*

(f) 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

The following premises have been established to complete the information below.

- *GMO: VCN-01*
- *Receptor: HAd5*
- *Donor: synthetic and human genome fragments inserted in HAd5 to generate VCN-01 replication selective adenovirus.*

1. Indicate whether it is a:

viroid	(.)	
RNA virus	(.)	
DNA virus	(.)	
bacterium	(.)	
fungus	(.)	
animal		
- mammals	(X)	<i>Homo sapiens (for PH20)</i>
- insect	(.)	
- fish	(.)	
- other animal	(.)	
(specify phylum, class)		
other, specify		<i>Synthetic</i>

2. Complete name

(i) order and/or higher taxon (for animals)	<i>Primate</i>
(ii) family name for plants	<i>Hominidae</i>
(iii) genus	<i>Homo</i>
(iv) species	<i>Homo Sapiens</i>
(v) subspecies	<i>Homo Sapiens Sapiens</i>
(vi) strain	<i>Not applicable</i>
(vii) cultivar/breeding line	<i>Not applicable</i>
(viii) pathovar	<i>Not applicable</i>
(ix) common name	<i>Human Species</i>

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	(.)
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If yes, specify the following:

 - (a) to which of the following organisms: *Not applicable*

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

Not applicable

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)
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If yes, specify

5. Do the donor and recipient organism exchange genetic material naturally?

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

E. Information relating to the genetically modified organism

The following premises have been established to complete the information below.

- *GMO: VCN-01*
- *Receptor: HAd5*
- *Donor: synthetic and human genome fragments inserted in HAd5 to generate VCN-01 replication selective adenovirus.*

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 (X) No (.) Unknown (.)

Specify

Whereas HAd5 can replicate in any epithelial human cell, VCN-01 has been designed to only replicate in human tumour cells.

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

Specify

VCN-01 does not replicate in normal human cells, and is only pathogenic in human tumour cells. In this sense, the GMO does not generate the pathogenicity of the respiratory tracts typically caused by HAd5 wild-type.

2. Genetic stability of the genetically modified organism

The cell line used to propagate VCN-01 is A549 continuous cell line, which was selected because it has no sequence homology between the viral genome and the genome of the complementation cells. Moreover, the Master Cell Bank was confirmed to be free of viral

contaminants, including HAd5 by qPCR against E1A protein. Thus, the risk of recombination between them during the manufacturing process is minimized.

Additionally, the identity of the GMO by restriction enzyme analysis is monitored in different steps along the manufacturing process of VCN-01 (pre-Viral Seed Material, Master Viral Seed Stock, Propagation and Harvest, Purified Harvest and Final Lot) and the DNA viral sequence corresponding to the four genetic modifications are tested at pre-Viral Seed Material and Master Viral Seed Stock.

Moreover, the genetic stability of VCN-01 is assessed as part of the specification of the Purified Harvest, as recommended by the European Pharmacopoeia monograph. The method of choice for detecting genomic instability is the amplification in non-permissive cells since multiple serial passages in non-permissive cells applies selective pressure similar to that in vivo. By using a bioamplification assay, VCN-01 genome was proven to be stable even when submitted to a high selective pressure.

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 modifications contained in VCN-01 significantly reduce its pathogenesis compared to HAd5. In this sense, VCN-01 only undergoes productive replication in tumour cells, which results in significantly reduced toxicity after in vivo administration.

4. Description of identification and detection methods

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

VCN-01 DNA can be detected in the environment by quantitative RT-PCR methods.

- (b) Techniques used to identify the GMO

DNA sequencing. VCN-01 identity is analyzed by PCR and restriction enzyme analysis of the viral DNA purified with a selected set of restriction enzymes.

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 GMO release is the conduct of a phase I clinical trial with a single intravenous administration of VCN-01, in a maximum of 20 patients.
It is not expected any significant potential environmental benefits.*

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 GMO will be released under controlled conditions by trained medical staff in the specific sites of the hospital facilities that take part of the proposed clinical trial.

3. Information concerning the release and the surrounding area

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

- Servicio de Oncología Médica del Institut Català d'Oncologia (ICO) – Hospital Duran i Reynals. Gran Via 199-203. 08908 Hospitalet de Llobregat, Barcelona*
- Servicio de Farmacia del Hospital Vall d'Hebrón Ps.Vall d'Hebron 119-129, 08035 Barcelona*
- Hospital Sant Rafael (Germanes Hospitalaries). Passeig Vall d'Hebron 107-117, 2ª planta. 08035, Barcelona*

- (b) Size of the site (m²):

(i) actual release site (m²): *Please refer to section ii)*

(ii) wider release site (m²):

When referring to "release" it is considered the manipulation and administration of VCN-01. Therefore, the release site is the biosafety cabinet of the hospital pharmacy and the individual room of the hospital. In such places, and during the 24 hours hospitalization of the patient, they will be of controlled access and limited to the medical staff that has been previously trained in the measures and procedures for dealing with VCN-01.

- (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

Not applicable

4. Method and amount of release

- (a) Quantities of GMOs to be released:

In this proposed phase I study, two dose levels will be used (3.3x10¹² y 1x10¹³ vp/patient). Each patient will receive a single intravenous administration at the corresponding dose level. The total amount of VCN-01 administered to all the patients during the whole study will be 1.4x10¹⁴ vp.

- (b) Duration of the operation:

The duration of each intervention will be approximately 10 minutes.

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

Sealed and duly labeled vials containing VCN-01 will be supplied for clinical use only and the release of the GMO will be responsibility of trained medical staff and pharmacists, in accordance with the clinical protocol and the Good Clinical Practices.

The product must be prepared in the hospital pharmacy in aseptic conditions. Biosafety level-2 practices and equipment will be used, the safety cabinet will be disinfected before and after its use with a conventional disinfectant (bleach, soap or similar). The medical staff involved will receive training to work under the biosafety practices applicable to the storage, transport, preparation and administration of VCN-01, GMO removal and waste management and to be in contact with the patient treated.

Type III biohazardous waste containers will be used for all the material in contact with the GMO (needles, gloves, empty vials, etc.) and waste management will be conducted by a specialized Company. Methods and procedures in case of accidental spill will be contained in a protocol. Please refer to section J.1.

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

Mediterranean climate and controlled climatic conditions inside the hospital facilities.

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.

VCN-01 is currently being administered in other ongoing phase I clinical trials in Spain, but information on shedding is not yet available from all of them. However, a brief summary of available presence of VCN-01 genomes in blood and its shedding in biological fluids from study B/ES/13/04 (VCN-01 intravenously administered) is provided as follows.

The shedding profile of VCN-01 after systemic administration is observed mainly within the first 8 days post-treatment in all biologic fluids analysed (sputum, faeces and urine).

In blood there are two peaks of VCN-01 genomes, at the day of administration (day 1, 4h post-administration, 100% positive samples), with a nadir at day 2, and at the highest doses a secondary peak of VCN-01 genomes is observed at day 3 corresponding to viral replication (95% positive samples). VCN-01 genomes in blood remain positive (10-1000-fold lower with respect to day 1) in the majority of patients at day 28.

The main fluids for VCN-01 shedding are sputum and faeces. In sputum, viral genome levels correlate with viral genome peaks in blood at days 1 and 3, and the highest peak is detected at day 8 (82% positive samples analysed on that day). In faeces, the peak of viral genomes is detected at day 8 (90% positive samples analysed on that day). The least important fluid for shedding is urine, where the peak occurs between day 3 and 8 (20% positive samples analysed).

In any case, during the conduct of these trials, it has never been reported any case of infection of health care professionals, or virus spread to family member/thirds in contact with these patients once they are discharged after having received VCN-01 in the clinical trial facilities. Therefore, no adverse effects have been detected to have an impact on the environment and human health.

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) | <i>Primate</i> |
| (ii) | family name for plants | <i>Hominidae</i> |
| (iii) | genus | <i>Homo</i> |
| (iv) | species | <i>Homo Sapiens</i> |
| (v) | subspecies | <i>Homo Sapiens Sapiens</i> |
| (vi) | strain | <i>Not applicable</i> |
| (vii) | cultivar/breeding line | <i>Not applicable</i> |
| (viii) | pathovar | <i>Not applicable</i> |
| (ix) | common name | <i>Human Species (suffering from cancer)</i> |

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

The released GMO in the framework of a phase I clinical trial in patients suffering from head and neck tumours seeks to cause the elimination of the tumour masses. After the infection VCN-01, it will recognize whether the cell is tumoural according to the high levels of expression of E2F-1 transcription factor and, only if positive, start replication. By doing this, the infected tumour cell will die and VCN-01 will have generated approx. 10000 copies of itself, which will subsequently infect the neighboring cells, where, in turn, they will recognize whether the cell is tumoural or not. Additionally, the hyaluronidase expression of the virus will increase the intratumoral dissemination of the oncolytic effect.

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

The host range of HAd is restricted to humans, and VCN-01, compared to the wild-type HAd5, is attenuated and thus can only replicate in human tumour cells. Therefore, there are not expected any potentially significant interactions with other organisms in the environment.

Moreover, according to the proposed GMO release it is highly unlikely that VCN-01 interacts with any other species.

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

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

Give details

VCN-01 does not replicate in normal human cells and can only replicate in human tumour cells.

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

According to the proposed conditions for the GMO release it is highly unlikely that VCN-01 interacts with any untargeted population. Being the host range of HAd5 restricted to humans, and that the genetic modifications contained in VCN-01 do not modify the species-

specificity, the likelihood of productive transmission to animals is negligible. And in the unlikely event of unwanted administration to untargeted population, the intrinsic selectivity would avoid dissemination to non-tumour 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

The likelihood of effective transmission of VCN-01 to other species is negligible.

- | | | |
|--------|---|-----------------------|
| (i) | order and/or higher taxon (for animals) | <i>Not applicable</i> |
| (ii) | family name for plants | <i>Not applicable</i> |
| (iii) | genus | <i>Not applicable</i> |
| (iv) | species | <i>Not applicable</i> |
| (v) | subspecies | <i>Not applicable</i> |
| (vi) | strain | <i>Not applicable</i> |
| (vii) | cultivar/breeding line | <i>Not applicable</i> |
| (viii) | pathovar | <i>Not applicable</i> |
| (ix) | common name | <i>Not applicable</i> |

7. Likelihood of genetic exchange in vivo

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

The likelihood of horizontal transmission of VCN-01 to other species is very low.

- (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.):

No data available.

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

Not applicable

H. Information relating to monitoring

1. Methods for monitoring the GMOs

The analytical technique to monitor the shedding of the GMO is the RT-PCR (real-time Polymerase Chain Reaction) with specific oligonucleotides for VCN-01 genome. By means of the RT-PCR technique the levels of viral genomes can be evaluated in different types of

sample, in which it can be both assessed the presence of virus (punctual measure) and its replication capabilities (consecutive measures).

In parallel, the monitoring of the direct or indirect effects of the GMO in the patients during the clinical trial will be conducted in accordance with the clinical protocol and will include physical explorations and oncologic evaluations, etc. Adverse events notification and clinical lab assessments for all the patients.

2. Methods for monitoring ecosystem effects

Based on the low probability of transmission to thirds and the inability of VCN-01 to amplify or replicate in any other species, and taking into consideration the controlled conditions for the GMO release, the likelihood of any environmental impact due to the shedding to untargeted population is considered negligible. Additionally, in the currently ongoing clinical trials with VCN-01, it has never been reported any case of infection of health care professionals, or virus spread to family member/thirds in contact with these patients once they are discharged after having received the GMO in the clinical trial facilities.

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

Not applicable. The stable transfer of genetic material to any other species different to human is not possible. Additionally, the likelihood of interaction between the GMO and any untargeted population is negligible due to the limited environmental shedding under the proposed conditions of the release, the transitory presence of VCN-01 genomes in non-human populations (based in the specie-specificity of human adenoviruses) and the replication selectivity of VCN-01 for tumour cells.

4. Size of the monitoring area (m²)

The GMO will be administered intravenously to patients in the individual room of a hospital. The monitoring after the administration will be performed in an individual room of the corresponding hospital facility, until the patient is discharged the next day.

5. Duration of the monitoring

The duration of the treatment is 28 days and the patient monitoring will be every four weeks until disease progression or unacceptable toxicity.

6. Frequency of the monitoring

According to the clinical protocol, samples will be taken from the patients and will be handled by the nurses in charge of the study, who will also be responsible of the appropriate transport to the corresponding lab for their analysis.

Virus levels in blood and other biological fluids will be measured by PR-PCR technique for the presence of VCN-01 genomes in a centralized laboratory responsible for the analysis.

I. Information on post-release and waste treatment

1. Post-release treatment of the site

When referring to “release” it is considered the manipulation/preparation and administration of VCN-01 to the patient.

Treatment of the site post-release:

All equipments and working surfaces will be disinfected with a conventional disinfectant (bleach, soap or similar).

All the material in contact with the VCN-01 (needles, empty vials, gloves, disposable lab coat, etc.) will be removed in the type III medical waste containers handled by a specialized company.

All the sheets and clothes in contact with the patient should be preferably disposable, and once the administration is finished, they should be removed to a type III medical waste container handled by a specialized company.

The non-disposable material considered contaminated will be removed to a type III medical waste container (as if disposable) handled by a specialized company. Non-disposable material without evidence of contamination will be cleaned and disinfected according to the procedures in place in the hospital for the management of such material including a washing treatment at 90°C for 30 min using a disinfectant including soap.

In the event of an accidental spill or release, the measures and procedures that have been described in section J.1. will take place.

2. Post-release treatment of the GMOs

For the post-release treatment of the GMO, please refer to section I.3.b.

3. (a) Type and amount of waste generated

The type of waste generated during the clinical trial will be:

- *Waste from the preparation and administration of VCN-01 (empty vials, needles, gloves, etc.) and from the protection material used by the medical staff. All these materials will be disposed into type III medical waste containers, which will be handled by a specialized Company.*
- *Specimens from the patients: the material in contact with the samples recollected from the patients within the first 24 hours post-administration will be disposed into type III medical waste containers.*

(b) Treatment of waste

Any material in contact with VCN-01 will be considered as a type III medical waste (biological/biohazardous) and will be disposed accordingly. Type III medical waste containers will be duly labeled and managed by a specialized company.

The inactivation of VCN-01 is best achieved by autoclaving at 121 °C for 15 minutes (or for longer times / higher temperatures); also conventional disinfectants (bleach, soap or similar) are effective.

J. Information on emergency response plans

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

All the personnel involved in the manipulation of VCN-01 will be provided with a detailed description of the preparation, as well as the treatment of waste and the measures and procedures to follow in the event of an accident.

In the event of an accidental spillage or release, the following steps will be taken, as it is established in the corresponding protocol. The person in charge must use the adequate protective clothes and complements. The affected area will be isolated and contented and will be treated with a solution of 5% bleach and a Sodium Hydroxide 0.5% solution or with a Virkon solution for at least 20-30 min. Afterwards, the liquid will be absorbed with disposable towels or other absorbent material, which will be disposed into a plastic bag inside a type III medical waste container. The affected area will be then cleaned with water and soap using additional disposable towels, and, again, all this material will be disposed in the same container. Finally, once it has been checked that no material in contact with the GMO removal is left in the room, the gloves and other personal protection will be disposed in the same container and it can then be closed and managed by the specialized Company.

In the event of accidental inoculation, the standard procedures for such an accident should be followed, and notify the internal department of Occupational Health, and the doctor responsible for the study.

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

Please refer to section J.1.

The removal of GMO in areas potentially affected can be achieved using a conventional disinfectant (bleach, soap or similar) kept for 20-30 min before cleaning the area.

All the sheets/clothes in contact with the patient and considered contaminated will be removed to a type III medical waste container (whether or not disposable) handled by a specialized company.

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

Please refer to section J.1 and J.2.

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

All the patients treated with VCN-01 will be monitored during the clinical trial. Any serious adverse event will be registered and evaluated by the medical staff involved in the trial together with the sponsor and the Health Authorities will be accordingly notified.

VCN-01 is not able to lead productive infections in other hosts than humans, it does not integrate in the host cell genome and is attenuated compared to the parental virus in their life cycle and interaction with the natural host. The likelihood that the application of VCN-01 will lead to disturbance of the population dynamics in the natural environment is negligible. The likelihood of accidental exposure of non-target population to VCN-01 is negligible, taking into account the reduced levels of infectious virus shedding from target population (cancer patients), the limited thermal stability of the virus in the environment and the high levels of virus required for effective infection.

In the event that any accidental release occurs, the sponsor will be immediately informed and must, subsequently, immediately notify the Comisión Nacional de Bioseguridad (Ministerio para la Transición Ecológica) and to the Consejo Interministerial de Organismos Modificados Genéticamente (Ministerio de Agricultura, Pesca y Alimentación) of Spanish Government.