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/13/04

(c) Date of acknowledgement of notification: *04/10/2012*

(d) Title of the project
"A phase I, multicenter, open-label, dose escalation study of intravenous administration of VCN-01 oncolytic adenovirus alone and in combination with intravenous gemcitabine in patients with advanced solid tumors"

(e) Proposed period of release
Last patient inclusion: April 2013. Last patient last visit: December 2014

2. Notifier
Name of institution or company: *VCN Biosciences S.L.*
BioIncubadora I. Biopol'H
BioPol'H
Av Gran Via de l'Hospitalet 199-203
08908 - L'Hospitalet de Llobregat (Barcelona, SPAIN)

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 (as HAd5) 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 (X) No (.)

If yes, insert the country code(s): *ES*

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/13/05*

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 transcomplementation between the genomes of VCN-01 and wild-type adenoviruses by co-infection at cellular level is highly unlikely, since inability of VCN-01 to replicate in non-tumor cells decreases substantially the risk of co-infection. In spite of that, if co-infection occurs, an eventual transcomplementation event between both viruses will result in an infection identical to basal HAd5 infection, since VCN-01 pathology does not differ from HAd5. Additionally, most of the human population is sero-positive for adenovirus, 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 and all adenoviruses are non-integrative viruses, therefore the likelihood of integration in the host cell DNA is very low and no adverse effects are known.*
- *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.*
- *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 characterisation:

(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 (.)

(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
Mediteranean	X
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-codifying 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.

(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. HAdV infections are mostly asymptomatic but may cause diseases of the respiratory, ocular and gastro-intestinal system, especially in children. The incubation period for getting disease is 1-10 days. Most of the human population is sero-positive for adenovirus, and HAdV-C is a widespread species, which implies that any adenoviral infection is easily neutralized.

Unlike retroviruses and lentiviruses, adenoviruses are non-integrative viruses. It has been described that lymphoid organs could generate persistence, but such extreme is considered very unusual; even if adenovirus are able to integrate in the host cell DNA, no adverse effects are known. While considerable efforts have been put to create transgenic mice by means of direct injection of adenoviruses in the testis, male germ cells appear to be resistant to infection by adenovirus.

8. Information concerning reproduction

(a) Generation time in natural ecosystems:
Not applicable. HAd5 is not found in natural ecosystems, its only reservoir are 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	

Not applicable

(b) relevant factors affecting survivability:

Adenoviruses rapidly lose their bioactivity at room temperature. HAd5 is susceptible to 1% sodium hypochlorite, 2% glutaraldehyde and sodium dodecyl sulfate (SDS) 0.25%. And it is also sensitive to heat (> 56 °C). Inactivation of HAd5 is best achieved by autoclaving at 121 °C for 15 minutes; also conventional disinfectants (bleach, soap or similar) are effective against HAd5 if kept in contact for 20-30 min.

10. (a) Ways of dissemination

Adenovirus enters its host via the respiratory tract or the eye through aerosols generated by an infected individual (coughing or sneezing). Adenoviral transmission can also take place by contact with saliva, or via the oral-faecal route. According to the public health agency of Canada the lower limit for infection by inhalation is 150 plaque forming units. Nonetheless adenoviral infections are usually self-limiting. Studies with live HAdV vaccines have shown that after enteric administration transmission may take place, presumably via the oral-faecal route, and that transmission requires intimate physical contact. Infection of casual contacts after enteric administration is unlikely, even with fully virulent adenoviruses. Besides humans and chimpanzees, 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 Δ24 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 (.)

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.

Composition of the insert	*Source*	*Intended function*
Tumour-specific synthetic promoter composed by 4 human E2F-1 transcription factor-binding boxes and 1 Sp-1-binding box	*Synthetic*	*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).*
Mutation Δ24 (deletion of 24 bp in E1A gene)	*Not applicable*	*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.*
90RGDK93 substitution of 90KKTK93 in the adenovirus fiber protein	*Synthetic*	*Such amino acids are involved in adenovirus interaction with heparin sulfate proteoglycans of the cell membrane. 90RGDK93 substitution reduces the liver uptake after systemic administration, which improves the virus bioavailability, enhancing the tumour infectivity.*
Insert of an expression cassette for the human sperm hyaluronidase (PH20) cDNA	*Synthetic (promoter and signals) and human (cDNA)*	*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.*

(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).

(d) Location of the insert in the host organism

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

(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

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)	*Homo sapiens (for PH20)*
- insect	(.)	
- fish	(.)	
- other animal	(.)	
(specify phylum, class)		
other, specify		*Synthetic*

2. Complete name

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

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: *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 (.)

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)

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

Amplification in non-permissive cells is the method of choice for detecting genomic instability, since multiple serial passages in non-permissive cells applies selective pressure similar to that in vivo, such that unstable viruses may be subject to modifications that affect biological activity detectable using an in vitro cytotoxicity assay. By using a bioamplification assay, VCN-01 genome was proven to be stable even when submitted to a high selective pressure.
Additionally, the DNA viral sequence corresponding to the four genetic modifications is monitored in different steps along the manufacturing process of VCN-01 (pre-Viral Seed Material, Master Viral Seed Stock, Purification and Harvest, Purified Harvest and Final Lot). Moreover, the genetic stability is assessed as part of the specification of the Purified Harvest, as recommended by the European Pharmacopoeia monograph.

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 GMO will be released under controlled conditions in an individual room of a hospital according to the clinical protocol of a national, multicentric clinical trial. The proposed phase I clinical trial is aimed at treating patients with advanced solid tumours. VCN-01 will be administered intravenously. Only those patients enrolled with pancreatic cancer in first-line will receive concomitantly gemcitabine (via intravenous administration), which is the current standard authorized by Health Authorities for pancreatic cancer as a palliative treatment.

The release will be responsibility of trained medical staff and pharmacists and the potential for virus dissemination will be strictly controlled. All the methods and procedures to control GMO dissemination and removal in case of accident have been taken into account and are contained in a series of protocols, that all the medical staff involved in the manipulation of the product, as well as patients and familiars, will be provided with.

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 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):

- *Centro Integral Oncológico Clara Campal (CIOCC) – Hospital Universitario Madrid Sanchinarro.*
  C/Oña nº10, 28959 Madrid
- *Hospital Duran i Reynals.*
  Gran Via 199-203. 08908 Hospitalet de Llobregat, Barcelona

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

(i) actual release site (m^2): *Please refer to section ii)*

(ii) wider release site (m^2):

When referring to "release" it is considered the manipulation and administration of VCN-01. Therefore, the release site is, on the one hand, the biosafety cabinet of the hospital pharmacy, and on the other hand, 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, four dose levels will be used (1xE11 vp, 1xE12 vp, 3.3xE12 vp and 1xE13 vp). 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 1.7xE14 vp.

(b) Duration of the operation:

The whole treatment will be 28 days. Each patient will receive a single intravenous administration at the corresponding dose level. The duration of each intervention will be approximately 30-45 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 to the hospital facility where the GMO administration will take place. The release will be responsibility of trained medical staff and pharmacists.

- *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). 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.*
  The transport of the prepared GMO for injection will take place in an airtight container, according to policies for transport of live virus vaccines.
- *The release will be responsibility of trained medical staff, in accordance with the clinical protocol and the Good Clinical Practices, in an individual room. The medical staff must wear disposable lab coat, gloves, glasses and FFP3 masks and cover-shoes. Before the administration, a warning sign on the door will inform about the treatment, restrictions and potential risks, and inside the individual room, type III medical waste containers will be put.*
  To avoid the GMO dissemination out of the release site, all the material in contact with the VCN-01 will be removed in the type III type III medical waste containers. After the administration, all equipments and working surfaces will be disinfected with a conventional disinfectant (bleach, soap or similar).
- *Once VCN-01 has been administered, the patient will remain in the same individual room, where he/she will be monitored for the next 24 hours. The room management will be supervised by nurses from the Unidad de Investigación Clínica. It will keep the warning sign on the door, which will remain closed. The patient visits will be*

restricted to one familiar. The procedures for the medical staff and visitors that come into contact with the patients treated with VCN-01 will be contained in a protocol, and it will be mandatory that they wear disposable lab coat, gloves, glasses and mask.

According to the clinical protocol, samples will be taken from the patient. There will be written methods and procedures for the safe handling of biological samples (which will be recollected within the 24h post-administration) and the nurses involved in the study will be responsible of the appropriate transport to the corresponding lab for their analysis. The specimens from the patient will be put in a double sealed bag inside an airtight container labeled as biohazardous material, and will be transported as biological/biohazardous samples.

- *Once the patient is discharged after the treatment, the room will be disinfected with a conventional disinfectant (bleach, soap or similar). Patients and familiars will receive preventive measures instructions to avoid the spread of the GMO beyond the site of release.*
- *Methods and procedures for GMO removal in case of accidental spill will be contained in a protocol. Please refer to section D.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.

Data not available.

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

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 advanced solid tumours seeks to cause the removal of the tumour mass. Once VCN-01 infects a cell, it recognizes according to the high levels of expression of E2F-1 transcription factor whether the cell is tumoural and, only if positive, starts 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 its turn, they will recognize whether the cell is tumoural or not. Additionally, the hyaluronidase expression of the virus will increase the intratumour dissemination of the oncolytic effect, and enhance the permeability of the tumour to chemotherapy, if administered concomitantly.

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 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)	*Not applicable*
(ii)	family name for plants	*Not applicable*
(iii)	genus	*Not applicable*
(iv)	species	*Not applicable*
(v)	subspecies	*Not applicable*
(vi)	strain	*Not applicable*
(vii)	cultivar/breeding line	*Not applicable*
(viii)	pathovar	*Not applicable*
(ix)	common name	*Not applicable*

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, ECGs, vital signs, adverse events notification, histology 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.

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 (m^2)

The GMO will be administered intravenously to patients in the individual room of a hospital. The monitoring after the administration will be performed in the same room of the corresponding hospital facility.

5. Duration of the monitoring

The duration of the monitoring will last for a minimum of 3 weeks after VCN-01 administration and until the end of treatment (day 28). Additionally, a follow-up of each patient has been proposed until 6 months after the end of treatment.

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. The specimens from the patient will be put in a double sealed bag inside an airtight container labeled as biohazardous material, and will be transported as biological/biohazardous samples.

The frequency of the monitoring will be the following:

- *VCN-01 shedding will be evaluated in blood, sputum, urine and faeces at days 1, 2, 7, 14, 21 and 28 (end of treatment).*
- *Presence of neutralizing antibodies (anti-adenovirus) will be evaluated at days 0, 14 and 28 (end of treatment), and also at the monthly follow-up visits up to 6 months after the end of treatment.*

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 and administration of VCN-01. Therefore, the release site is, on the one hand, the biosafety cabinet of the hospital pharmacy, and on the other hand, the individual room of the hospital. In such places (where VCN-01 is manipulated or administered), there should always be a disinfectant at hand (bleach, soap or similar), and all equipments and working surfaces will be disinfected with a conventional disinfectant (bleach, soap or similar) before and after its use. All the material in contact with the VCN-01 (needles, gloves, empty vials, etc.) will be removed in type III medical waste containers, which will have been put in the room before the treatment starts.

All the sheets and clothes in contact with the patient should be preferably disposable, and once the patient is discharged, they should be removed to a type III medical waste container.

After the administration, the patient will remain in the same individual room, where he/she will be monitored for 24 hours. According to the clinical protocol, samples will be taken from the patient and the nurses involved in the study will be responsible of the appropriate transport to the corresponding lab for their analysis. The specimens from the patient will be put in a double sealed bag inside an airtight container labelled as biohazardous material, and will be transported as biological/biohazardous samples.

Once the patient is discharged after the treatment, the room will be disinfected with a conventional disinfectant (bleach, soap or similar).

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 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 against VCN-01 if kept in contact for 20-30 min.

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 with a SOP with the administration procedure, 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.

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, with a follow-up of six months after the end of treatment. 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 Ministerio de Medio Ambiente of Spanish Government.