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	Sweden (SE)
(b)	Notification number	B/SE/14/000614-64
(c)	Date of acknowledgement of notification	2015-09-28
(d)	Title of the project	Phase I/IIa Study of recombinant adenovirus in patients with neuroendocrine tumors; safety and efficacy
(e)	Proposed period of release	From 01/01/2016 until 30/06/2018

2. Notifier

Name of institution or company: Uppsala University, Department of Immunology, Genetics and Pathology, Uppsala, Sweden

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 …

(b) Identity of the GMO (genus and species)
AdVince (also known as Ad5PTD(CgA-E1AmiR122) is a neuroendocrine-specific replicating oncolytic adenovirus designed to treat patients with neuroendocrine tumors and

their liver metastases. It is a human group C (serotype 5) adenovirus, that features a human chromogranin A promoter and mouse H19 insulator controlling expression of the adenoviral E1A gene for selective replication of the virus in neuroendocrine cells. It also contains microRNA target sequences inserted in the 3'UTR of E1A for reduced virus replication in normal hepatocytes. Furthermore, a peptide transduction domain (PTD) motif is inserted in the capsid (hexon protein, hypervariable region 5) for increased infectivity (Leja, Dzojic et al. 2007, Leja, Nilsson et al. 2010, Yu, Jin et al. 2011). It does not contain the E1B region.

(c) Genetic stability – according to Annex IIIa, II, A(10)
AdVince was subjected to sequential passages (from pre-master seed to production level) in human embryonic kidney (HEK) 293 cells, which contains the adenoviral E1 region in its genome. Recombination between AdVince sequence and the E1 region in the 293 genome, during virus production, can result in the generation of E1B-containing replication competent adenovirus (RCA) recombinants. The estimated level of RCA in the clinical product is less than 1 RCA particle per million vector particles. The contribution of recombinants will be most likely minimal due to infection competition of AdVince particles, which are present in a 1,000,000-fold excess. Potential leakage of wild-type-like recombinants should be compared to the effects of systemic infection of Ad5. However levels of recombinants can not reached levels associated with a fatal outcome in severely immune-compromised individuals (5x10E12-5x10E13 vp).

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 B/../../…

Please use the following country codes:
Austria AT; Belgium BE; Germany DE; Denmark DK; Spain ES; Finland FI; France FR; United Kingdom GB; Greece GR; Ireland IE; Iceland IS; Italy IT; Luxembourg LU; Netherlands NL; Norway NO; Portugal PT; Sweden SE

6. Has the same GMO been notified for release or placing on the market outside the Community by the same or other notifier?

Yes (.) No (X)

If yes:
- Member State of notification …
- Notification number B/../../…

7. Summary of the potential environmental impact of the release of the GMOs.
AdVince is derived from human adenovirus type 5 (Ad5). Wild type Ad5 is ubiquitous and causes self-limiting infections of the upper respiratory tract and give cold-like symptoms. Ad5 is a class 2 microorganism, which means it can cause a disease in humans but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment.

AdVince mimics some of the characteristics of the wild-type but it may have a broader tropism than Ad5, which potentially means that cells normally not infected by Ad5 can become infected. However, AdVince can only replicate in neuroendocrine cells, which significantly reduces the risk of unwanted spread. Therefore, the risk associated is low since human adenoviral infection is very common with mild symptoms and the majority of adults are already seropositive.
AdVince will be injected into the hepatic artery to target NET metastases in the liver. After injection, it is expected that AdVince will be present transiently in patients' blood and possibly, but unlikely in urine. Blood and urine samples will be analyzed by quantitative PCR to monitor virus shedding. Spreading of this virus outside the cancer patients is extremely unlikely. AdVince will be handled as biohazardous agent to avoid exposure to personnel and environment. All procedures with AdVince such as preparation and dilution will take place in a biosafety cabinet. Personnel will be advised of special hazards and will receive instructions regarding virus preparation and will follow safety measures during AdVince administration to minimize exposure of the hospital environment to high titers of virus. Remaining virus will be collected in containers with Virkon to inactivate virus particles.

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

1. Recipient or parental organism characterisation:

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

(select one only)

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

other, specify …

2. Name

(i)	order and/or higher taxon (for animals)	Adenoviridae
(ii)	genus	Mastadenovirus
(iii)	species	Human Adenovirus
(iv)	subspecies	subgroup C
(v)	strain	serotype 5
(vi)	pathovar (biotype, ecotype, race, etc.)	-

(vii) common name Ad5

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:

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 (.) 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, specify	man

(b) If the organism is an animal: natural habitat or usual agroecosystem:
…

5. (a) Detection techniques
Quantitative PCR

(b) Identification techniques
Quantitative PCR

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

Adenovirus is characterized as GMM/GMO class 2 by the Swedish Work Enviroment Authority. The applicable guidelines for handling, protection, labeling and destruction of AdVince will be followed and the study personnel will be trained to handle GMM. Handling of the AdVince at restricted areas at the Pharmacy (-80°C Freezer), class II laboratories at Rudbeck Laboratory, Uppsala University and Uppsala University Hospital must be approved by the Swedish Work Environment Authority and/or by the Swedish Medical Products Agency before start of the study.

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

Adenovirus serotype 5 infects humans and causes mostly mild non-chronic disease of upper respiratory tract. Individuals with a normally functioning immune system are not endangered by this virus. Most people have been exposed to Ad5 and have developed immunity against the virus. Infections in animals, caused by human Ad have been observed only under experiments in laboratory environment.

AdVince is a laboratory product, which is not found in the natural environment. Adenovirus vector may cause serious immunogenic reactions if administered systemically in high titers as it was in 1999 when a patient died after injection of a high-dose adenoviral vector into the hepatic artery. However, adenoviral vectors and adenoviruses have been used without toxicity in a variety of clinical trials. Nearly 200 infusions of recombinant competent adenovirus ONYX-015 (Ad2/5 chimera, E1B-deleted) were administered directly into the hepatic artery without significant toxicity (Au, Thorne et al. 2007). Patients received intrahepatic injections of ONYX-015 at the highest dose 2×10^{12} vp, up to eight times without dose-limiting toxicities. Intravenous infusions of conditionally replication competent adenoviruses ONYX-015 and CG7870 were performed at doses 2×10^{13} and 6×10^{12} particles, respectively, without identification of a maximally tolerated dose to date (Reid, Warren et al. 2002). Dose-limiting toxicity in humans was reached in a trial using hepatic artery infusion of a non-replicating adenovirus expressing p53, rAd.p53. The virus was well-tolerated following hepatic artery infusion at doses of up to and 2.5×10^{13} particles. At a dose of 7.5×10^{13} particles, rAd.p53 was associated with dose-limiting cardiac output suppression (Reid, Warren et al. 2002).

Replication of AdVince is restricted to take place in normal and neoplastic neuroendocrine cells. Possible off-target effects are normal neuroendocrine cells in the mucosa (cells from

which the cancer may arrive). This risk should be low due to the route of injection (virus will be injected into the hepatic artery). Another possible off-target cells are endocrine cells in the pancreas, which do express CgA and could therefore provide virus replication. This risk is also considered to be minimal due to the site of injection, since the hepatic artery is separate from the pancreatic artery. Virus replication in tumor cells can give rise to a second wave of viremia due to the release of progeny virus into the circulation. Other clinical trials indicate that a level of 10^3-10^4 vp per ml blood can be expected. This is associated with a moderate risk of toxicity. Addition of cyclophosphamide as an adjuvant to suppress antiviral immunity will potentially increase the therapeutic effect and thereby also the putative toxicity. However, cyclophosphamide has been used together with injection of adenoviruses and increased toxicity has not been reported (Cerullo, Diaconu et al. 2011).

The addition of the PTD sequence into the capsid of the adenovirus is increasing the transduction efficiency of tumor cells but potentially also of normal cells (Yu, Jin et al. 2011). This is expected to lead to increased efficiency but possibly also to increased toxicity. However, PTD-modified Ad5 showed a favorable biodistribution profile in mice after intravenous injection compared to non-modified Ad5 (Yu, Jin et al. 2011). Liver toxicity has been reported in mouse models but has not been a severe dose-limiting effect in human trials. Nevertheless, since AdVince will be injected into the hepatic artery to target liver metastases, microRNA target sequences are added for the liver-specific miR122 in the virus genome. This leads to specific degradation of virus E1A in hepatocytes and a higher safety profile that a virus without miR122 target sequences (Cawood, Chen et al. 2009, Leja, Nilsson et al. 2010, Ylosmaki, Lavilla-Alonso et al. 2013).

8. Information concerning reproduction

(a) Generation time in natural ecosystems:
…

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

(c) Way of reproduction: Sexual .. Asexual ..

(c) Factors affecting reproduction:
AdVince replicates in human hosts. It preferentially replicates in neuroendocrine cells and/or in cells, where the human CgA promoter is active. Replication of AdVince is suppressed in liver cells, where microRNA 122 molecules are expressed. AdVince is produced in the permissive cell line HEK 293, which contains the adenoviral E1 region in its genome. Therefore recombination events between the AdVince E1A region and the HEK293 E1 region are possible. The final clinical AdVince product was assessed for presence of wild-type E1B-contatining recombinant adenoviruses. The estimated level of RCA in the clinical product is less than 1 RCA particle per million vector particles.

9. Survivability

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

(i)	endospores	(.)
(ii)	cysts	(.)
(iii)	sclerotia	(.)
(iv)	asexual spores (fungi)	(.)

(v)	sexual spores (funghi)	(.)
(vi)	eggs	(.)
(vii)	pupae	(.)
(viii)	larvae	(.)
(ix)	other, specify …	

(b) relevant factors affecting survivability:
Storage in buffer at -80°C to +4°C allows survival of virus outside hosts.

10. (a) Ways of dissemination
Direct contact, aerosol formation, possible spread through the fecal-oral route**.**

(b) Factors affecting dissemination

11. Previous genetic modifications of the recipient or parental organism already notified for release in the country where the notification is made (give notification numbers)
…, B/../../…

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

1. Type of the genetic modification

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

2. Intended outcome of the genetic modification
The native E1A promoter region has been deleted and replaced by short mouse H19 core insulator and the human chromogranin A (CgA) promoter to make AdVince replication neuroendocrine selective. Six copies of target sequences for the liver-specific microRNA miR122 have been inserted within the 3' UTR of the E1A gene. A cassette with the modified E1A region is in the pShuttle(i/CgA-E1A-miR122) (Leja, Nilsson et al. 2010). The adenoviral genome is contained in the bacmid Ad5PTD(wt) (Yu, Jin et al. 2011). The Tat-PTD sequence is inserted within hexon sequence, in the so called hypervariable region (HVR) 5. AdVince was generated based on λ-phage mediated-recombineering in *Escherichia coli* strain SW102 by replacing the E1 region with the i/CgA-E1A-miR122 cassette.
AdVince will be injected into hepatic artery in patients with neuroendocrine liver metastases. After infecting neuroendocrine cancer cells, AdVince will initiate replication process, which ends with cell lysis and the release of progeny viruses into the tumor environment. Progeny viruses infect new cancer cells and virus spreads within the tumor. Normal cells, including hepatocytes, can be infected by AdVince. However virus replication will be suppressed due to presence of selective CgA promoter and miR122 target sequences.

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	bacmid

(b) Identity of the vector
1) pAd5PTD(wt)
2) pShuttle (i/CgA-E1A-miR122)

(c) Host range of the vector
Escherichia coli

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

Yes (X) No (.)

antibiotic resistance	(X)
other, specify	…

Indication of which antibiotic resistance gene is inserted: kanamycin in pShuttle (i/CgA-E1A-miR122) and chloramphenicol in pAd5PTD(wt)
Note that none of the antibiotic resistance gene are present in the final viral construct AdVince.

(e) Constituent fragments of the vector

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

(i)	transformation	(.)
(ii)	electroporation	(.)
(iii)	macroinjection	(.)
(iv)	microinjection	(.)
(v)	infection	(.)
(vi)	other, specify …	λ-phage mediated-recombineering

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
The short mouse core H19 insulator
Human CgA promoter
miR122 target sequences

(b) Source of each constituent part of the insert
Short mouse *H19 insulator*: PCR amplified from pShuttle (I/PPT-E1A). The long insulator (I) was cloned into pShuttle from pGEM-ICR, a kind gift from Dr. R. Ohlsson (Uppsala University, Uppsala, Sweden).
Human CgA promoter: PCR amplified from human genomic DNA (Roche, Indianapolis, IN) was cloned from the human DNA.
miR122 target sequences: a synthetic dsDNA sequence containing six tandem repeats complementary to the mature has-mir-122 target sequences (http://microrna.sanger.ac.uk) with six nucleotide spacers between each repeat, flanked by XbaI, HpaI and SmaI sites was purchased in a pGA4 plasmid (GeneArt, Regensburg, Germany)

(c) Intended function of each constituent part of the insert in the GMO
H19 insulator: shields the CgA promoter from the background transcriptional interference
CgA promoter: active in neuroendocrine cells, drives expression of adenoviral E1A gene
miR122 target sequences: recognized by homologous sequences to liver-specific miR122 molecules, which leads to degradation of E1A mRNA

(d) Location of the insert in the host organism

-	on a free plasmid	(.)
-	integrated in the chromosome	(.)
-	other, specify	integrated into the virus 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

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 …

2. Complete name

(i) order and/or higher taxon (for animals) …
(ii) family name for plants …
(iii) genus Homo and Mus
(iv) species Homo sapiens; Mus musculus
(v) subspecies …
(vi) strain …
(vii) cultivar/breeding line …
(viii) pathovar …
(ix) common name …

3. Is the organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?
Yes (.) No (X) Not known (.)
If yes, specify the following:

(b) to which of the following organisms:

humans (.)
animals (.)
plants (.)
other ..

(b) are the donated sequences involved in any way to the pathogenic or harmful properties of the organism
Yes () No (X) Not known (.)

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

4. Is the donor organism classified under existing Community rules relating to the protection of human health and the environment, such as Directive 90/679/EEC on the protection of workers from risks to exposure to biological agents at work?
Yes (.) No (X)
If yes, specify …

5. Do the donor and recipient organism exchange genetic material naturally?
Yes (.) No (X) Not known (.)

E. Information relating to the genetically modified organism

1. Genetic traits and phenotypic characteristics of the recipient or parental organism which have been changed as a result of the genetic modification

(a) is the GMO different from the recipient as far as survivability is concerned?
Yes (.) No (X) Not known (.)
Specify …

(b) is the GMO in any way different from the recipient as far as mode and/or rate of reproduction is concerned?
Yes (X) No (.) Unknown (.)
Specify AdVince replicates only in neuroendocrine cells or cells where the human CgA promoter is active. Replication is suppressed in cells expressing miR122 molecules.

(c) is the GMO in any way different from the recipient as far as dissemination is concerned?
Yes (X) No (.) Not known (.)
Specify AdVince contains tat-PTD motif on the capsid, which increase infectivity of the GMO in comparison to wild-type Ad5.

(d) is the GMO in any way different from the recipient as far as pathogenicity is concerned?
Yes (X) No (.) Not known (.)
Specify AdVince is replication-selective and it replicates in neuroendocrine cells or cells where CgA promoter is active. AdVince contains miR122 target sequences and therefore replication of AdVince is suppressed in cells expressing miR122 molecules. This makes the GMO less pathogenic that its wild-type counterpart. The addition of the PTD sequence into the capsid of the adenovirus is increasing the transduction efficiency of tumor cells but potentially also of normal cells (Yu, Jin et al. 2011). This is expected to lead to increased efficiency but possibly also to increased toxicity.

2. Genetic stability of the genetically modified organism
AdVince does not integrate into the host genome and survive only transiently in the host cell.

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)

AdVince DNA does not integrate into the host cell genome. Replication of AdVince is restricted to take place in normal and neoplastic neuroendocrine cells where the human CgA promoter is active. Possible off-target effects are normal neuroendocrine cells in the mucosa (cells from which the cancer may arrive) and endocrine cells in the pancreas. This risk should be low due to the route of injection (virus will be injected into the hepatic artery). Virus replication in tumor cells can give rise to a second wave of viremia due to the release of progeny virus into the circulation. Other clinical trials indicate that a level of 10^3-10^4 vp per ml blood can be expected. However, due to preformed anti-adenovirus antibodies long-term shedding of this virus is highly unlikely. The addition of the PTD sequence (the Peptide Transfer Domain from the HIV Tat protein) into the capsid of the adenovirus is increasing the transduction efficiency of tumor cells but potentially also of normal cells. This is expected to lead to increased efficiency but possibly also to increased toxicity. However, PTD-modified Ad5 showed a favorable biodistribution profile in mice after intravenous injection compared to non-modified Ad5. Liver toxicity has been reported in mouse models but has not been a severe dose-limiting effect in human trials. Nevertheless, AdVince contains liver-specific miR122 target sequences and this leads to specific degradation of virus E1A in hepatocytes and a higher safety profile that a virus without miR122 target sequences.

AdVince injections may induce flu-like symptoms, including fever, myalgia, asthenia, chills, and dry cough. However the symptoms are expected to be mostly mild (grade 1 and 2). Intrahepatic injections of adenovirus may increase levels of liver enzymes (ALT, ALP, AST), bilirubin and proinflammatory cytokines. Humoral and cytokine responses after AdVince treatment will be carefully monitored in blood samples. Liver function test will be assessed before and for 3 days following each treatment.

4. Description of identification and detection methods

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

(b) Techniques used to identify the GMO
Quantitative PCR and Sequencing

F. Information relating to the release

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

The initative for this Phase I/IIa study is based on basic and preclinical research in animal cancer models. AdVince is designed to target neuroendocrine tumors, in particular neuroendocrine liver metastases. Oncolytic viruses as AdVince have some advantages as anti-cancer agents over conventional chemotherapy drugs and small molecules. Virus propagates inside neuroendocrine tumor cells before lysing them, leading to the release of large amounts of progeny virus, which can infect neighbouring cells, thus amplifying the initial inoculum. AdVince kills through oncolysis and is not dependent on the apoptotic property of the target neuroendocrine cells and can therefore kill drug-resistant cancer cells.

Furthermore, introduction of a virus within a tumor will introduce danger signals that can alter the suppressive environment and induce strong anti-tumoral immune responses. The immune response raised against tumor-associated antigens can last long after the immune system has cleared the virus.

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 AdVince will be injected into hepatic artery to target neuroendocrine tumor metastases in the liver.

3. Information concerning the release and the surrounding area

(a) Geographical location (administrative region and where appropriate grid reference):
AdVince will be diluted in normal saline before administration at the University Hospital in Uppsala, Sweden. AdVince will be transported from the storage site (Pharmacy freezer) to administration site in sealed cryovials. AdVince will be handled only in pre-specified, restricted areas and biohazard cabinets approved by the Swedish Medical Product Agency.

(b) Size of the site (m^2): … m^2
(i) actual release site (m^2): … m^2
(ii) wider release site (m^2): … m^2

The patients will be kept in the restricted area (hospital room) during treatment.

(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:
Patients will be treated with four doses: (2x10e10 vp/ml; 2x10e11 vp/ml; 6x10e11 vp/ml or 2x10e12 vp/ml). A frozen vial with pre-diluted virus at a dose specified for the cohort is thawed and maintained at 2 to 8°C until dilution. The virus solution is then mixed with normal saline to a final volume of 10 ml. An SOP for preparation of infusion solution is written for hospital personnel and describes preparation of virus before tumor injection.

(b) Duration of the operation:
A single infusion of 10 ml of viral solution is administered slowly followed by a 10-ml normal saline flush given at the same speed and site. This procedure takes approximately 10-20 min.

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

Transfer of the GMO will be done in sealed containers. The hospital personnel will wear protective clothes and gloves. Detailed procedures regarding AdVince preparation and inactivation is described in the SOPs.

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

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

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) …
   (ii) family name for plants …
   (iii) genus …
   (iv) species …
   (v) subspecies …
   (vi) strain …
   (vii) cultivar/breeding line …
   (viii) pathovar …
   (ix) common name …

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

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

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?
   Yes (.) No (X) Not known (X)
   Give details
   …

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

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

   (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 expected

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

   (c) likely consequences of gene transfer:
   possible stabilization or regress of neuroendocrine mestastases in the liver

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 available

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)
   Not expected

H. Information relating to monitoring

1. Methods for monitoring the GMOs
   AdVince will be monitored in tumor biopsies, in blood by quantitative PCR and in urine by quantitative PCR. Serum or plasma will be analyzed for anti-adenovirus antibodies.

2. Methods for monitoring ecosystem effects
   Not applicable

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms
   Not applicable. There is no risk of transfer of donated genetic material from patients to other organisms.

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

5. Duration of the monitoring
   According to protocol.

6. Frequency of the monitoring

According to protocol.

I. Information on post-release and waste treatment

1. Post-release treatment of the site
1% Virkon and 70% ethanol will be used to disinfect working area and inactivate eventual spillage.

2. Post-release treatment of the GMOs
Remaining empty cryvials and virus solution will be collected into a container with 1% Virkon solution for at least 30 min before disposing in the biohazard waste box.

3. (a) Type and amount of waste generated
Cryovials of virus stock solution, syringes, tubing set, catheter and other material such as gloves that may come in contact with the virus.

3. (b) Treatment of waste
All waste material will be placed in sealed containers and inactivated with 1% Virkon before disposal as biohazardous material according to the hospital routines.

J. Information on emergency response plans

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

Virus suspension leakage: Inactivate virus solution with 1% Virkon for at least 30 minutes before cleaning with paper. Dispose the paper in boxes for biohazardous waste.

Virus suspension on clothes: Small (up to 2 mL) volumes of diluted virus solution: Pour small volume of ethanol 70%, rinse with water and clean the clothes according to hospital routine. Large volumes or highly concentrated virus: Pour 1% Virkon on the contaminated area and dispose the clothes in a biohazard waste box.

Virus suspension on skin: Rinse with water. Wash with soap and rinse with water. Use disinfectant. Contact medical care if inflammation or irritation develops.

Virus suspension in the eyes: Rinse with eye-wash. Contact medical care if inflammation or irritation develops.

The Sponsor and Principal Investigator will be informed about any accidents that occur when using AdVince.

2. Methods for removal of the GMO(s) of the areas potentially affected
Section J1

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

Not applicable

4. Plans for protecting human health and the environment in the event of an undesirable effect: Patients will be monitored according to protocol and any clinically adverse events will be evaluated, followed-up and reported according to the procedures described in the protocol.

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