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/17/2017-002565-22
(c)	Date of acknowledgement of notification	2017/07/03
(d)	Title of the project	Phase I/II trial investigating an immunostimulatory oncolytic adenovirus for cancer
(e)	Proposed period of release	From Oct 1 2017 until Nov 2020

2. Notifier

Name of institution or company: Lokon Pharma AB

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)

LOAd703 is an adenovirus group C serotype 5 with changed fiber to serotype 35 in L5. It has deletions in E1 and E3 regions: E1Adelta24, E3deltagp19K and E3delta6.7K. Ahead of E1A it has 8 E2F sites (4 palindromes) and one Sp-1 site. A transgene cassette is inserted after L5 including a CMV promoter, human full length 4-1BBL and an extracellular and intracellular domain of human

CD40L fused to an isoleucine zipper domain (TMZ-CD40L) (Eriksson et al, Clin Cancer Res, 2017, EPub ahead of print).

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

Adenovirus has a stable genome. LOAd703 has been expanded on both 293 and A549 cells in several passages, thereafter sequenced and confirmed that no mutations, insertions or deletions have occurred.

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 (x) No (.)

If yes:

- Member State of notification US
- Notification number not applicable (RAC/FDA)

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

Adenovirus is a common cold virus that infects humans giving rise ti mild nonchronic disease. Individuals with normally functioning immune system are not endangered by this virus. Most people have been exposed to adenovirus and have developed immunity to this pathogen.

LOAd703 can only replicate in cells with continuous hyper-phosphorylated retinoblastoma protein, which occurs in tumor cells but not in normal cells. The virus will be injected into the tumor of cancer patients and the shedding of replicating virus is not expected since similar adenovirus-based oncolytic viruses can be shedded but usually not as viable particles. Those studies also administered virus intravenously. In former studies using a similar virus (AdCD40L) that was injected intratumorally, we did not detect AdCD40L in blood after intratumoral injection. However, the anti-adenovirus antibodies are increased after repeated intratumoral injection, especially in patients with liver metastases that has frequent blood contact. Hence, some virus-blood interaction certainly take place even upon intratumoral injection (Loskog et al Br J Cancer 2016,114:872). Spreading of the LOAd703 virus outside of the cancer patient is, hence, very unlikely. At the hospital, the virus will be handled in low volumes 500-1500ul, they will carry eye protection, gloves and protective coats. After injecting the virus, the syringe and needle is inactivated using Vircon® or similar disinfectant

prior disposal in biohazard containers. The staff is educated and experienced to handle GMO. It is our opinion that upon possible unwanted exposure of LOAd703 to the environment, it will die without a tumor host to infect, an infection of healthy humans will lead to rapid destruction by the host immune system. LOAd703 virus infects cells via human CD46 not present on animals or plants. Hence, infection will not occur outside the human population.

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	human

(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
Adenoviral vectors are categorized as a GMM, level II, by the Swedish Work Environment Authority. The applicable guidelines for handling, protection, labeling and destruction of Ad will be followed and the study personnel is trained to handle GMM/GMO. Handling of LOAd703 must be approved by the Swedish Work Environment Authority and by the Swedish Medical Products Agency prior start of 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 is a common cold virus that infects humans giving rise to mild non-chronic disease. Individuals with a normally functioning immune system are not endangered bu this virus. Most people have been exposed to adenovirus serotype 5 and have developed immunity to this pathogen. LOAd703 can only replicate in tumor cells and spreading within the human population is therefore unlikely. Infection of a healthy human will result in rapid destruction by the host immune system. The immunostimulatory transgenes 4-1BBL and TMZ-CD40L will support the immunity to the virus, besides having immunostimulatory effects against the tumor when injected intratumorally.

Expression of 4-1BBL and TMZ-CD40L will to great extent be confined in the cells of the needle tract. Systemic exposure of recombinant, high dose, 4-1BBL or CD40L can give transient upregulation of liver transaminases. Hence, treated patients are monitored for blood chemistry. Similar treatment using AdCD40L therapy has shown safe with 0 SAEs reported attributed to the virus (>40 patients, 3-8 treatments each), Malmström et al Clin Cancer Res, 2010, 16:3279; Loskog et al Br J Cancer, 2016,114:872; Ireaneaus et al, manuscript.

8. Information concerning reproduction

(a) Generation time in natural ecosystems:
na

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

(c) Way of reproduction: Sexual na Asexual na

(c) Factors affecting reproduction:
Adenoviruses replicate in human host, LOAd703 replicates in cancer cells.

9. Survivability

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

(i)	endospores	(na)
(ii)	cysts	(na)
(iii)	sclerotia	(na)
(iv)	asexual spores (fungi)	(na)
(v)	sexual spores (funghi)	(na)
(vi)	eggs	(na)
(vii)	pupae	(na)
(viii)	larvae	(na)

(ix) other, specify na

(b) relevant factors affecting survivability:
storage outside human host cells in TRIS buffer at -80C.

10. (a) Ways of dissemination
Direct contact or aerosol formation

(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/../../… (not by Lokon Pharma)

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

LOAd703 is an adenovirus group C serotype 5 with changed fiber to serotype 35 in L5. This shift of fiber enables LOAd703 to infect only cells expressing human CD46. The E1Adelta24 removes the capacity of replication in healthy human cells. Ahead of E1A it has instead 8 E2F sites (4 palindromes) and one Sp-1 site that drives replication of the virus in cells with free E2F. E2F is free in cells with continuously hyper-phosphorylated retinoblastoma (i.e. cancer cells). E3deltagp19K and E3delta6.7K are removed to reduce the virus capacity to avoid the immune system since these genes otherwise results in entrapment of MHC in the endoplasmatic reticulum and downregulation of death receptors TRAIL 1/2. A transgene cassette is inserted after L5 including a CMV promoter, human full length 4-1BBL and an extracellular and intracellular domain of human CD40L fused to an isoleucine zipper domain (TMZ-CD40L) (Eriksson et al, Clin Cancer Res, 2017, EPub ahead of print). These genes are translated into immunostimulatory proteins that hopefully will support anti-tumor immune responses and increase survival of cancer patients.

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 (.) No (n)

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

(b) Identity of the vector
…

(c) Host range of the vector
…

(d) Presence in the vector of sequences giving a selectable or identifiable phenotype
Yes (.) No (.)

antibiotic resistance (.)
other, specify …

Indication of which antibiotic resistance gene is inserted
…

(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 …

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 Homologous recombination

6. Composition of the insert

(a) Composition of the insert
CMV promoter - - 4-1BBL – TMZ-CD40L
serotype 35 fiber domain

E2F/Sp-1 site

(b) Source of each constituent part of the insert

The CMV promoter is the genetic identical sequence to the commonly used cytomegalo virus gene promoter. The 4-1BBL is the genetic identical sequence to the human wild type 4-1BBL gene. The TMZ-CD40L is the genetic identical sequence to the human extracellular and intracellular domain of human wild type CD40L fused to the genetic identical sequence to an isoleucine zipper domain. The serotype 35 fiber domain is the genetic identical sequence to the adenovirus serotype 35 fiber. The E2F/Sp-1 site is the genetic sequence of known E2F and Sp-1 binding sites. The genetic sequences were generated in vitro by Genscript Inc.

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

- The CMV promoter intend to drive expression of the 4-1BBL and TMZ-CD40L genes in infected cells.
- 4-1BBL and TMZ-CD40L are intended to stimulate antigen presenting dendritic cells and promote expansion of cytotoxic T cells as well as NK cells.
- The serotype 35 fiber directs infection of LOAd703 to CD46 positive cells (i.e. human cells).
- E2F/Sp-1 binding sites bind E2F and Sp-1 to the virus genome which initiates virus replication in tumor cells.

(d) Location of the insert in the host organism

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

(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 they are in vitro generated but theoretical gene sequences built on genes from the human genome.

2. Complete name

(i)	order and/or higher taxon (for animals)	…
(ii)	family name for plants	…
(iii)	genus	Homo
(iv)	species	Homosapiens
(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 (x) No (.) Not known (.)

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

Replication of LOAd703 in tumor cells will kill the infected tumor cell. Expression of 4-1BBL and TMZ-CD40L can activate the immune system and possible lead to cytokine release syndrome.

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 (x) No (.) Not known (.)

Specify LOAd703 can only replicate/survive in human tumor cells while normal adenoviruses can replicate in any human cell.

(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 LOAd703 can only replicate/survive in human tumor cells while normal adenoviruses can replicate in any human cell.

(c) is the GMO in any way different from the recipient as far as dissemination is concerned?

Yes (x) No () Not known (.)

Specify Ad5 viruses infect CAR expressing cells which includes some animal cells and human cells. LOAd703 has an Ad35 fiber that restricts infection to human cells since it needs human CD46 for binding and entry into host cells.

(d) is the GMO in any way different from the recipient as far as pathogenicity is concerned?

Yes (x) No (.) Not known (.)

Specify Since LOAd703 cannot replicate in normal cells and express immunostimulatory transgenes, it is likely less pathogenic than normal Ad5 viruses.

2. Genetic stability of the genetically modified organism

LOAd703 does not integrate into the host genome and the virus genome is stable.

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)

LOAd703 can only replicate in tumor cells and spreading within the human population is therefore unlikely. Infection of a healthy human will result in rapid destruction by the host immune system. The immunostimulatory transgenes 4-1BBL and TMZ-CD40L will support the immunity to the virus, besides having immunostimulatory effects against the tumor when injected intratumorally.

Expression of 4-1BBL and TMZ-CD40L will to great extent be confined in the cells of the needle tract. Systemic exposure of recombinant, high dose, 4-1BBL or CD40L can give

transient upregulation of liver transaminases. Hence, treated patients are monitored for blood chemistry. Similar treatment using AdCD40L therapy has shown safe with 0 SAEs reported attributed to the virus (>40 patients, 3-8 treatments each), Malmström et al Clin Cancer Res, 2010, 16:3279; Loskog et al Br J Cancer, 2016,114:872; Ireaneaus et al, manuscript.

Expression of 4-1BBL and TMZ-CD40L could lead to cytokine release syndrome but since the expression is limited (infected cells are killed by the host immune system), such responses are likely self-limiting but can be managed by corticosteroids or tocilizumab (anti-IL6R antibody known to block IL6 driven cytokine release syndrome).

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

F. Information relating to the release

1. Purpose of the release (including any significant potential environmental benefits that may be expected)
LOAd703 will be used in a clinical Phase I/II trial treating patients suffering from colorectal cancer, ovarian cancer, pancreatic cancer or biliary cancer.

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 LOAd703 will be injected into tumor lesions

3. Information concerning the release and the surrounding area

(a) Geographical location (administrative region and where appropriate grid reference):
Uppsala university hospital, Uppsala, Sweden

(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 treated in the radiology facility.

(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:
Maximum 12 injections per patient dose escalating from 1x10e11 to 1x10e12 virus particles per injection. Maximum 50 patients will be treated.

(b) Duration of the operation:
The treatment takes a few minutes per patient

(c) Methods and procedures to avoid and/or minimise the spread of the GMOs beyond the site of the release
The virus will be kept in a syringe, injected and then remaining virus, syringe, needle and gloves etc that have been in contact with virus will be incubated in Vircon ® or similar disinfectant prior disposal in biohazard boxes. Staff will have protective eye ware, gloves and protective clothes.

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	Homo
(iv)	species	Homosapiens
(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)
Hopefully LOAd703 will increase tumor cell killing and result in longer survival of the treated cancer patients

3. Any other potentially significant interactions with other organisms in the environment
Not likely

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

Not applicable

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:
Local inflammation, replication of virus in tumor cells, killing of tumor cells.

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.):
LOAd703 can activate human dendritic cells and expand cytotoxic T cells as well as NK cells. Infection of tumor cells leads to LOAd703 replication while normal cells cannot replicate the virus. Replication leads to oncolysis – tumor cell death.

A similar virus, but without replication capacity, AdCD40L, was able to decrease tumor metabolism in 50% of patients as studied by positron emission tomography (PET) Loskog et al Br J Cancer, 2016,114:872.

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
Quantitative PCR in blood, urine, biopsies and oral swabs

2. Methods for monitoring ecosystem effects
Not applicable

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms
Quantitative PCR, but unlikely that this would occur.

4. Size of the monitoring area (m^2)
… m^2

Monitoring of treated patients

5. Duration of the monitoring
At several time points during a maximum 50 weeks trial participation per patient

6. Frequency of the monitoring
Before treatment, at the 6^{th} and 12^{th} injection and at final follow up (4 months post final virus injection).

I. Information on post-release and waste treatment

1. Post-release treatment of the site
1% Virkon, or similar disinfectant, will be used to disinfect working area and to inactivate eventual spillage.

2. Post-release treatment of the GMOs
Remaining vector solution will be collected into a container with 1% Virkon solution, or similar disinfectant. The container will be sealed and disposed of as biohazardous material according to the hospital routines.

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

3. (b) Treatment of waste
All waste material will be placed in sealed containers and inactivated with 1% Virkon, or similar disinfectant, 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 the virus suspension with Virkon® or equivalent disinfectant by gently pouring Virkon®/disinfectant on the virus. Let it inactivate for at least 10 minutes before cleaning with paper using gloves. Dispose of the paper and gloves in a biohazard box.

Virus suspension on clothes:
Soak the contaminated item in Virkon®/disinfectant and inactivate the virus for 10 minutes. Dispose the clothes in a biohazard box.

Virus suspension on skin:
Rinse with water. Wash with soap and rinse with water. Repeat washing. Soak the skin in disinfectant and let air-dry. Contact medical care if inflammation or irritation develops. Report the incident accordingly to internal routines accordingly to Hospital routine.

Virus suspension in the eyes:
Rinse with eye-wash. Contact medical care if inflammation or irritation develops. Report the incident accordingly to internal routines accordingly to Hospital routine.

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

Inactivate the virus suspension with Virkon® or equivalent disinfectant by gently pouring Virkon®/disinfectant on the virus. Let it inactivate for at least 10 minutes before cleaning with paper using gloves. Dispose of the paper and gloves in a biohazard box.

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

Inactivate the virus suspension with Virkon® or equivalent disinfectant by gently pouring Virkon®/disinfectant on the virus. Let it inactivate for at least 10 minutes before cleaning with paper using gloves. Dispose of the paper and gloves in a biohazard box.

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

See point J1 above.
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.