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	**Slovenia**
(b)	Notification number	**B/SI/09/01**
(c)	Date of acknowledgement of notification	**March 4, 2009**
(d)	Title of the project	**Intratumoral application of naked DNA, encoding gene for human interleukin 12, followed by electrically-assisted gene transfer into tumor cells of canine patients**
(e)	Proposed period of release	**From 1/11/2009 until 1/10/2012**

2. Notifier

Name of institution or company: **University of Ljubljana, Veterinary faculty, Gerbičeva 60, Ljubljana, Slovenia**

3. GMO characterisation

(a) Indicate whether the GMO is a:

viroid	(.)
RNA virus	(.)
DNA virus	(.)
bacterium	(.)
fungus	(.)
animal:	
- mammals	(.)
- insect	(.)
- fish	(.)
- other animal	(.)

specify phylum, class

Plasmid pORF-hIL12 is a commercially available plasmid, intended for research use only. It can not be found outside research laboratories and does not have any

characteristics of a living organism. Therefore it can not be called "genetically modified organism" per se.

(b) Identity of the GMO (genus and species)
Not relevant. The plasmid DNA does not have a taxonomic classification.

(c) Genetic stability – according to Annex IIIa, II, A(10)
Annex IIIa, II, A(10): Verification of the genetic stability of the organisms and factors affecting it.
Avilable data indicates that the prokarytic and eukaryotic sequence elements of the pORF-hIL12 are genetically stable under controlled conditions used for plasmid manufacture and will not become unstable via intratumoral application.
The ampicilin resistance gene of plasmid (pORF-hIL12) originates from the naturally occurring bacterial transposon Tn3, and includes promoter and encoding synthesis of the enzyme beta-lactamase. The encoded gene product is expressed in bacteria and confers resistance to ampicilin or similar antibiotics from the penicillin group. The ampicilin resistance gene and flanking sequences of pORF-hIL12 do not contain any of the inverted repeat sequences necessary for classical transposition of the gene into bacterial chromosome or other plasmids

Pharmaco-kinetic studies indicates that pDNA is rapidly degraded in blood (half-life < 5 minutes) and does not persist in gonadal or other tissues following intravenous injection (Lew, 1995, Parker, 1995). Intravenous injection was in this study considered as a "worst-case" scenario relative to the intended clinical route of cutaneous/subcutaneous tumors in dogs administration. Data from other nonclinical biodistribution studies of intramuscularly injected e.g. pDNA vaccines indicate that the injected pDNA is progressively cleared from tissues and not integrate into host cell genomic DNA at the site of injection (Vilalta et al, 2005; Sheets et al, 2006, Martin et al, 1999). In addition, the results of the study, where electroporation was used to facilitate the transport of plasmid DNA into the muscle cells, demonstrated that electroporation increased the proportion of integration of plasmid DNA into genomic DNA, but this event is still ~1000x lower than occurrence of spontaneous mutations (Wang et al, 2004)

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.

Plasmid pORF-hIL12 is a DNA molecule which has no properties of a living organism or virus and cannot be considered as a GMO. All genes, which allow mobilisation of the plasmid, are deleted, and therefore it can not be transferred between transfected and not-transfected cells. It can not replicate in mammalian organism and it doesn't insert into host's genome.

In the instance, that plasmid is released into environment; possibility of transfer into human or animal organism is negligible small, especially in quantities, which would allow expression of gene product in any significant concentrations. Vertical transmission of plasmid is not possible. The probability of bacterial uptake of the plasmid, facilitating replication, is very small. Bacteria can not express gene product. Transfer of plasmid between bacteria is not possible. Replication of plasmid is possible only in *E. coli* due to specific origin of replication.

Plasmid pORF carries genes, encoding resistance to ampicillin. Transcription of these genes is possible only in *E. coli*, due to specific promotor. Therefore, expression of resistance is not possible in mammalian cells.

The potential environmental risk of pORF-hIL12 is therefore considered to be minimal or negligible and acceptable in view of the potential clinical benefit to dogs with cutaneous/subcutaneous tumors.

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	(.)
bacterium	(.)
fungus	(.)
animal	
- mammals	(.)
- insect	(.)
- fish	(.)

- other animal (.)
  (specify phylum, class) …

other, specify

pORF (plasmid "open reading frame") (InvivoGen, Tolouse, France -commercially available) is a pDNA, an artificial bacterial plasmid which does not exist in the natural environment and does not have a taxonomic classification.

2. Name

(i)	order and/or higher taxon (for animals)	…
(ii)	genus	…
(iii)	species	…
(iv)	subspecies	…
(v)	strain	…
(vi)	pathovar (biotype, ecotype, race, etc.)	…
(vii)	common name	**Plasmid pORF (open reading frame)**

3. Geographical distribution of the organism

(a) Indigenous to, or otherwise established in, the country where the notification is made:
Yes (.) No (.) Not known (.)

It does not have natural environment and is used in research laboratories worldwide.

(b) Indigenous to, or otherwise established in, other EC countries:
(i) Yes (.)

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

Atlantic ..
Mediteranean ..
Boreal ..
Alpine ..
Continental ..
Macaronesian ..

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

It does not have natural environment and is used in research laboratories worldwide.

(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

It is artificially developed and does not have natural habitat.

(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 …	

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

5. (a) Detection techniques
Polymerase Chain Reaction (PCR) assays

(b) Identification techniques
Polymerase Chain Reaction (PCR) assays

6. Is the recipient organism classified under existing Community rules relating to the protection of human health and/or the environment?
Yes (.) No (x)
If yes, specify
…

7. Is the recipient organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?
Yes (.) No (x) Not known (.)

If yes:

(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) of Directive 2001/18/EC
…

8. Information concerning reproduction

(a) Generation time in natural ecosystems:

The generation time of pORF in natural ecosystems is not known. The pORF is capable of replication in permissive bacteria *E.coli*. In laboratory cultures, doubling times of 24-71 minutes have been reported (Bremer H et al, 1986).

(b) Generation time in the ecosystem where the release will take place:
Relevant nonclinical data suggest that plasmid such as pORF or pORF-hIL12 will not undergo episomal replication in mammalian cells (Wolf JA et al, 1990)

(c) Way of reproduction: Sexual .. Asexual (**x**)
Replication in bacteria (possible only in *E. coli*)

(d) Factors affecting reproduction:
Reproduction (i.e. replication) is possible only in *E. coli*, due to specific origin of replication (pMB1 Ori). These bacteria are not common in tissues, which will be transfected with plasmid pORF-hIL12 (i.e. tumor tissue and possibly skin of dogs). In the unlikely case, that the plasmid comes into contact with intestinal microflora, uptake of these bacteria is very low (uptake is possible only into competent strains of bacteria, which do not appear in natural environment).
In addition, the injected plasmid DNA would be rapidly degraded in the blood and other tissues, if it would disseminated to these tissues. Data demonstrated that in muscles most of the DNA is degraded in first 30 min after electroporation (Bureau et al, 2004). In blood, the half-life of plasmid DNA is less than 5 min (Lew et al, 1995, Parker et al, 1995). Study quantifying the amount of plasmid DNA incorporated in cationic-lipid in the gonads demonstrated that no plasmid DNA could be detected in the gonads using available methods (PCR) (Vilalta et al, 2005). An integration study of electroporation transferred plasmid DNA in the genomic DNA at the site of application (muscle) or in other tissues found that probably 1 copy of plasmid DNA could integrate in the genomic DNA after injection of 50 mg od plasmid DNA in the muscle. Due to the technical limits of purifying process more accurate data could not be provided (Wang et al, 2004).
Furthermore, the plasmid can not be transferred between bacterial cells (deletion of genes, allowing mobilisation) and in the absence of ampicillin, transfected bacteria will have no advantage for growth, which will lead to loss of plasmid. Plasmid will be used in low quantities (depending on size of tumor, not exceeding 0,5 mg of plasmid).

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	

It does not for any structures, listed above.
The pORF-hIL12 is not known to form any of the structures listed above. Instances of inadvertent environmental release would generally be expected to lead to rapid

degradation of the pDNA due to the abundance of deoxyribonucleases in the natural environment. However, based on experimental findings related to the presence of free DNA in the environment (Lorenz et al, 1994), trace amounts of pDNA could persist in soils or wastewater.

(b) relevant factors affecting survivability:
Instances of inadvertent environmental release would generally be expected to lead to rapid degradation of the pDNA due to the abundance of deoxyribonucleases in the natural environment.

10. (a) Ways of dissemination
Possibility that the plasmid will be taken up by competent *E. coli*, which could replicate it, is negligibly small. Vertical transmission is not possible.

(b) Factors affecting dissemination
Plasmids pORF and pORF-hIL12 have "pMB1 Ori" origin of replication, which is specific for *E. coli*. These bacteria are not common on canine skin and cutaneous/subcutaneous tumors in dogs, where the plasmid will be administered. Plasmid will be injected in the middle of treated tumor nodule, precluding it from coming into contact with germinal cells.

All genes, which allow mobilisation of the plasmid pORF-hIL12, are deleted, and therefore it can not be transferred between transfected and not-transfected cells. It can not replicate in mammalian organism and it doesn't insert into host's genome.

Introduction of pORF-hIL12 into tumor cells will be facilitated using electroporation of host tissue (tumor nodules), which allows local transfection of only electroporated tissue. Intratumoral injection of naked DNA, without use of electric pulses, is a very inefficient method of transfection, enabling poor transgene expression (transfecion efficiency of less than 1%). Use of electric pulses (i.e. electrotransfection, electrically-assisted gene delivery) significantly increases transfection efficiency (Čemažar, 2002). Naked DNA, which doesn't cross cell membrane and stays in extracellular matrix, is rapidly degraded with endonucleases in less than 30 minutes (Lew, 1995). Therefore, possibility of entry of plasmid into circulation and transfer into other organs, which would allow dissemination into environment (e.g. respiratory, digestive, urinary, reproductive tract), is negligible small.

Furthermore, one week after the last treatment, the treated tumour nodule will be surgically resected with wide surgical margins, which will mechanically remove all transfected tissue from patients. Treated tumour nodules will be protected from any possible contact with humans or animals with adhesive tapes, bandages or similar mechanical protection. Changing if bandages will be only performed at the clinic. Dogs will wear elizbethan collars, if necessary (in case of licking treated area). Written instructions, will be provided to the owner at the time of release of patient into home environment.

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)

None.

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

1. Type of the genetic modification

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

2. Intended outcome of the genetic modification

Plasmid pORF-hIL12 is commercially available plasmid, which was created by cloning of gene, encoding human IL12 into plasmid pORF ("open reading frame" plasmid).

3. (a) Has a vector been used in the process of modification?
Yes (.) No (x)

If no, go straight to question 5.

(b) If yes, is the vector wholly or partially present in the modified organism?
Yes (.) 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	(.)
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 …	

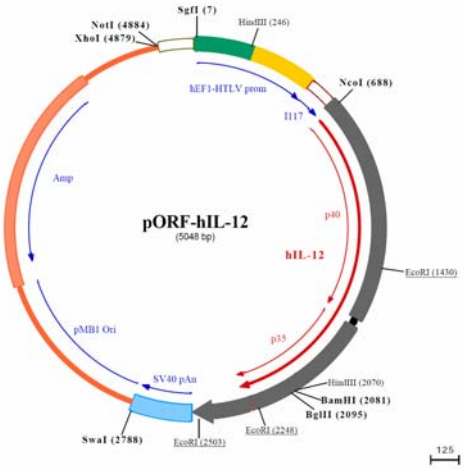
5. If the answer to question B.3(a) and (b) is no, what was the method used in the process of modification?

(i)	transformation	(.)
(ii)	microinjection	(.)
(iii)	microencapsulation	(.)
(iv)	macroinjection	(.)
(v)	other, specify	

pORF-hIL12 was constructed commercially, using standard cloning methods.

6. Composition of the insert

(a) Composition of the insert



http://www.invivogen.com/PDF/pORF-hIL-12_G2_08A11-SV_TDS.pdf

EF-1a / **HTLV hybrid promoter** is a composite promoter comprised of the Elongation Factor-1a (EF-1a) promoter1 and 5' untranslated region of the Human T-Cell Leukemia Virus (HTLV). EF-1a

utilizes a type 2 promoter that encodes for a "house keeping" gene. The promoter is stronger than CMV and is expressed at high levels in all cell cycles and lower levels during G0 phase. The promoter is also non-tissue specific; it is highly expressed in all cell types. The R segment and part of the U5 sequence (R-U5') of the HTLV Type 1 Long Terminal Repeat2 has been coupled to the EF-1a promoter to enhance stability of DNA and RNA. This modification not only increases steady state transcription, but also significantly increases translation efficiency possibly through mRNA stabilization.

• **Intron I117 5'UTR:** InvivoGen utilizes an inducible promoter for the second transcriptional unit that is spliced out as an intron in mammalian cells. *LacI* expression causes overproduction of Lac repressor protein acting on the bacterial promoter to repress the expression of the gene. This safeguard is essential when the second transcription gene product is toxic to *E. coli*. Treatment with IPTG enables the expression of the second transcription unit in bacteria constitutively expressing *LacI*.

• ***hIL-12 Fusion Gene*** *Human IL-12 gene (intronless ORF) from the ATG to the stop codon. Size: 1611 bp*

• ***SV40 pAn:*** *The Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA. The efficiency of this signal was first described by Carswell et al.3*

• ***pMB1 Ori*** *is a minimal E. coli origin of replication with the same activity as the longer Ori.*

• ***Amp (ampicillin resistance gene):*** *The ampicillin resistance gene allows the selection of bacteria carrying the pORF plasmid.*

(b) Source of each constituent part of the insert

Data is not available, because it is commercially available product.

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

- - hIL-12 fusion gene - gene encoding human IL12

(e) Location of the insert in the host organism

- on a free plasmid (x)
- integrated in the chromosome (.)
- other, specify …

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

Yes (.) No (x)

If yes, specify …

D. Information on the organism(s) from which the insert is derived

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)	Primates, Hominidae
(ii)	family name for plants	…
(iii)	genus	Homo
(iv)	species	H.sapiens
(v)	subspecies	…
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	human

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 () Not known (X)
Specify …
Survival of pORF or pORF-hIL12 in the natural environment has not been studied. The potential for survival of the pORF-hIL12 in the natural environment as a consequence of the intended use is considered remote.

(b) is the GMO in any way different from the recipient as far as mode and/or rate of reproduction is concerned?
Yes (.) No (x) Unknown (.)
Specify …

(c) is the GMO in any way different from the recipient as far as dissemination is concerned?
Yes (.) No (x) Not known (.)
Specify …

(d) is the GMO in any way different from the recipient as far as pathogenicity is concerned?
Yes (.) No (x) Not known (.)
Specify …
The pathogenicity of pORF in humans and animals is not known. The component of pORF-hIL12 is hIL-12 which is considered nonpathogenic in the amounts that are expressed in animals as demonstrated in non-clinical study in dogs (Pavlin et al 2008).

2. Genetic stability of the genetically modified organism
See A (3) (c) above

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)
Annex III A, point II(A)(11)(d): Pathogenicity: infectivity, toxigenicity, virulence, allergenicity, carrier (vector) of pathogen, possible vectors, host range including non-target organism. Possible activation of latent viruses (proviruses). Ability to

colonise other organisms.…
Annex III A, point II(C)(II)(i):

Gene therapy of tumor nodules in dogs will lead into expression of encoded product (hIL12), which will have therapeutic antitumor effect on tumour tissue. IL12 stimulates proliferation of peripheral mononuclear blood cells, activation of natural killer cells and stimulation of interferon-gamma production. High amount of IL-12 can cause side effect, such as fever, nausea (Leonard et al, 1997). However, with doses of plasmid, which will be used, it is not possible to reach toxic concentrations of IL12 in any human or animal organism (Pavlin et al, 2008, Daud et al, 2008).

4. Description of identification and detection methods

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

Polymerase Chain Reaction (PCR) assays

(b) Techniques used to identify the GMO

Polymerase Chain Reaction (PCR) assays

F. Information relating to the release

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

The purpose of release is phase I/II of clinical trial, studying antitumor effects of electrogene therapy, using plasmid pORF-hIL12 in dogs with spontaneously occurring tumors.

Patients, which will be included into study, will be released to home environment two hours after performed gene therapy. The purpose of our study is to achieve antitumor effect of gene therapy on treated tumor nodules, enabling improvement of quality of life and prolonging survival in treated subjects.

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

Yes (.) No (.)

If yes, specify

Not relevant. There is no natural habitat or ecosystem for the plasmid pORF-hIL12.

3. Information concerning the release and the surrounding area

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

Not relevant - the extent of planned clinical trial is very limited with small number of participating animals (up to 20 in 3 years) from different locations in Slovenia. Therefore geographical locations of release can not be determined in advance.

(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

Not relevant, can not be defined

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

Not relevant (see below)

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

Not relevant (see below).

Sections (b) - (d) are not considered relevant due to the fact, that the release is not intended beyond very limited extent (treatment of individual trial subjects). Transfection of tumor nodules in patients will be only transient (up to 2 weeks after the first therapy, followed by surgical removal of transfected tissue). Gene therapy will be carried out at Veterinary faculty, Clinic for companion animals, Cesta v Mestni log 47, Ljubljana. Dogs, which will be included into clinical trial, will receive 2 consecutive applications of plasmid in one week interval. Dose of used plasmid will vary according to size of treated tumor, and will not exceed 500 µg of plasmid per one treatment. One week after the last treatment, the tumor nodule (and all transfected tissue) will be surgically removed with wide surgical margins.

Our patients will be mostly older dogs, which are physically impaired due to their age and disease (advanced stages of different types of cancer), and therefore can not tolerate normal physical exercise. Daily walks are usually carried out only on short distances with animals on leash.

4. Method and amount of release

(a) Quantities of GMOs to be released:
Up to maximum amount of 50 µg of plasmid per patient. (2 consecutive applications of maximum 500 µg of plasmid; electroporation yields transfection of approximately 5%: 2 x 500µg x 5% = 50 µg). Expected number of patients, which will be included in the study, is around 20 dogs in 3 years.

(b) Duration of the operation:
1.11.2009 – 1.10.2012

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

Treated tumor nodules will be protected from any possible contact with humans or animals with adhesive tapes, bandages or similar mechanical protection. Dogs will wear elizabethan collars, if necessary (in case of licking treated area). Written instructions will be provided to the owner at the time of release of patient into home environment.

5. Short description of average environmental conditions (weather, temperature, etc.)
…
The pORF-hIL12 will be administered to trial subjects at clinical sites in an enclosed facility at ambient temperature.

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.

No data available because no previous releases have been carried out with the same plasmid.

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)	**Mammalia, Carnivora, Canidae**
(ii)	family name for plants	…
(iii)	genus	**Canis**
(iv)	species	**C. lupus**
(v)	subspecies	**C. lupus familiaris**
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	**Dog**

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

Gene therapy of tumor nodules in dogs will lead into expression of encoded product (hIL12), which will have therapeutic antitumor effect on tumour tissue. IL12 stimulates proliferation of peripheral mononuclear blood cells, activation of natural killer cells and stimulation of interferon-gamma production.

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

Other potentially significant interactions with other organisms in the environment are not expected for the reasons, given under A7 in B 8-10.

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

Plasmid pORF-hIL12 (not a GMO) could potentially disseminate into soils through accidents in transport or improper handling. The possibility of dissemination and establishment in soil or other ecosystems in these cases is considered remote, due to plasmid properties, described above, and presence of naturally occurring endonucleases in environment.

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)	**Primates, Hominidae**
(ii)	family name for plants	…
(iii)	genus	**Homo**
(iv)	species	**H. sapiens**
(v)	subspecies	…
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	**Human**
(j)	order and/or higher taxon (for animals)	
(ii)	family name for plants	**Enterobacteriaceae**
(iii)	genus	**Escherichia**
(iv)	species	**E. coli**
(v)	subspecies	…
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	**E. coli**

7. Likelihood of genetic exchange in vivo

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

The likelihood of genetic exchange between pORF-hIL12 and any non-target organism is considered remote for the reasons stated under A7 and B 8-10.

(b) from other organisms to the GMO:

Not relevant, because the plasmid is not a GMO.

(c) likely consequences of gene transfer:

Human - in case of transfection of any human tissue and consequently expression of transgene, the level of expression would be very low, due to low doses of used plasmid and low efficiency of transfection. With doses of plasmid, which will be used, it is not possible to reach toxic concentrations of IL12 in any human or animal organism.

***E. coli* - in the unlikely case the plasmid will come into contact with competent bacteria in urinary or digestive tract and dissemination into environment, it can not be transferred between transfected and not-transfected bacterial cells. Replication of plasmid is possible only in *E. coli* due to specific replication origin. Expression of transgene product is not possible in bacterial cells. The uptake of plasmid in the absence of ampicillin will not have any advantage for growth and will lead to loss of plasmid.**

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 studies of pORF-hIL12 have been performed in simulated natural environments.

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

No significant interactions with biogeochemical processes are anticipated.

H. Information relating to monitoring

1. Methods for monitoring the GMOs

2. Methods for monitoring ecosystem effects
…

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

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

5. Duration of the monitoring

…

6. Frequency of the monitoring

…

Dogs, which will be included into clinical trial, will be examined once a week at the Veterinary faculty, Clinic for companion animals. Examination will include taking blood samples from treated animals. Due to very limited extent of clinical trial and negligible possibility of any negative effect of plasmid release, other methods of monitoring are not anticipated.

I. Information on post-release and waste treatment

1. Post-release treatment of the site

No post-release treatment of the site is necessary.

2. Post-release treatment of the GMOs

In dogs, where therapy with pORF-hIL12 will be performed, treated tumour nodules will be surgically removed one week after last treatment, which means that all transfected tissue will be mechanically removed from the animals. Until removal, tumour nodules will be protected from any possible contact with humans or animals with adhesive tapes, bandages or similar mechanical protection. Dogs will wear elizabethan collars, if necessary (in case of licking treated area). Written instructions will be provided to the owner at the time of release of patient into home environment. The owners will also be instructed to make daily walks with dogs on leashes and on short distances in close proximity of homes and to avoid any contact with other animals.

Disposal of used electrodes, scalpels syringes, gloves or other items that may potentially contain residual amounts of plasmid will be performed in accordance with institutional procedures of the clinical site.

3. (a) Type and amount of waste generated

The amount of plasmid DNA in disposable medical equipment (electrodes, scalpels, syringes, suture material, needles, surgical gloves, etc) that may contain plasmid DNA will not exceed 50 μg.

Generated waste will not contain any amounts of plasmid at all.

3. (b) Treatment of waste

All disposable material, which will come into contact with patients, will be placed into plastic containers (sharps container). All waste will be incinerated in accordance with guidelines for good laboratory practice and according to European standard 17025. Disposal of waste will be performed by accredited contractor Saubermacher Slovenija

d.o.o. Surgical instruments, which will come into contact with patients, will be disposed into plastic containers with disinfection fluid (Sekusept plus, 1% solution) and promptly autoclaved. Urine and excrement of treated dogs won't undergo any special treatment, since treated animals do not excrete any kinds of toxins, allergens or other potentially harmful substances.

J. Information on emergency response plans

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

Personnel, which will cooperate in the study, will be notified about procedures concerning cleaning and disposal of plasmid solution in case of unintentional spilling of the liquid (mechanical removal of DNA solution, disposal in waste container for hazardous material, use of personal protective equipment).

In case that one of dogs, which will be included in the study, runs away and gets lost either from Veterinary faculty, or from its owner, leader of the project should be notified. He/she will activate personnel of Clinic for companion animals to start searching in the neighbourhood of Clinic. If the patient isn't found, animal shelters, Veterinary administration of Republic of Slovenia and Ministry of the environment and spatial planning will be informed. Due to the limited extent of our clinical study, protective measures and specific properties of our patients (pets with at least some obedience training), we think that possibility of any harmful effects on people or environment is negligible. Therefore a notification of above mentioned institutions is only informative.

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

Mechanical removal of material, which came into contact with plasmid solution.
In addition, spilled liquids will be absorbed with common absorbent materials and placed in appropriate containers for disposal.

3. Methods for disposal or sanitation of plants, animals, soils, etc. that could be exposed during or after the spread
Disposal or sanitation of plants, animals, soils, etc. exposed to the product is not necessary due to the low risk of resultant harm.

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

Because the used plasmid does not appear to pose a significant risk to human health or the environment, specific plans for protection have not been prepared.

Oblikovano: Tabulatorji: Ne pri 5,71 cm

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