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	*Germany*
(b)	Notification number	*B/DE/10/PEI1173*
(c)	Date of acknowledgement of notification	*06/09/2010*
(d)	Title of the project	*GL-ONC1-004/TUE: Phase I/II study of intraperitoneal administration of GL-ONC1, a genetically modified vaccinia virus, in patients with peritoneal carcinomatosis*
(e)	Proposed period of release Related to proposed length of study	*From 01/June/ 2011 until 31/12/2013*

2. Notifier

Name of institution or company:	*Sponsor:* *Genelux GmbH* *Am Neuland 1* *82347 Bernried*

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 *Virus, double stranded DNA virus, Poxviridae, Chordopoxvirinae*

(b) Identity of the GMO (genus and species)

Orthopoxvirus, LIVP strain of vaccinia virus that has been modified by inserting ruc-gfp (a fusion gene of Renilla luciferase and green fluorescent protein), LacZ (beta-galactosidase), and gusA (beta-glucuronidase) expression cassettes into F14.5L (located between F14L and F15L), thymidine kinase (TK), and hemagglutinin (HA) loci, respectively..

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

Vaccinia viruses are of high genetic stability. The whole life cycle is restricted to the cytoplasm of infected cells thus recombination with host genomes located in the nucleus are very unlikely to occur. Since poxviruses are not endemic in human populations, it is further unlikely that these viruses would recombine with a wild-type virus to produce a more virulent strain; and, despite worldwide use of the live virus vaccine, no reported adverse events due to mutations to a more aggressive phenotype have ever been reported.

4. Is the same GMO release planned elsewhere in the Community (in conformity with Article 6(1)), by the same notifier?

Yes (.) No (x)

If yes, insert the country code(s)

5. Has the same GMO been notified for release elsewhere in the Community by the same notifier?

Yes (x) No (.)

If yes:

- Member State of notification *GB*
- Notification number *N/A*

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.

GL-ONC1 virus is only stable in suspension. If droplets escape and air-dry, the virus is unstable, infectivity will decrease exponentially (decreased by 99.99% within 24 hrs when released into the environment at room temperature; by days 6-7, all viruses disintegrate).

Effects if escaped in hospital or wider environment: dependent on form of escape. As dried particles viruses are very unstable. Within an infected patient virus is stable. Mainly

immunocompromized (small children and the elderly) could be at risk if in direct contact with infectious 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)	Poxviridae
(ii)	genus	Orthopoxvirus
(iii)	species	Vaccinia virus
(iv)	subspecies	Vaccinia virus Lister
(v)	strain	LIVP (Lister from the Institute for viral preparations in Moscow)
(vi)	pathovar (biotype, ecotype, race, etc.)	…
(vii)	common name	Vaccinia virus LIVP

3. Geographical distribution of the organism

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

(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 (x)
(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

Exists only in the laboratory, amplification through cell culture.

(b) If the organism is an animal: natural habitat or usual agroecosystem:
N/A

5. (a) Detection techniques
Electron microscopy, PCR, immunohistochemistry

(b) Identification techniques
Electron microscopy, PCR, sequencing

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

Handling under BSL 2 conditions is required.

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:

Virus is not able to survive on its own. After accidental or intended exposure (vaccination) it may replicate within an infected animal/human.

The virus is not endemic and does not cause any known disease in humans or animals.

Consequences of accidental exposure as auto-inoculation of mucous membranes or abraded skin may result in a benign rash; needle sticks may most likely be restricted to local reactions as development of vesicular or pustular lesion, area of induration or erythema surrounding a scab or ulcer at inoculation site. Flu like symptoms may occur.

Most adverse reactions are mild to moderate complications that resolve on their own.

Serious reactions are rare and may only occur in immunocompromized: encephalitis, progressive vaccinia, eczema vaccinatum, generalized vaccinia with multiple lesions. Secondary infections may be possible; complications are serious for those with eczema or who are immunocompromized.

<u>Note:</u> Consequences will always be dose and immune status dependent AND depicted here is the worst case scenario for less attenuated vaccinia viruses as formerly widely used smallpox vaccines, which will probably not apply to the highly attenuated GMO.

(a) to which of the following organisms:

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

(b) give the relevant information specified under Annex III A, point II. (A)(11)(d) of Directive 2001/18/EC

GL-ONC1 virus is not able to survive on its own.

After accidental or intended exposure (vaccination) it may replicate within an infected animal/human. The life cycle is about 10 hours in cell culture.

The virus is only transmissible from one animal/human to another by direct contact of infected material with mucous or open skin; if infected animal/human has no pox lesions on skin, probability of transmissibility is very low.

The virus is not endemic and does not cause any known disease in humans or animals.

Please refer also to B7.

8. Information concerning reproduction

(a) Generation time in natural ecosystems:

One replication cycle in cell culture is approximately 10 hours.

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

N/A.

In vaccinated patients the GMO should only replicate in tumors.

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

(c) Factors affecting reproduction:

Cidofovir (CDV): Because it is a dCMP analog, CDV is thought to act by inhibiting viral DNA polymerases.

Vaccinia immune globulin (VIG): Upon administration VIG neutralizes free viral particles, blocking spread of infection.

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:

GL-ONC1 virus is only stable in suspension (at room temperature or frozen) or as freeze dried material. If droplets escape and air-dry, the virus is unstable (no more infectivity after up to 6 -7 days), infectivity will decrease exponentially.

10. (a) Ways of dissemination

As the virus is a laboratory strain, dissemination will only occur accidentally, e.g. as auto-inoculation of mucous membranes, abraded skin or by needle sticks.
After application in the clinic into patients virus may be contracted by direct contact with infected tissues (pox lesions - very low probability of occurrence; peritoneal fluid – only if positive for virus).

(b) Factors affecting dissemination

GL-ONC1 virus is only stable in suspension. If droplets escape and air-dry, the virus is unstable, infectivity will decrease exponentially (decreased by 99.99% within 24 hrs when released into the environment at room temperature; by days 6-7, all viruses disintegrate). Virus is susceptible to 1% sodium hypochlorite, 2% glutaraldehyde, formaldehyde, household bleach and heat by autoclaving.

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)

N/A

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

Attenuation and enhancement of tumor specific targeting by disruption of the nonessential LIVP genes F14.5L, HA and TK with the three diagnostic marker genes RUC-GFP, LacZ, gusA and the non expressed sequence for hTfR.

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 (x)

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

Virus infected cells were consecutively transfected with plasmid vectors carrying the respective inserts. The inserts were integrated into the virus by site directed recombination between virus specific homologous DNA fragments present within the vectors (flanking the inserts) and the virus genome. Subsequently recombinant virus plaques were isolated and purified.

6. Composition of the insert

(a) Composition of the insert

Four different inserts have been inserted into 3 different loci of the recipient virus. Each insert is under control of a vaccinia virus promoter. RUC-GFP was inserted into F14.5L locus, Lac Z and hTfF (the latter in reverse orientation and thus not expressed) into TK locus and gus A into the HA locus.

(b) Source of each constituent part of the insert

- *RUC-GFP: Synthetic vaccinia early/late promoter (pE/L), followed by sea pansy Renilla reniformis luciferase and humanized Aequorea victoria green fluorescent protein fusion gene*
- *Lac Z: Vaccinia virus Western reserve (WR) early/late p7.5 promoter, followed by the bacterial E. coli beta-galactosidase gene*

- *gus A: Vaccinia virus WR late p11 promoter followed by the bacterial E. coli beta-D-glucuronidase gene*
- *hTfR: Synthetic vaccinia early/late promoter (pE/L), followed by human transferrin receptor sequence in reverse orientation to the promoter.*

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

The inserted genes serve several purposes:

Disruption of the viral nonessential genes (F14.5L, TK and HA) by insertion of the respective genes not only attenuated the virus but also enhanced its tumour-specific targeting.

Furthermore, the three inserted diagnostic marker genes (RUC-GFP, lacZ, gusA) will be used to monitor virus replication in vivo and in vitro for the detection of virus in biopsies:

- *RUC-GFP allows for direct in vivo monitoring of tumour targeting, e.g. in the peritoneal cavity and the detection of metastases, as well as the staging of human subjects by imaging of regional lymph nodes to determine degree of metastases.*
- *X-Gal and X-Glu allow for histopathological staining of tumour biopsies, and thus for monitoring of tumour targeting and oncolytic effects of the virus, which can be carried out in tumours that are not easily accessible by fluorescence. The procedures can also be performed in clinics that do not have this special equipment.*
- *And will be used also for monitoring the presence/release of the drug.*
- *In addition, virus-encoded beta-galactosidase and beta-glucuronidase could be applied as Gene-Directed Enzyme Prodrug Therapy (GDEPT) and as an activator of contrast agents for deep tissue imaging.*

The vaccinia specific promoter sequences are needed for expression of the different markers.

(d) Location of the insert in the host organism

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

(e) Does the insert contain parts whose product or function are not known?
Yes (.) No (x)
If yes, specify …

D1. Information on the organism(s) from which the insert is derived

1. Indicate whether it is a:

viroid (.)
RNA virus (.)
DNA virus (.)
bacterium (x)

fungus (.)
animal
- mammals (.)
- 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	*Enterobacteriaceae*
(iii)	genus	*Echerichia*
(iv)	species	*Echerichia coli*
(v)	subspecies	…
(vi)	strain	*K12*
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	*E. coli*

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 (.) 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 (.)

D2. 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	(.)
- insect	(.)
- fish	(.)
- other animal	(x)

(specify phylum, class) …

other, specify *Renilla: sea pansy: soft sea coral*

2. Complete name

(j)	order and/or higher taxon (for animals)	*Pennatulaceae*
(ii)	family name for plants	*Renillidae*
(iii)	genus	*Renilla*
(iv)	species	*Renilla reniformis*
(v)	subspecies	…
(vi)	strain	...
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	*sea pansy*

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:

(c) 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 (.) 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 (.)

D3. 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 (.)
- insect (.)
- fish (.)
- other animal (x)
(specify phylum, class) …

other, specify *Aequorea: jellyfish*

2. Complete name

(k)	order and/or higher taxon (for animals)	*Hydroida*
(ii)	family name for plants	*Aequoreidae*
(iii)	genus	*Aequorea*
(iv)	species	*Aequorea victoria*
(v)	subspecies	…
(vi)	strain	...
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	*jelly fish*

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:

(d) 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 (.) 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 (.)

D4. 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

(l)	order and/or higher taxon (for animals)	*Primates*
(ii)	family name for plants	*Hominidae*
(iii)	genus	*Homo*
(iv)	species	*Homo 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:

(e) 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

Survivability for the GMO when brought into the laboratory environment has been tested and showed that GL-ONC1 virus is only stable in suspension. If droplets escape and air-dry, the virus is unstable, infectivity will decrease exponentially (decreased by 99.99% within 24 hrs when released into the environment at room temperature; by days 6-7, all viruses disintegrate).

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

In vivo, dissemination within mice or rats is mainly restricted to tumors.

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

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

Specify

In vivo, in mice and rats the virus is highly attenuated in comparison to the parental strain, i.e. much higher doses can be tolerated without side effects.

2. Genetic stability of the genetically modified organism

Since poxviruses are not endemic in human populations, it is unlikely that these viruses would recombine with a wild-type virus to produce a more virulent strain; and, despite worldwide use of the live vaccinia vaccine, no reported adverse events due to mutations to a more aggressive phenotype have ever been reported.

3. Is the GMO significantly pathogenic or harmful in any way (including its extracellular products), either living or dead?

Yes (.) No (.) Unknown (x)

(a) to which of the following organisms?

humans (x)
animals (x)
plants (.)
other …

(b) give the relevant information specified under Annex III A, point II(A)(11)(d) and II(C)(2)(i)

GL-ONC1 virus is not able to survive on its own.

After accidental or intended exposure (vaccination) it may replicate within an infected animal/human. The life cycle is about 10 hours in cell culture.

The virus is only transmissible from one animal/human to another by direct contact of infected material with mucous or open skin; if infected animal/human has no pox lesions on skin and does not shed virus in any body fluids, probability of transmissibility is very low.

The virus is not endemic and does not cause any known disease in humans or animals.

Please refer also to B7.

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment
Electron microscopy, PCR, immunohistochemistry

(b) Techniques used to identify the GMO
PCR, beta-galactosidase and beta-glucuronidase assay, sequencing

F. Information relating to the release

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

The GMO is an oncolytic virus and will be administered to patients in a phase I/II clinical trial, entitled "Phase I/II study of intraperitoneal administration of GL-ONC1, a genetically modified vaccinia virus, in patients with peritoneal carcinomatosis", with the purpose

- *To determine the safety and feasibility of the intraperitoneal administration of a genetically attenuated vaccinia virus, GL-ONC1.*
- *To determine and recommend a Phase II dose.*
- *To evaluate anti-vaccinia virus immune response.*
- *To document possible anti-tumor efficacy.*

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

Vaccinia viruses are laboratory strains that have no known host in nature and cause no known disease in humans or animals. Here, the virus will be administered to patients.

3. Information concerning the release and the surrounding area

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

Universitätsklinikum Tübingen, Abteilung Innere Medizin I, Hepatologie, Gastroenterologie, Infektiologie, Otfried-Müller-Str. 10, D-72076 Tübingen. Here the GMO will be administered to patients in the Infectious Disease Ward and patients will be housed in this facility until viral shedding tests are negative.

(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

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

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

4. Method and amount of release

(a) Quantities of GMOs to be released:

Dosage starts with $1x10^6$ pfu/cycle and is intended to reach 5×10^9 pfu/cycle if no adverse events are encountered in patients. Each patient will receive up to 4 cycles of treatment.

(b) Duration of the operation:

For the duration of the proposed clinical trial (up to 30 months or more).

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

The GMO will exclusively be used in clinical trials. GL-ONC1 will be administered to patients under the responsibility of the Principal Investigator in accordance to the clinical protocol and in compliance with Good Clinical Practice. Treatment of patients will occur in the Infectious Disease Ward and patients will be housed in this facility until viral shedding tests are negative.

Protection of the family members and visitors:

Until study patients can be dismissed from Tübingen University Clinic due to absence of viral shedding clinic staff will ensure that the risk of study drug transmission to visitors (family members, other third party people) is kept at a minimum. For this purpose, isolation on the Infectious Disease Ward as well as the stringent application of our special vaccinia virus-oriented hygiene instructions are instrumental.

Following dismissal from Tübingen University Clinic all study patients are instructed to keep hygiene performance at a high standard. For example, patients are advised to perform thorough hand-hygiene with soap and water or disinfecting agents and to use separate eating utensils and toilets, if possible, to not prepare any food, and to use bleach in the toilet after every visit. Furthermore, patients are advised that they should not have any physical contact with pregnant women, immunocompromised people and children. This information is provided in the patient information sheet for reference.

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

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 previous hazardous releases have been found.

G. Interactions of the GMO with the environment and potential impact on the environment, if significantly different from the recipient or parent organism

The GMO only differs in its therapeutic potential, improved attenuation and tumor specifcity from its parent but has main basic characteristics of a poxvirus.

1. Name of target organism (if applicable)

(m)	order and/or higher taxon (for animals)	Primates
(ii)	family name for plants	Hominidae
(iii)	genus	Homo
(iv)	species	Homo sapiens
(v)	subspecies	…
(vi)	strain	...
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	human

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

After application into the patient, GL-ONC1 should preferentially colonize tumors and be cleared from the rest of the body.

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

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

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

Give details

Vaccinia viruses are of high genetic stability. The whole life cycle is restricted to the cytoplasm of infected cells thus recombination with host genomes located in the nucleus are very unlikely to occur. Since poxviruses are not endemic in human populations, it is further unlikely that these viruses would recombine with a wild-type virus to produce a more virulent strain; and, despite worldwide use of the live virus vaccine, no reported adverse events due to mutations to a more aggressive phenotype have ever been reported.

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

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	…

N/A

7. Likelihood of genetic exchange in vivo

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

   (b) from other organisms to the GMO:
   N/A

   (c) likely consequences of gene transfer:
   N/A

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.):
N/A

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

H. Information relating to monitoring

1. Methods for monitoring the GMOs

   PCR, virus plaque assay, immunohistochemistry

2. Methods for monitoring ecosystem effects

   Not planned, as vaccinia viruses and GL-ONC1 are not naturally found in the environment. Patients will be continually monitored at the clinic and during follow up visits until the end of the trial.

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

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

5. Duration of the monitoring

   Patients will be monitored in the clinic after administrations of the GMO, and will be followed up for one year during the follow-up phase of the clinical study.

6. Frequency of the monitoring

   Patients will be monitored daily during first week after application then weekly.

I. Information on post-release and waste treatment

1. Post-release treatment of the site
N/A.

2. Post-release treatment of the GMOs

Residues will be disposed by autoclaving.

3. (a) Type and amount of waste generated

Residues of virus in original vials, syringes, catheters, etc.

3. (b) Treatment of waste

Residues of GL-ONC1 virus and all material that came in contact will be inactivated by autoclaving.

J. Information on emergency response plans

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

Patients will be housed in an Infectious Disease Ward until they have been tested negative for viral shedding. Emergency treatment with vaccinia immune globulin (VIG) will be made available.

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

If spilled accidentally before administration into patient: Allow aerosols to settle; wearing protective clothing, gently cover spill with paper towel and apply 1% sodium hypochlorite, starting at perimeter and working towards the centre; allow sufficient contact time before clean up (30 min). UV irradiation of contaminated surface or instrument (>15 min).

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

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

Patients will be housed in an Infectious Disease Ward until they have been tested negative for viral shedding.

If patients are shedding the GMO, there may be a risk of infection especially for immunocompromized, but transmission should only occur by direct contact with the shed virus. Hospitalisation of the patient will be arranged until negative shedding is confirmed.

Staff is required to wear protective clothing as referenced above to clean a spill. Floors. work surfaces or equipment contaminated by a spill are cleaned and disinfected with bleach. Contaminated clothing or linen are placed in biohazard Iabelied bags and laundered or incinerated (i.e.• disposal items).