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	NL
(b)	Notification number	B/NL/11/002
(c)	Date of acknowledgement of notification	07/04/2011
(d)	Title of the project	

Cancer immunotherapy of human papillomavirus-induced (pre)malignant lesion using replication-incompetent Semliki Forest Virus replicon particles encoding human papillomavirus antigens.

(e)	Proposed period of release	From 01/01/2012 until 01/01/2022

2. Notifier
Prof Dr H.W.Nijman

Name of institution or company: …
University Medical Center Groningen, Groningen, The Netherlands

3. GMO characterisation

(a) Indicate whether the GMO is a:

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

specify phylum, class

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

Togaviruses, Genus Alphavirus, Semliki Forest virus (SFV)

…

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

The GMO is not replication competent therefore genetic stability is not applicable for the GMOs introduced into the environment.

The chances that the GMO will become persistent and invasive in natural habitats under the conditions of the proposed release is negligible. First, the GMO is replication-incompetent. It is a suicide virus particle that lacks the genetic information encoding the structural proteins of the virus. It has just the capacity to initiate a single round of infection in target cells. It will not produce progeny virus precluding the possibility of the GMO becoming persistent. The only realistic possibility for the GMO to persist longer than a single round of infection would have to rely on recombination of the genome of the GMO with alphavirus RNA sequences carrying the structural genes. The chance that this will happen, however, is negligible since the target population to be treated with the GMO is extremely unlikely to carry alphavirus RNA sequences.
Finally, upon recombination with an alphavirus RNA sequence, the worse, and very unlikely, possibility is that a variant wt SFV would arise, which would not persist under the conditions of the proposed release for several reasons including the fact that the mosquito vector required for prolonged transmission is not present in the habitat involved.

The GMO has no selective advantage over a wt SFV, or other alphavirus, under the conditions of the proposed release, on the contrary. As indicated above, the GMO is replication-incompetent. Even in the very unlikely event of a recombination, there is no reason to assume that the resulting gene arrangement would possess any selective advantage over wt SFV.

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.

The potential environmental impact of the GMO, SFVeE6,7 replicon particles, is negligible.
During the production phase of the GMO (SFV particles) in the 'producer' cells recombination and mutation events may occur that could result in the production of infectious SFV. For this to occur, two RNA recombination events have to occur simultaneously and the capsid protein mutation (preventing necessary autoprotease activity of the capsid) has to be reversed. The theoretical frequency of the generation of replication-proficient virus (RPV) during the *in vitro* production phase of the SFV replicon particles is less than 4.2×10^{-17}. If any, replication-competent SFV present in the vaccine will spread in the patient comparable to a wild-type infection. There is no evidence documented on human-to-human virus transmission.

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

other, specify …

2. Name

(i)	order and/or higher taxon (for animals)	Togaviridea
(ii)	genus	Alphaviruses
(iii)	species	Semliki Forest virus
(iv)	subspecies	
(v)	strain	SFV L10
(vi)	pathovar (biotype, ecotype, race, etc.)	…
(vii)	common name	SFV

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	Rodents, mosquito's

(b) If the organism is an animal: natural habitat or usual agroecosystem:
SFV was first isolated from mosquitoes in the Semliki Forest , Uganda in 1942. It is found in central, eastern and southern Africa.

5. (a) Detection techniques
SFV can be detected by virus plaque assay

(b) Identification techniques
SFV can be identified by standard molecular techniques such as sequencing, restriction analysis and 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

The pathogenicity category of the wild-type SFV in Europe is 2 (Directive 2000/54/EC of the European Parliament and of the Counsil (2000)).

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	(X)
plants	(.)
other	(.)

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

Pathogenic characteristics

In contrast to most other Togaviruses, SFV is not considered to be dangerous for humans. SFV is spread by mosquito bites and thus can also infect humans. SFV infection is not associated with any disease in humans but it may cause mild symptoms such as headache, fever and rash. Human infections are common in certain parts of Africa where up to 40% of the population has been shown to be seropositive; however direct human-to-human virus transmission has not been documented. This Old World Alphavirus is common in Angola and Nigeria where up to 37% seroprevalence rate has been found. The available data suggests that the vast majority of European population does not have pre-existing antibodies to SFV.

SFV is not able to infect mammals through inhalation or gastrointestinal exposure although rodents in the laboratory can be infected by intranasal instillation. The virus can cause encephalitis in rodents and horses. The syndrome in these animals depends on the age of the animal, the route of infection and the strain used. Antibody-mediated clearance of infectious virus is completed around 7 to 10 days with viral RNA and protein persisting for months.

In humans, one possible case involving a lethal human infection has been reported. This occurred in an immunocompromised individual (immunodeficiency disorder involving antibody production), who worked with large amounts of wild-type SFV in the laboratory. It is however to date uncertain whether lethality was caused by the SFV infection.

8. Information concerning reproduction

(a) Generation time in natural ecosystems:

Wild-type SFV replicates in approximately 6 hrs.

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

Unknown

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

Virus shedding

(c) Factors affecting reproduction:
Mosquitoes are required to transmit the virus

…

9. Survivability

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

(i)	endospores	(.)
(ii)	cysts	(.)
(iii)	sclerotia	(.)
(iv)	asexual spores (fungi)	(.)
(v)	sexual spores (funghi)	(.)
(vi)	eggs	(.)
(vii)	pupae	(.)
(viii)	larvae	(.)
(ix)	other, specify	…not applicable

(b) relevant factors affecting survivability:
Alphaviruses commonly have a low chemical resistance and survive poorly outside a host. Temperature, humidity, air and direct light strongly affect the survival of the viruses.
In phosphate-buffered saline without serum the infectivity of SFV decreases within 1 to 3 hrs to less than 0.1%.

10. (a) Ways of dissemination
Mosquito bites

(b) Factors affecting dissemination
The theoretical frequency for the generation of replication-proficient virus (RPV) during the production phase of the vaccine is less than approximately $4x10^{-17}$.
For the phase I study a maximum of two dosages of $5x10^{8}$ particles will be administered, thus the risk that a replication-proficient SFV particle is administered is considered extremely low.
Semliki Forest virus has been assigned to the 'Low' category in the relative environmental risk table. While SFV can be pathogenic in mice, hardly any pathology has been associated with this virus in those regions with a high prevalence in the population (e.g. Africa). Thus, in the extremely unlikely possibility that RPV is present in the vaccine, the environmental risk remains low.

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

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

1. Type of the genetic modification

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

2. Intended outcome of the genetic modification
High level expression of the protein (tumor antigen) encoded by the transgene.

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

If no, go straight to question 5.

(b) If yes, is the vector wholly or partially present in the modified organism?
Yes (X) No (.)

If no, go straight to question 5.

4. If the answer to 3(b) is yes, supply the following information

(a) Type of vector

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

(b) Identity of the vector

A full-length cDNA clone of SFV4 is placed in a plasmid containing the SP6 RNA polymerase promoter to allow *in vitro* transcription of full-length and infectious RNA transcripts. The plasmid is called pSP6-SFV4.
From this plasmid the following plasmids have been generated.

pSFV1-3
The pSP6-SFV4 was used to construct a SFV DNA expression vector by deletion of the coding region of the 26S structural genes to make way for heterologous inserts. However, the non-structural coding region, which is required for the production of the nsP1-4 replicase complex is preserved. The 26S coding region has been replaced with a polylinker sequence allowing insertional cloning of desired cDNA sequences under the 26S promoter, resulting in the pSFV1-3. The vectors pSFV1, 2 and 3 differ as to the position where the polylinker cassette has been inserted. In pSFV3 (used in this application) the cassette is placed immediately after the initiation codon (AUG) of the original capsid gene.

pSFV-helper-C and pSFV-helper-S1 (two helper plasmids)

pSFV-helper-C: lacks the replicase gene (and thus the packaging signal), but maintains the 5' and 3' replications signals and the subgenomic promoter followed by the gene coding for the capsid protein (C).

pSFV-helper-S1: lacks the replicase gene (and thus the packaging signal) and maintains the 5' and 3' replications signals. This plasmid contains the subgenomic promoter followed by the translational enhancer sequence contained in the capsid gene, a very small (17 amino acids) sequence derived from the 2A sequence of foot-and-mouth-disease virus (providing release of functional envelope proteins from the polyprotein), and the envelope genes p62, 6K and E1.

In the original virus the structural proteins are produced as a polyprotein precursor which is co-translationally cleaved by the capsid protease and signal peptidase to produce the capsid protein and the envelope proteins. In the pSFV-helper-C a serine-alanine mutation is introduced in the capsid protein gene that abolishes the autoprotease activity of the capsid, which is dispensable when using a two-helper system where the capsid protein and the envelope proteins are expressed separately. However the autoprotease activity would be required for production of replication-competent virus in the extremely unlikely event of a "double" RNA recombination. The serine-alanine mutation is induced through mutation of three nucleotides, and thus provides yet another level of biosafety to the vector system.

…

(c) Host range of the vector

The plasmid vectors replicate in E.coli bacteria.

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

Yes (X) No (.)

antibiotic resistance (X)
other, specify …

Indication of which antibiotic resistance gene is inserted
Ampicillin

(e) Constituent fragments of the vector
As described in 4b

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

(i) transformation (.)
(ii) electroporation (X)
(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 …

6. Composition of the insert

(a) Composition of the insert

rSFVeE6,7 vector
The genes coding for a translational enhancer and the HPV type 16 proteins E6 and E7 are inserted in pSFV3 (see above).

HPV16 E6 and E7
The HPV16 E6 and E7 genes are obtained from the plasmid pRSV-HPV16E6E7. In this plasmid the HPV16 E6 and E7 genes are present in tandem, with a stop codon after the E6 gene. To generate the SFV vector coding for the HPV fusion protein of E6 and E7 one base pair between E6 and E7 was inserted and the stop codon TAA of E6 was changed to GAA. In addition a translational enhancer was cloned in front of the gene coding for the E6,7 fusion protein.

Translational enhancer
The 5' end of the capsid gene of SFV coding for the first 34 amino acid residues has been shown to function as a translational enhancer. This translational enhancer sequence was cloned in front of the E6,7 fusion gene. In addition, the sequence coding for the 2A of foot-and-mouth disease virus (51 base pairs; bp) was cloned in frame between the translational enhancer and the E6,7 fusion gene in order to provide cleavage between the proteins. Please note that the entire FMDV sequence is appr. 8.000 bp. The 51 bp sequence only encodes for one of the many proteins of this virus. The 51 bp sequence only prohibits a peptide bond between the translational enhancer sequence and the E6,7 fusion protein.

(b) Source of each constituent part of the insert
HPV16 E6 and E7 genes: obtained from the plasmid pRSV-HPV16E6E7.
Translational enhancer: the 5' end of the capsid gene of SFV coding for the first 34 amino acid residues.

(c) Intended function of each constituent part of the insert in the GMO
HPV16 E6,7 tumor antigen expressed by the GMO against which an immune response should be elicited.
Translational enhancer: to enhance protein expression of the transgene E6,7.

(d) Location of the insert in the host organism

- on a free plasmid (.)
- integrated in the chromosome ()
- other, specify integrated in the pSFV3 vector

(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	(X)
bacterium	(.)
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	…
(iii)	genus	Papillomaviridae
(iv)	species	papillomavirus
(v)	subspecies	human papillomavirus
(vi)	strain	HPV16 and 18
(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 (X) No (.) Not known (.)

If yes, specify the following:

(b) to which of the following organisms:

humans	(X)
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):

E7 is a cytoplasmic serine phosphoprotein, and has been shown to be a transcriptional transactivator. E7 binds Rb protein and then presumably moves to the nucleus. E6 binds to a cellular protein called E6-Ap. This complex then binds to p53. E6-Ap is a ubiquitin ligase. Cellular enzymes load it with activated ubiquitin molecules which are transferred to p53. Ubiquitin loading of p53 targets it for degradation by proteasome mediated proteolysis. The forced entry into S-phase in conjunction with genomic instability, resulting from p53 degradation may lead to cell transformation.

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 (x) No (.)

If yes, specify …

The pathogenicity category of human papillomavirus in Europe is 2 (Directive 93/88/EC of the European Parliament and of the Counsil).

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

The GMO is replication-deficient. Upon infection of an organism or cell with the GMO the GMO will replicate its RNA and produce large amounts of the transgene but no new virus particles can be produced as the RNA encoding the structural proteins of the virus are not incorporated in the GMO. Infected cells die through apoptosis within a week after infection.

(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

The GMO cannot reproduce itself

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

Yes (X) No (.) Not known (.)

Specify

Frequencies and probability of recombinations and mutations of the GMO that are required to generate replication proficient SFV (recipient):

Using the two-helper system, high-titer recombinant SFV replicon particles can be generated. The replicon particles are fully infectious, producing a single burst of RNA replication and heterologous protein gene expression. The theoretical frequency of the generation of replication-proficient virus

(RPV) during the *in vitro* production phase of the SFV replicon particles is less than $4.2x10^{-17}$, as explained below.

Recombination of two RNA species occurs with relatively high frequency (10^{-6}), but by splitting the capsid and envelope helper regions into two independent helper RNAs, a recombination of all three RNA species is needed to form RPV within the packaging cells. Such "double" RNA recombination frequency is therefore app. 10^{-12} (10^{-6} x 10^{-6}).

In order to further reduce the probability to generate RPVs a mutation was introduced in the capsid protein gene in helper-C that abolishes the autoprotease activity of the capsid, an activity which is not required in the two-helper expression system, but would be required for production of replication-competent virus in the extremely unlikely event of a "double" RNA recombination. The maximum frequency of reversion of this mutation is $4.2x10^{-5}$, as determined by Smerdou and Liljestrom.
Thus, the theoretical frequency for the occurrence of a combination of a "double" RNA recombination event (10^{-6} each) and reversion of the capsid protein mutation would be less than $10^{-6}x10^{-6}x4.2x10^{-5}= 4.2x10^{-17}$. This frequency is too low to be evaluated experimentally.
Extensive analysis has failed to demonstrate the formation of any RPVs in $2x10^{13}$ particles.

(d) is the GMO in any way different from the recipient as far as pathogenicity is concerned?
Yes (X) No (.) Not known (.)
Specify The GMO is not pathogenic

2. Genetic stability of the genetically modified organism

SFV replicon particles at room temperature and diluted in buffer rapidly loose infectivity. At -80°C the virus and the replicon particles diluted in phosphate-buffered saline are stable over 2 years.

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)
…

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment
Not appplicable

(b) Techniques used to identify the GMO

Identification of replicase or transgene expression in cells infected with the GMO using immunohistochemistry.

F. Information relating to the release

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

Therapeutic immunization is an attractive alternative to the current options for treating precancerous lesions and (invasive) cancer. The immune cells induced by cancer immunotherapy can target the tumour cells and kill them. When long-lasting immunity is induced the immunotherapy may prevent recurrence of the disease.

This application describes an immunization methodology with replicon particles based on an attenuated Semliki Forest Virus (SFV) encoding tumour antigens. Immunization with these replication-incompetent SFV replicon particles is aimed at eliciting a therapeutic anti-tumour response. The general goal of the work will be to develop a new immunotherapeutic treatment strategy using replicon particles from Semliki Forest Virus that induces a strong and long-lasting immunological anti-tumour response.
To show the efficacy of this methodology, a first specific application will be clinically tested. This application is targeted against (pre)malignant disease, induced by persistent infection with an oncogenic human papillomavirus (HPV).
HPV infection has been recognized as the necessary cause of premalignant genital and oro-pharyngeal lesions, cervical cancer, vulvar cancer, anal cancer, penile cancer and oro-pharyngeal cancer. HPV-induced cancer is the second largest cause of cancer deaths in women worldwide.

Current treatment for premalignant HPV-induced genital lesions primarily relies on surgery, which is highly discomforting and carries a risk of complications like bleeding, stenosis and/or cervical incompetence which may lead to infertility. Above all, it does not eradicate the underlying HPV infection.

The proposed immunotherapeutic strategy targets HPV-infected and HPV-transformed cells and is expected to induce long-lasting immunity, thus preventing recurrence. This treatment may be an attractive alternative to the current surgical options for treating HPV-induced (pre)malignant diseases.

The primary purpose of the project is to perform safety and immune efficacy studies (phase I and II clinical trials) with replication-incompetent rSFV replicon particles encoding a fusion protein of the E6 and E7 proteins of HPV type 16 in patients with HPV-induced premalignant cervical lesions (CIN II/III).

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 This Old World Alphavirus is common in Angola and Nigeria where up to 37% seroprevalence rate has been found. The available data suggests that the vast majority of European population does not have pre-existing antibodies to SFV.

3. Information concerning the release and the surrounding area

(a) Geographical location (administrative region and where appropriate grid reference):
University Medical Center Groningen, Groningen, The Netherlands

(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:
Not applicable

(d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO
Not applicable

4. Method and amount of release

(a) Quantities of GMOs to be released:
In the first trials two injections of maximally $5x10^8$ i.u. rSFVeE6,7 per injection will be administered. In subsequent trials maximally four injections of $5x10^8$ i.u. rSFVeE6,7 will be administered.

(b) Duration of the operation:
Time needed for an intramuscular injection (approximately 1 minute), Patients will be observed for 4 hr after administration of the vaccine to monitor possible adverse events.

(c) Methods and procedures to avoid and/or minimise the spread of the GMOs beyond the site of the release
Not applicable

5. Short description of average environmental conditions (weather, temperature, etc.)
Standard 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 available

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

1. Name of target organism (if applicable)

(i)	order and/or higher taxon (for animals)	Hominidae…
(ii)	family name for plants	…
(iii)	genus	Homo
(iv)	species	Homa sapiens
(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)
The GMO will be injected intramuscularly. The virus locally infects cells and within the infected cell the GMO will replicate its RNA and produce the tumor antigen (E6,7 protein) encoded by the transgene. The infected cell dies through apoptotic cell death within 1-3 days. The apoptotic cells will be processed by antigen presenting cells (immune cells) and the tumor antigen will be presented to other cells of the immune system giving rise to a (tumor)antigen-specific immune response. The immune cells can recognize the specific cancer cells and kill them.

3. Any other potentially significant interactions with other organisms in the environment
no

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

7. Likelihood of genetic exchange in vivo

(a) from the GMO to other organisms in the release ecosystem:
Exchange between the GMO and other organisms is highly unlikely

(b) from other organisms to the GMO:
Exchange between the GMO and other organisms is highly unlikely

(c) likely consequences of gene transfer:
Integration of the E6E7 transgene into the human genome

The SFV viral system is especially suited to safely induce cellular immune responses against HPV E6 and E7. Firstly, SFV is an RNA virus replicating in the cell cytosol. Up to now no evidence has been reported suggesting that the SFV genome, upon the hypothetical event of reverse transcription, can integrate in the host cell genome. To our knowledge no studies have been reported on the possibility of integration of genomes of other positive-strand viruses. Thus, the risk of integration of the E6 and E7 genes in the cellular genome is negligible.

Secondly, we demonstrated that in the hypothetical event of integration of the sequence into the host genome the transforming activity of the fusion protein is strongly impaired compared to the transforming activity of the separate E6 and E7 proteins

Finally, since SFV infection is cytolytic by apoptosis, no genetic information of E6 and E7 is likely to persist for more than 10 days after injection.

HPV infections are common infections in the human population. High-risk HPV-types such as HPV16 and 18 are co-factors in the development of genital cancers and head-and-neck cancers. During their life-time an estimated 80% of men and women are exposed to an infection with these viruses. The vast majority of HPV-infected people clear the virus and virally infected cells within 1 year after infection. During their life-time appr. 1-2 % of women develop HPV-induced genital (pre)malignant lesions.

Thus the chance on the induction of an HPV-induced malignancy by the administration of SFVeE6,7 particles depends on the following events that all have to occur .
First, the very unlikely event (never documented) of integration of the rSFV RNA encoding the fusion protein E6,7 in the host cell genome.
Second, the unlikely event that the rSFV-infected cell does not die through apoptotic cell death.
Third, the unlikely possibility that despite the strongly reduced transforming capacity of the E6,7 fusion protein, the protein remains functional as a co-factor in the transformation of the cell.
Fourth, the unlikely possibility that the immune system does not eradicate the infected/transformed cell.

The chance that these events occur simultaneously within one cell without the immune system eradicating this cell is negligible.

8. Give references to relevant results (if available) from studies of the behaviour and characteristics of the GMO and its ecological impact carried out in stimulated natural environments (e.g. microcosms, etc.):
Not available

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

H. Information relating to monitoring

1. Methods for monitoring the GMOs
In the clinical trial the GMOs will not be monitored
The rSFV replicon particles will not be determined after administration since they are not regarded as harmful.

2. Methods for monitoring ecosystem effects
Not applicable

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms
Not applicable

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

5. Duration of the monitoring
…

6. Frequency of the monitoring
…

I. Information on post-release and waste treatment

1. Post-release treatment of the site
Material exposed to the GMO will be disinfected with1% sodium hypochlorite or 70% ethanol and if applicable autoclaved. Waste will be discarded in special containers and destroyed according to procedures for contaminated hospital waste.

2. Post-release treatment of the GMOs
Material exposed to the GMO will be disinfected with1% sodium hypochlorite or 70% ethanol and if applicable autoclaved and discarded in special containers and destroyed according to procedures for contaminated hospital waste.

3. (a) Type and amount of waste generated
Waste includes syringes and needles, for virus administration containing the remaining volume virus aliquot that is not injected, disposable gloves and clothing of personnel, plaster, cotton wool to clean the injection site.
Blood samples and biopsies from the HPV-lesions will be taken for immunomonitoring. Cell culture and disposables from these analysis.

3. (b) Treatment of waste
Waste will be disinfected with1% sodium hypochlorite or 70% ethanol and if applicable autoclaved. Waste will be discarded in special containers and destroyed according to procedures for contaminated hospital waste.

The patient samples will be taken by properly trained personnel and will be transported to the laboratory and stored in qualified containers. The in vitro analysis of this material will be performed under ML-1 conditions. All materials that have

been in contact with the human samples will be disinfected with 1% sodium hypochlorite or 70% ethanol, autoclaved and discarded in special containers and destroyed according to procedures for contaminated hospital waste.

J. Information on emergency response plans

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread
Spilled GMO will be wiped up with dry tissues. Subsequently the surface will be disinfected with tissues soaked in active chlorine solution (10%). All collected materials and gloves worn during execution of the cleaning procedure are disposed according to procedures for contaminated hospital waste.

2. Methods for removal of the GMO(s) of the areas potentially affected
See J.1.

3. Methods for disposal or sanitation of plants, animals, soils, etc. that could be exposed during or after the spread
Not applicable

4. Plans for protecting human health and the environment in the event of an undesirable effect
Patients will be monitored for 4 hrs post-injection for acute side effects of vaccine-induced immune responses. The patient will also be monitored for the occurrence of adverse events following the vaccination according to the clinical protocol. Serious adverse events will be registered and evaluated and health authorities will be notified when relevant. Special plans for protecting the environment are not considered necessary, for the reasons stated above.