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 Spain
(b) Notification number B/ES/06/42
(c) Date of acknowledgement of notification 22/06/2006
(d) Title of the project: An international, randomised, double blind, placebo controlled, parallel group study to investigate whether TroVax®, added to first-line standard of care therapy, prolongs the survival of patients with locally advanced or metastatic clear cell renal adenocarcinoma. (TV3/001/06)
(e) Proposed period of release: Maximum of 3,5 years From ../../…. until ../../….

2. Notifier

Name of institution or company:
Oxford BioMedica UK Ltd.,
Medawar Centre,
Robert Robinson Avenue,
The Oxford Science Park,
Oxford
OX4 4GA, United Kingdom
Contacts: Mrs Julia Sutton (Biological Safety Officer)
Dr Fiona Ellard (Deputy Biological Safety Officer)
Telephone +44 (0)1865 783000
…

3. GMO characterisation:

(a) Indicate whether the GMO is a:

| | |
|---|---|
| viroid | (.) |
| RNA virus | (.) |
| DNA virus | (X) Modified Vaccinia Ankara Virus |
| bacterium | (.) |
| fungus | (.) |
| animal | |
| - mammals | (.) |
| - insect | (.) |
| - fish | (.) |
| - other animal | (.) |

specify phylum, class …

(b) Identity of the GMO (genus and species):
Poxviridae, Vaccinia
…

(c) Genetic stability – according to Annex IIIa, II, A(10)
There was no evidence of MVA reversion to virulence or complications reported when MVA was administered to over 120,000 recipients, many of which were at risk from vaccine complications (Mayr and Danner, 1979. Berliner und Münchener tierärztliche Wochenschrift 92, p251-256)

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

Yes (X) No (.)

If yes, insert the country code(s) FR, DE, GB ,NL, Poland…

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, FR, NL, DE, Poland …
- Notification number B/ES/06/…

**Please use the following country codes:**

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

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

Yes (X) No (.)

If yes:

- Member State of notification Federation of Russia, Romania, Ukraine, Israel, USA
- Notification number B/../../…

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

The environmental impact is likely to be low for the following reasons:

The GMO (TroVax) is based on MVA which is non-pathogenic in animals including suckling mice, rabbits and primates. TroVax is replication defective (replication only occurs in primary chicken embryo cells or BHK-21 cells) and hence is unlikely to survive in the environment.due to an inability to replicate. Although poxviruses are very stable to normal environmental conditions (Paragraph 19 ACDP & ACGM 'Vaccination of laboratory workers handling vaccinia and related poxviruses infectious for humans': 1990), in the absence of replication TroVax inaction will occur by UV irradiation or with standard hospital disinfectants, e.g. Virkon.

The lack of persistence may be implied from the following information based on MVA. Although replication competent vaccinia viruses are known to persist in the vaccination scab after scarification (paragraph 69 in part 2B Annex III of the ACGM 'Compendium of Guidance from the Health and Safety Commissions Advisory Committee on the genetic modification'), no replication and no scab formation at the inoculation site has been observed after vaccination with MVA or TroVax. In addition MVA has been used in an extensive vaccination program (Mayr and Danner, 1979.Berliner und Műnchener tierärztliche Wochenschrift 92, p251-256) without any reports of spread into the environment. The US Centres for Disease Control and Protection (CDC) state that although no formal surveillance system has been established to monitor laboratory workers, no laboratory-acquired infections resulting from exposure to these highly attenuated strains or recombinant vaccines derived from these strains (MVA, NYVAC,

ALVAC, or TROVAC) have been reported in the scientific literature or to CDC. They also state that although the risk for transmission of recombinant vaccinia viruses to exposed health-care workers is unknown, no reports of transmission to health-care personnel from vaccine recipients have been published.

When TroVax is used the administration site is swabbed with alcohol and an occlusive dressing is also used as an extra precaution to protect healthcare workers. However this risk is considered low as studies have shown that there is no shedding of TroVax in the treated patients that could pose a risk to anything or anyone.

7.1. genetic transfer capability

(a) post-release transfer of genetic material from GMOs into organisms in affected ecosystems;

Recombination between closely related poxviruses may occur during virus replication under natural conditions. However although MVA can infect most mammalian species, the cells that it infects die of a result of the infection. During this infection unless the cells are permissive for the virus (primary chicken embryo cells or BHK-21 cells) no infectious particles are produced (Sancho et al. 2002 J. Virology 76 (16) p8318-8334). Due to the short duration of this non-productive infection it is extremely unlikely that recombination will occur under natural conditions between MVA and other poxviruses and hence that genetic material will be transferred to other organisms.

(b) post-release transfer of genetic material from indigenous organisms to the GMOs;
Unlikely due to the reasons quoted in (a).

7.2. likelihood of postrelease selection leading to the expression of unexpected and/or undesirable traits in the modified organism,

Since the GMO (TroVax) will not replicate in the patients this is very unlikely. This view is supported by the fact that there is no evidence that such selection occurs in the parental MVA.

7.3. measures employed to ensure and to verify genetic stability. Description of genetic traits which may prevent or minimise dispersal of genetic material. Methods to verify genetic stability.

The following measures were used to assess genetic stability between the original virus master seed stock and the clinical batch; sequencing, southern blot and PCR analysis of the final clinical lots. Sequencing and southern blot analysis of the master viral seed stock and the final clinical lots produced up to 10 passages later have demonstrated the transgene to be genetically stable after multiple passages. Measurements of the percentage of foci expressing 5T4 on chicken fibroblast monolayers have detected no changes over passage. Similarly, Western blot analysis also indicates no detectable change in the level of transgene protein expression after multiple passages.

The fact that MVA only replicates in permissive cells (CEFs and BHKs), thus dispersal will be limited to the first organism infected will prevent or minimise the dispersal of genetic material.

7.4. routes of biological dispersal, known or potential modes of interaction with the disseminating agent, including inhalation, ingestion, surface contact, burrowing, etc.

The route of infection by replication competent vaccinia is usually by skin lesions, accidental injection or the respiratory system. In the clinical setting with vaccinia it has been reported that persistance in the vaccination scab after scarification occurs which could lead to a risk of dispersal (paragraph 69 in part 2B Annex III of the ACGM 'Compendium of Guidance from the Health and Safety Commissions Advisory Committee on the genetic modification'). However, since MVA does not replicate in mammals (there is no replication and no scab at the inoculation site) it is unlikely that biological dispersal will occur with TroVax. When TroVax is used the administration site is swabbed with alcohol and an occlusive dressing is also used as an extra precaution to protect healthcare workers. However this risk is considered low as studies have shown that there is no shedding of TroVax in the treated patients that could pose a risk to anything or anyone.

7.5. description of ecosystems to which the GMOs could be disseminated.

The GMO (TroVax) will be confined to the hospital test site, including the hospital pharmacy, clinical lab, and autoclaving/incineration area.

7.6. potential for excessive population increase in the environment,

The GMO (TroVax) is replication defective in organisms likely to be encountered in the hospital test site and hence is unlikely to survive in the environment and hence there is no

potential for population increase in the environment.

7.7. competitive advantage of the GMOs in relation to the unmodified recipient or parental organism(s),

As both MVA and the GMO (TroVax) are replication defective unless in a very small range of permissive cell lines, they do not have a competitive advantage over natural poxviruses. There is no evidence that TroVax has any significant competitive advantage over the parental MVA.

7.8. identification and description of the target organisms if applicable,

The GMO is to be used in cancer patients participating in clinical trials.

7.9. anticipated mechanism and result of interaction between the released GMOs and the target organism(s) if applicable,

The GMO (TroVax) is designed to express the 5T4 protein. 5T4 is a 72 kDa oncofoetal glycoprotein that is expressed on over 70% of carcinomas of the breast, gastrointestinal tract, colon and ovaries. Unlike other self-antigen TAAs e.g. CEA, 5T4 expression appears to be tumour specific with only low level expression reported in the gut. Immunohistochemical analysis indicates that 5T4 expression is an indicator of poor prognosis in colorectal cancer. Additionally, when tumour cells are transfected with the cDNA encoding for 5T4, they display increased motility, suggesting that expression of this molecule may induce metastatic properties in a tumour. The GMO will be injected into the patients. The patients cells injected with the GMO will express the 5T4 protein. This should result in the patients raising an immune response against the 5T4 protein and potentially a treatment for their cancer.

7.10. identification and description of non-target organisms which may be adversely affected by the release of the GMO, and the anticipated mechanisms of any identified adverse interaction,

The GMO is replication defective in mammals including humans. It is possible that hospital staff may be injected by accident, but this would be unlikely to have any ill effects. No reports of MVA infections in healthcare workers handling MVA in the US have been reported to the US CDC. Since the 5T4 protein is expressed on the placenta,

during the trials pregnant healthcare workers will not administer the GMO. However pre-clinical toxicology studies in pregnant mice using the murine version of the 5T4 protein have not indicated any risks to either the fetus or mother. No other organisms are likely to be injected.

7.11. likelihood of post-release shifts in biological interactions or in host range,

As the GMO is replication defective, this is extremely unlikely.

7.12. known or predicted interactions with non-target organisms in the environment, including competitors, preys, hosts, symbionts, predators, parasites and pathogens,

None.

7.13. known or predicted involvement in biogeochemical processes,

None.

7.14. other potential interactions with the environment.

None.

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) Modified Vaccinia Ankara Virus |
| 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 | … |
| (iv) | subspecies | … |
| (v) | strain | … |
| (vi) | pathovar (biotype, ecotype, race, etc.) | … |
| (vii) | common name | Modified Vaccinia Ankara Virus (MVA) |

3. Geographical distribution of the organism

(a) Indigenous to, or otherwise established in, the country where the notification is made:

Yes (.) No (.) Not known (X) Vaccinia and derivatives of vaccinia such as MVA were used extensively as vaccines in the eradication of smallpox worldwide. However the natural host is unknown.

(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 (X)

Vaccinia and derivatives of vaccinia such as MVA were used extensively as vaccines in the eradication of smallpox worldwide. However the natural host of vaccinia is unknown.

(c) Is it frequently used in the country where the notification is made?

Yes (X) No (.)

(d) Is it frequently kept in the country where the notification is made?

Yes (X.) No (.)

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 | (X) |

The natural host of vaccina virus is unknown. Modified Vaccinia Ankara Virus is an artificial laboratory produced strain that is cultured in baby hamster kidney cells (BHK21) or primary chick embryo fibroblasts

(b) If the organism is an animal: natural habitat or usual agroecosystem:
Not applicable

5. (a) Detection techniques
In previous clinical trials using recombinant MVA, viral shedding was monitored in patient samples using quantitative PCR. MVA may be detected in tissue culture media using specific antibodies.

(b) Identification techniques
MVA can be identified using PCR or antibody techniques. The GMO TroVax may be distinguished from the parental MVA using antibodies or PCR assys specific to the 5T4 protein.

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 UK HSE classifies MVA as a biosafety level 1 organism.
The German authorities indicate that BL1 may be possible for MVA usage if the inserted gene does not alter the host range of the MVA or increase the risk.

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

The directive asks for information on the pathogenicity: infectivity, toxigenicity, virulence, allergenicity, carrier (vector) of pathogen, possible vectors, host range including non-target organism or the GMO and any possible activation of latent viruses (proviruses) by the GMO and its ability to colonise other organisms;

The GMO and parental MVA are non-pathogenic in animals including suckling mice, rabbits and primates. They are unlikely to survive in the environment as replication is restricted to BHK21 and primary avian cells. MVA has been used in an extensive vaccination program (Mayr and Danner, 1979. Berliner und Münchener tierärztliche Wochenschrift 92, p251-256) with no reports of pathogenicity or replication in the participants. CDC states that it has have no reports of either healthcare or laboratory workers becoming infected. Colonisation of other organisms has not been reported.

8. Information concerning reproduction

MVA is replication defective in all mammalian cell lines tested except Baby Hamster Kidney cells (BHK-21). During its development it was adapted to replicate in primary chicken embryo fibroblast cells. MVA is non-pathogenic in animals including suckling mice, rabbits and primates. It is unlikely to survive in the environment.

(a) Generation time in natural ecosystems:

The vector will not replicate in natural ecosystems.

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

Not applicable.

(c) Way of reproduction: See text from section 8

Sexual .. Asexual X ..

(c) Factors affecting reproduction:

See text from section 8

9. Survivability

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

MVA does not form structures for 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:

MVA is replication defective (replication only occurs in primary chicken embryo cells or BHK-21 cells) and hence is unlikely to survive in the environment due to an inability to replicate. Although poxviruses are very stable to normal environmental conditions (Paragraph 19 ACDP & ACGM 'Vaccination of laboratory workers handling vaccinia and related poxviruses infectious for humans': 1990), in the absence of replication TroVax inaction will occur by UV irradiation or with standard hospital disinfectants, e.g. Virkon. It has been used in an extensive vaccination program (Mayr and Danner, 1979. Berliner und Münchener tierärztliche Wochenschrift 92, p251-256) without any reports on survivability in the environment.

10. (a) Ways of dissemination

The route of infection by replication competent vaccinia is usually by skin lesions, injection or the respiratory system. There is no reason to suggest that entry of MVA into an organism would occur by a different route. However as MVA will not replicate in mammals, dissemination from organism to organism will not occur. Survival in the environment is unlikely.

(b) Factors affecting dissemination

Since MVA will only replicate in a limited range of tissue culture cells, it is unlikely to survive in the environment. Therefore it is difficult to identify a factor that would cause dissemination.

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

Not applicable in Spain.

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

The GMO (TroVax) is designed to express the 5T4 protein. 5T4 is a 72 kDa oncofoetal glycoprotein that is expressed on over 70% of carcinomas of the breast, gastrointestinal tract, colon and ovaries. Unlike other self-antigen TAAs e.g. CEA, 5T4 expression appears to be tumour specific with only low level expression reported in the gut. Immunohistochemical analysis indicates that 5T4 expression is an indicator of poor prognosis in colorectal cancer. Additionally, when tumour cells are transfected with the cDNA encoding for 5T4, they display increased motility, suggesting that expression of this molecule may induce metastatic properties in a tumour. The GMO will be injected into the patients. The patients cells injected with the GMO will express the 5T4 protein. This should result in the patients raising an immune response against the 5T4 protein and potentially a treatment for their cancer.

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 | (.) |

A plasmid vector containing the human 5T4 cDNA, mH5 promotor, and sequences homologous to MVA (Flank 1 and Flank 2) was used to produce a recombinant MVA virus.

(b) Identity of the vector

Description of genetic trait(s) or phenotypic characteristics and in particular any new traits and characteristics which may be expressed or no longer expressed;

The plasmid vector:
Construction of the TroVax transfer vector pTRV-1b:
This vector was constructed using PCR to amplify the flanking regions around del III from an MVA template and linking this to the modified H5 promoter that was produced as a synthetic oligonucleotide. These fragments were inserted into the basic cloning vector pNEB 193 (obtained from New England BioLabs). The 5T4 coding region was inserted downstream of the mH5 promoter. The H5 promoter directs expression at both early and late phases of the viral replication cycle. The H5 promoter that is used is a synthetic derivative that has been modified by the incorporation of a base change that has been shown to enhance early promoter activity. This mH5 promoter is 70 nucleotides long. The pNEB 193 plasmid possesses the ampicillin antibody selection gene. Since the inserts are cloned downstream of eukaryotic promoters they are unlikely to be expressed in E.coli. The insert is cloned into part of the β-galactosidase gene as so the activity of this gene will be lost.

The MVA vector:
The modified MVA vector will now express the human 5T4 protein. The modification of the MVA vector will not affect its tropism, survival or infectivity

characteristics since the human 5T4 gene has been inserted into a non-coding region of the genome. MVA virus replication has been compared to MVA–5T4 (TroVax) replication in permissive CEF cells and the replication kinetics of both were found to be similar.

(c) Host range of the vector

- Plasmid vector
  The plasmid vector will replicate in E.coli.

- MVA
  MVA will replicate in primary chicken embryo fibroblasts and BHK21 cells.

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

Yes (X) No (.)

antibiotic resistance (X) The plasmid vector contains a antibiotic resistance marker (ampicillin). The insert is cloned into part of the β-galactosidase gene as so the activity of this gene will be lost.

other, specify MVA does not contain a selectable marker. …

Indication of which antibiotic resistance gene is inserted

(e) Constituent fragments of the vector

The plasmid vector:

The plasmid vector is the basic cloning vector pNEB 193 (obtained from New England BioLabs) a derivative of pUC.. The pNEB 193 plasmid possesses the ampicillin antibody selection gene.

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

| | | |
|---|---|---|
| (i) | transformation | (X) |
| (ii) | electroporation | (.) |
| (iii) | macroinjection | (.) |
| (iv) | microinjection | (.) |
| (v) | infection | (X) |
| (vi) | other, specify … | |

The plasmid vector is replicated by transformation into E.coli

A recombinant MVA vector was produced by the following procedure.
Monolayers of primary chicken embryo fibroblast (CEF) cells were infected with MVA p581. After 1 hour at 37$^{o}$C, virus was removed and the cells were transfected with the transfer plasmid (pTRV-1b) and the cells were incubated at 37$^{o}$C. Recombination between the MVA virus and the MVA sequences in the plasmid vector was allowed to occur. The cells were harvested 48 hours post-transfection, frozen on dry ice and thawed at 37$^{o}$C 3 times with vigorous vortexing between freeze-thaw cycles, serially diluted, and inoculated onto CEF cells. After 1hr adsorption, the cells were washed and incubated in MEM containing 2% FBS at 37$^{o}$C. Two days later, recombinant h5T4 positive foci were visualised by live immuno-staining of CEF cells on Concanavalin A coated 6-well plates similar to that described by Earl et al. 1998. (Earl P, Wyatt LS, Moss B *et al*. Generation of vaccinia virus recombinant viruses. In: Current Protocols in Molecular Biology. John Wiley & Sons Inc, 1998; Suppl 43: Units 16.17.1-16.17.19.). Plaque purification was performed six times.

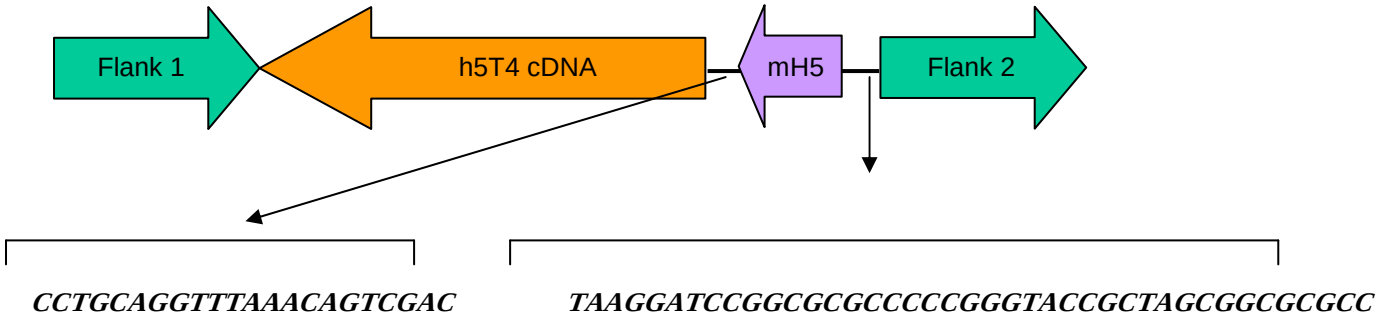
5. If the answer to question B.3(a) and (b) is no, what was the method used in the process of modification?

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

6. Composition of the insert

(a) Composition of the insert

The insert as present in the bacterial plasmid is shown in figure 1.




- There are two short regions of plasmid sequences remaining in the insert that is transferred into the GMO (TroVax) (see figure 1).
- Flank 1 and 2 sequences are from around del III from MVA.
- The region marked mH5 is a modified vaccinia H5 promotor (Wyatt et al. 1996. Vaccine 14, 1451-1458). The H5 promoter directs expression at both early and late phases of the viral replication cycle The H5 promoter that is used is a synthetic derivative that has been modified by the incorporation of a base change that has been shown to enhance early promoter activity This mH5 promoter is 70 nucleotides long. The sequence is as follows:
  ATTAAAAATTGAAAATAAATACAAAGGTTCTTGAGGGTTGTGTTAA
  ATTGAAAGCGAGAAATAATCATAAATA
- The region marked h5T4 cDNA is the coding sequence for the human 5T4 72 kDa oncofoetal glycoprotein that is expressed on over 70% of carcinomas of the breast, gastrointestinal tract, colon and ovaries.
  The amino acid sequence is:

MPGGCSRGPAAGDGRLRLARLALVLLGWVSSSSPTSSASSFSSSAPFLASAVSAQPPLPDQCPAL
CECSEAARTVKCVNRNLTEVPTDLPAYVRNLFLTGNQLAVLPAGAFARRPPLAELAALNLSGS
RLDEVRAGAFEHLPSLRQLDLSHNPLADLSPFAFSGSNASVSAPSPLVELILNHIVPPEDERQNRS
FEGMVVAALLAGRALQGLRRLELASNHFLYLPRDVLAQLPSLRHLDLSNNSLVSLTYVSFRNL
THLESLHLEDNALKVLHNGTLAELQGLPHIRVFLDNNPWVCDCHMADMVTWLKETEVVQGK
DARLTCAYPEKMRNRVLLELNSADLDCDPILPPSLQTSYVFLGIVLALIGAIFLLVLYLNRKGIK
KWMHNIRDACRDHMEGYHYRYEINADPRLTNLSSNSDV

The nucleotide sequence is as follows

CTGTCTAAACGCGTTAGTAAAACATGGCGAGGAAATAAATCATATAAAAAATGATTTCATGATTAAACC

ATGTTGTGAAAAAGTCAAGAACGTTCACATTGGCGGACAATCTAAAAACAATACAGTGATTGCAGATTT

GCCATATATGGATAATGCGGTATCCGATGTATGCAATTCACTGTATAAAAAGAATGTATCAAGAATATC

CAGATTTGCTAATTTGATAAAGATAGATGACGATGACAAGACTCCTACTGGTGTATATAATTATTTTAAA

CCTAAAGATGCCATTCCTGTTATTATATCCATAGGAAAGGATAGAGATGTTTGTGAACTATTAATCTCA

TCTGATAAAGCGTGTGCGTGTATAGAGTTAAATTCATATAAAGTAGCCATTCTTCCCATGGATGTTTCC

TTTTTTACCAAAGGAAATGCATCATTGATTATTCTCCTGTTTGATTTCTCTATCGATGCGGCACCTCTCT

TAAGAAGTGTAACCGATAATAATGTTATTATATCTAGACACCAGCGTCTACATGACGAGCTTCCGAGTT

CCAATTGGTTCAAGTTTTACATAAGTATAAAGTCCGACTATTGTTCTATATTATATATGGTTGTTGATGG

ATCTGTGATGCATGCAATAGCTGATAATAGAACTTACGCAAATATTAGCAAAAATATATTAGACAATACT

ACAATTAACGATGAGTGTAGATGCTGTTATTTTGAACCACAGATTAGGATTCTTGATAGAGATGAGATG

CTCAATGGATCATCGTGTGATATGAACAGACATTGTATTATGATGAATTTACCTGATGTAGGCGAATTT

GGATCTAGTATGTTGGGGAAATATGAACCTGACATGATTAAGATTGCTCTTTCGGTGGCTGGTACTCG

AGTCAGACATCCGAGTTAGAACTGAGGTTTGTTAATCTGGGGTCCGCATTGATTTCATATCTGTAATGA

TACCCTTCCATGTGATCCCTGCAGGCATCTCTGATGTTATGCATCCACTTTTTTATCCCCTTGCGGTTC

AAATACAAAACCAGGAGGAAAATAGCGCCTATCAGGGCTAAAACAATACCCAGGAAGACATAAGAGG

TTTGCAGGGATGGGGGAAGAATCGGGTCACAGTCCAGGTCAGCACTGTTGAGTTCCAAGAGGACCC

GATTCCTCATTTTTTCCGGATATGCACAGGTGAGCCGGTCTTTGCCCTGCACTACCTCTGTTTCCTTG

AGCCAGGTCACCATGTCTGCCATGTGGCAGTCGCAGACCCAGGGATTGTTGTCCAGGAAAACCCTAA

TGTGGGGTAGACCTTGCAACTCAGCCAGGGTGCCATTGTGAAGGACCTTGAGGGCATTGTCCTCCAG

GTGGAGGCTTTCTAGATGTGTCAGGTTGCGGAAGGACACGTAGGTCAGGCTCACCAGCGAATTATTA

CTTAAGTCCAGGTGCCTGAGGCTGGGCAGTTGGGCCAGCACATCCCGCGGCAGGTAAAGGAAGTGG

TTGCTGGCCAGCTCCAAGCGGCGGAGCCCCTGCAGTGCACGGCCCGCCAGCAGGGCCGCCACCAC

CATGCCCTCGAAGCTCCGGTTCTGCCGCTCATCTTCAGGGGGCACGATGTGGTTCAGGATCAGTTCC

ACAAGGGGACTGGGGGCCGAGACGCTGGCATTGCTGCCCGAGAAAGCGAAGGGACTGAGGTCGGC

CAGTGGGTTGTGGCTGAGGTCGAGCTGGCGCAGGCTGGGCAGATGCTCGAAGGCGCCCGCGCGCA

CCTCGTCCAGGCGGCTGCCGCTGAGGTTGAGCGCGGCCAGCTCCGCCAGCGGCGGCCGGCGGGC

GAAGGCGCCGGCAGGGAGCACGGCCAGCTGGTTGCCGGTAAGGAAGAGGTTGCGCACGTAGGCGG

GCAGGTCCGTGGGCACCTCGGTCAGATTGCGGTTAACGCACTTGACTGTGCGCGCTGCCTCGGAGC

ACTCGCACAGCGCGGGGCACTGGTCCGGCAGCGGGGGCTGGGCGGACACGGCGGAAGCCAGGAA

CGGCGCCGAGGAGGAGAAGGAGGATGCCGAGGAGGTGGGAGAAGACGAGGAGACCCAGCCCAGG

AGTACCAGCGCTAGTCGCGCCAGCCGCAGACGCCCGTCCCCGGCGGCGGGGCCCCGGGAGCACC

CCCCAGGCATCGCGGCTCGCGGTTGCGGCCGCCTGCAGGTTTAAACAGTCGACTATTTATGATTATT

TCTCGCTTTCAATTTAACACAACCCTCAAGAACCTTTGTATTTATTTTCAATTTTTAATTAAGGATCCGG

CGCGCCCCCGGGTACCGCTAGCGGCGCGCCTTTGGAAAGTTTTATAGGTAGTTGATAGAACAAAATA

CATAATTTTGTAAAAATAAATCACTTTTTATACTAATATGACACGATTACCAATACTTTTGTTACTAATAT

CATTAGTATACGCTACACCTTTTCCTCAGACATCTAAAAAAATAGGTGATGATGCAACTTTATCATGTAA

TCGAAATAATACAAATGACTACGTTGTTATGAGTGCTTGGTATAAGGAGCCCAATTCCATTATTCTTTTA

GCTGCTAAAAGCGACGTCTTGTATTTTGATAATTATACCAAGGATAAAATATCTTACGACTCTCCATAC

GATGATCaTAGTTACAACTATCACAATTAAATCATTGACTGCTAGAGATGCCGGTACTTATGtATGTGCA

TTCTTTATGACATCGCCTACAAATGACACTGATAAAGTAGATTATGAAGAATACTCCACAGAGTTGATT

GTAAATACAGATAGTGAATCGACTATAGACATAATACTATCTGGATCTACACATTCACCGGAAACTAGT

TCTGAGAAACCTGATTATATAGATAATTCTAATTGCTCGTCGGTATTCGAAATCGCGACTCCGGAACCA

ATTACTGATAATGTAGAAGATCATACAGACACCGTCACATACACTAGTGATAGCATTAATACAGTAAGT

GCATCATCTGGAGAATCCACAACAGACGAGACTCCGGAACCAATTACTGATAAAGAAGAAGATCATAC

AGTTACAGACACTGTCTCATACACTACAGTAAGTACATCATCTGGAATTGTCACTACTAAATCAACCAC

CGATGATGCGGATCTTTATGATACGTACAATGATAATGATACAGTACCATCAACTACTGTAGGCGGTA

GTACAACCTCTATTAGCAATTATAAAACCAAGGACTTTGTAGAAA

(b) Source of each constituent part of the insert

The flank 1 and 2 regions and the H5 promoter regions are from vaccinia virus. Bacterial plasmid DNA sequences are detailed in figure 1. The region marked h5T4 cDNA is the coding sequence for the human 5T4 72 kDa oncofoetal glycoprotein

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

The flank regions in the plasmid vector are homologous to sequences in the MVA vector to allow the TroVax recombinant MVA to be produced by homologous recombination.

The modified H5 promoter directs expression of the human 5T4 gene at both early and late phases of the recombinant viral replication cycle.

The h5T4 cDNA sequence encodes for the human 5T4 protein. 5T4 is a 72 kDa oncofoetal glycoprotein that is expressed on over 70% of carcinomas of the breast, gastrointestinal tract, colon and ovaries. Unlike other self-antigen TAAs e.g. CEA, 5T4 expression appears to be tumour specific with only low level expression reported in the gut. Immunohistochemical analysis indicates that 5T4 expression is an indicator of poor prognosis in colorectal cancer. Additionally, when tumour cells are transfected with the cDNA encoding for 5T4, they display increased motility, suggesting that expression of this molecule may induce metastatic properties in a tumour. The GMO will be injected into the patients. The patients cells injected with the GMO will express the 5T4 protein. This should result in the patients raising an immune response against the 5T4 protein and potentially a treatment for their cancer.

(d) Location of the insert in the host organism

- on a free plasmid (X) the pTRV-1b transfer plasmid
- integrated in the chromosome (.)
- other, specify …

The insert is located around del III in the MVA vector. The MVA vector is a virus.

(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) Vaccinia Virus
bacterium (.)
fungus (.)
animal
- mammals (X.)
- insect (.)
- fish (.)
- other animal (.)

(specify phylum, class) Homo sapiens …

other, specify …

The insert present in the plasmid vector contains sequences from MVA, vaccinia and humans.

The insert in the MVA vector contains sequences from bacteria, vaccinia and humans.

2. Complete name

| | | MVA/vaccinia | human 5T4 gene |
|---|---|---|---|
| (i) | order and/or higher taxon (for animals) | Poxviridae | Homo |
| (ii) | family name for plants | … | |
| (iii) | genus | Orthopoxvirus | |
| (iv) | species | … | Sapiens |
| (v) | subspecies | … | |
| (vi) | strain | … | |
| (vii) | cultivar/breeding line | … | |
| (viii) | pathovar | … | |
| (ix) | common name | MVA | human… |

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

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

If yes, specify the following:

Yes The H5 promoter is derived from vaccinia which is pathogenic, however the sequence is only 70 nucleotides and does not code for any harmful genes.

No The 5T4 gene is human.

(a) to which of the following organisms:

Vaccina virus is harmful to humans and animals, however as stated above only 70 nucleotides of sequence are present.

humans (X)

animals (X)

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 (X) No (.)

If yes, specify

Vaccina virus is a biosafety level 2 organism, however as stated above only 70 nucleotides of sequence are present and these are not associated with pathogenicity.

5. Do the donor and recipient organism exchange genetic material naturally?

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

.

MVA is an attenuated form of vaccinia. Recombination between closely related poxviruses may occur during virus replication under natural conditions. However only 70 nucleotides of the vaccinia sequence is present and the nucleotide sequence was produced synthetically using olignucleotide synthesis.

Exchange of sequences between MVA and humans is unlikely.

## 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

The MVA vector will now express the human 5T4 protein. The modification of the MVA vector will not affect its tropism, survival or infectivity characteristics since the human 5T4 gene has been inserted into a non-coding region of the genome. MVA virus replication has been compared to MVA–5T4 (TroVax) replication in permissive CEF cells and the replication kinetics of both was found to be similar.

(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

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

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

Specify

The expression of the human 5T4 gene in cells infected with MVA vectors will have no effect on dissemination.

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

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

Specify

The 5T4 protein is not toxic or likely to effect the host range of MVA. The only potential effect could be in pregnant women since the 5T4 protein is expressed on the placenta. Therefore during the trials pregnant healthcare workers will not administer the GMO. However pre-clinical toxicology studies in pregnant mice using the murine version of the 5T4 protein have not indicated any risks to either the fetus or mother. No other organisms are likely to be injected.

2. Genetic stability of the genetically modified organism

Sequencing and southern blot analysis of the master viral seed stock and the final clinical lots produced up to 10 passages later have demonstrated the transgene to be genetically stable after multiple passages. Measurements of the percentage of foci expressing 5T4 on chicken fibroblast monolayers have detected no changes over passage. Similarly, Western blot analysis also indicates no detectable change in the level of transgene protein expression after multiple passages.

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 ;

The GMO (TroVax) will not replicate except in a limited number of cell lines in tissue culture so wide scale environment monitoring is unlikely to be required . Detection of potential shedding from treated patients has been carried out using GMO specific primers and PCR.

(b) Techniques used to identify the GMO

(i) The GMO can be identified by sequencing, Southern blotting or diagnostic PCR..

(ii) The expression of the donor gene (5T4) may be detected using either western blotting or immunostaining of GMO (TroVax) infected cells. The primary antibody used for detection is H8, a monoclonal antibody raised against human 5T4 protein purified from human placenta (Hole and Stern. 1988. Br. J. Cancer 57, p239-246). This antibody recognizes human 5T4 only in its native correctly glycosylated form.

## F. Information relating to the release

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

The purpose of the release is a phase III clinical trial to investigate the efficacy of a treatment for renal cancer. The exact title of the trial is "An international, randomised, double blind, placebo controlled, parallel group study to investigate whether TroVax®, added to first-line standard of care therapy, prolongs the survival of patients with locally advanced or metastatic clear cell renal adenocarcinoma. (TV3/001/06)".

This is a randomised, double blind, placebo controlled, parallel group, phase III, international study in patients with locally advanced or metastatic renal cell cancer designed to assess whether TroVax® when added to first line standard of care therapy prolongs the survival of patients.

700 patients will be recruited into the study who will each receive one of three defined standards of care (low dose IL2 injection, subcutaneous IFN-α injections or sunitinib tablets) and 13 intramuscular injections of TroVax®. All patients will be followed up for survival.

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 …

The natural habitat of vaccinia virius is unknown, MVA is a laboratory strain

3. Information concerning the release and the surrounding area

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

The trial is due to take place at many sites within Europe ( Spain FR, DE, GB ,NL, Poland) and the USA. The actual clinical sites to be utilised have not yet been finalized.

(b) Size of the site ($m^2$): … $m^2$

The sites are distinct rooms at the hospitals or areas away from the main ward. A size of the rooms cannot be given here in general until all of the sites have been specifically identified.

(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:

The dosage regimen will be 13 injections, given over a period of 65 weeks. Each injection will be a 1ml intramuscular injection. There will be a single dose level of $1x10^9$ $TCID_{50}$/ml per injection.

(b) Duration of the operation:

65 weeks

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

1. worker protection measures taken during the release,

The personnel at the study sites, the pharmacy and laboratory personnel involved will receive detailed training in the handling of TroVax®. The sites will also have adequate facilities for the handling and storage of TroVax® prior to receiving any TroVax®. All healthcare staff handling TroVax or materials contaminated by it must wear an apron, gloves, mask and protective goggles.

2. post-release treatment of the site,

The site of injection will be swabbed with ethanol and the injection will be given intramuscularly. Following this, the injection site will be swabbed with ethanol again

and then covered with an occlusive bandage. This bandage will be removed and disposed of before the patient is discharged.

3. techniques foreseen for elimination or inactivation of the GMOs at the end of the experiment,

All solid waste will be de-activated by autoclaving and then incinerated after autoclaving or according to local hospital site regulations for GMO waste.

All liquid spills on surfaces are to be treated with Virkon (Antec Int) or other viral inactivation products approved locally according to the manufacturer's instructions for the vaccinia & pseudo-cowpox viruses.

All other waste (bandages, swabs etc) will be incinerated at the hospital site as per standard clinical waste.

All solid material for repeated use, e.g. aprons, googles, will be de-activated by autoclaving or incineration.

Where local guidelines are more restrictive than the measurements described here, they should prevail (e.g. airlock before side room for injections).

5. Short description of average environmental conditions (weather, temperature, etc.)
Not applicable.

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.

TroVax® has been administered to over 100 patients with metastatic colorectal or renal cancer. Over 350 doses have been administered. No serious adverse events attributed to TroVax® by investigators or the sponsor have been reported. Mild transient injection site reactions are reported in the majority of patients together with mild transient pyrexia. No other notable, common or serious adverse events have been reported in studies using TroVax® as a single agent in heavily pretreated patients or in studies combining TroVax® with chemotherapy, (5FU and leucovorin combined with either oxaliplatin[1] or irinotecan[2]), IL-2 (high dose intravenous regimen[3] or low dose subcutaneous injections[4]).
TroVax® induced an immune response against the 5T4 antigen in >90% of patients treated in

all studies. An antibody or cellular immune response was observed in virtually all patients after the second or third injection of TroVax®. CD8+ve cellular responses were often higher than noted with other cancer vaccines reported in the literature. Of three phase I or II studies conducted in colorectal cancer two demonstrated a correlation between tumour response and the anti-5T4 immune response. Notably this correlation was specific to the 5T4 immune response and there was no correlation with other markers of general immunocompetence[5] [6]. No environmental impacts have been observed.

[1] Ongoing data from Oxford BioMedica Clinical Trial Phase II " An open label study of TroVax® given in conjunction with 5-fluorouracil/Leukovorin/Oxaliplatin: Safety and immunogenicity before during and after chemotherapy"

[2] Ongoing data from Oxford BioMedica Clinical Trial Phase II "An Open Label Study Of TroVax® Given In Conjunction With 5-Fluorouracil/Leukovorin (De Gramont Regimen) Plus Irinotecan: Safety And Immunogenicity Before During And After Chemotherapy"

[3] Ongoing data from Oxford BioMedica Clinical Trial Phase II "A preliminary study of the safety, immunogenicity and clinical efficacy of TroVax® given in conjunction with interleukin 2 (IL-2) in the treatment of stage IV renal cell cancer"

[4] Ongoing data from Oxford BioMedica Clinical Trial Phase II "Safety, immunology and biological activity evaluation of TroVax® in treatment of patients with locally advanced or metastatic renal carcinoma"

[5] Harrop R, et al. Poster presented at AACR 2005. Phase II studies in metastatic CRC with the Cancer Vaccine TroVax® in combination with chemotherapy: A productive partnership.

[6] Harrop R, et al. "Vaccination of Colorectal Cancer Patients with modified Vaccinia Ankara Delivering the Tumor Antigen 5T4 (TroVax) Induces Immune Responses which Correlate with Disease Control: A Phase I/II Trial" Submitted to Clinical Cancer Research Dec 2005

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

Not applicable

1. Name of target organism (if applicable)

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

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

The GMO (TroVax) is designed to express the 5T4 protein. 5T4 is a 72 kDa oncofoetal glycoprotein that is expressed on over 70% of carcinomas of the breast, gastrointestinal tract, colon and ovaries. Unlike other self-antigen TAAs e.g. CEA, 5T4 expression appears to be tumour specific with only low level expression reported in the gut. Immunohistochemical analysis indicates that 5T4 expression is an indicator of poor prognosis in colorectal cancer. Additionally, when tumour cells are transfected with the cDNA encoding for 5T4, they display increased motility, suggesting that expression of this molecule may induce metastatic properties in a tumour. The GMO will be injected into the patients. The patients cells injected with the GMO will express the 5T4 protein. This should result in the patients raising an immune response against the 5T4 protein and potentially a treatment for their cancer.

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

None anticipated.

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

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

Give details

Since the GMO is replication defective increased competitivness, or increased invasiveness of the GMO is highly unlikely to occur. No evidence of post-release selection has been observed in any previous trials with the GMO.

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

The GMO (TroVax) will be confined to the hospital test site, including the hospital pharmacy, clinical lab, and autoclaving/incineration area. Since the GMO is replication defective no type of ecosystem is likely to be supportive of the GMO.

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

It is possible that hospital staff may be injected by accident, but this would be unlikely to have any ill effects.. The only potential effect could be in pregnant women since the 5T4 protein is expressed on the placenta. Therefore during the trials pregnant healthcare workers will not administer the GMO. However pre-clinical toxicology studies in pregnant mice using the murine version of the 5T4 protein have not indicated any risks to either the fetus or mother. No other organisms are likely to be injected.

| | | |
|---|---|---|
| (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:
unlikely due to the GMO being replication defective.

(b) from other organisms to the GMO:
Unlikely

(c) likely consequences of gene transfer:
The inserted gene is not a pathogencity or virulence gene and hence there are unlikely to be any consequences.

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.):
Due to the nature and attenuation of the GMO such studies would not be applicable.

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)
None

## H. Information relating to monitoring

1. Methods for monitoring the GMOs

   In previous clinical trials using recombinant MVA, viral shedding was monitored in patient samples using quantitative PCR. MVA may be detected in tissue culture media using specific antibodies.

2. Methods for monitoring ecosystem effects

   Quantitative PCR.

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

   Quantitative PCR

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

   … $m^2$

   Monitoring of the patient or hospital treatment area is not planned in the light of the results of monitoring results obtained in previous clinical trials.

5. Duration of the monitoring

   See section 4 above.

6. Frequency of the monitoring

   See section 4 above.

## I. Information on post-release and waste treatment

1. Post-release treatment of the site

The site of injection will be swabbed with ethanol and the injection will be given intramuscularly. Following this, the injection site will be swabbed with ethanol again and then covered with an occlusive bandage. This bandage will be removed and disposed of before the patient is discharged. All other clinical waste will be treated as described in 3b.

2. Post-release treatment of the GMOs

Since the GMO is replication defective, the only source of GMO will be clinical waste which will be treated as described in 3b.

3. (a) Type and amount of waste generated

Type: Needles, syringes, vials, swabs, patient dressings, aprons for single use, googles for single use (if applicable) and cleansing material.

Quantity: Low (only material in direct contact with TroVax will need autoclaving/incineration),

(b) Treatment of waste

**Details of how the waste is to be deactivated**

**Autoclaving – for solid items containing waste**

Waste will be de-activated by autoclaving and is then routinely incinerated after autoclaving or is disposed according to local hospital regulations for GMO waste.

**Disinfectant – for liquid spills and surface disinfection**

Virkon (Antec Int) or locally approved equivalent will be used to disinfect surfaces according to the manufacturer's instructions for the vaccinia & pseudocowpox viruses.

In validation studies two representative examples from the poxviridae have been tested, these being vaccinia (the parent of TroVax) and bovine pseudocowpox virus. For vaccinia, a 10min exposure to both 0.5% and 1% solutions of Virkon caused a 1000-fold reduction in infectious titre, with complete inactivation being demonstrated after 30 minutes. For pseudo-cowpox virus, a greater dilution range was explored and complete inactivation was demonstrated after a 30min exposure to a 1:300 w/v solution. Virkon Disinfectant or equivalent is used following manufacturer's guidelines. There is a colour indicator built into Virkon that facilitates monitoring the deactivating efficacy of the solution in use. Solutions of Virkon are routinely changed following the Standard Operating Procedure in the treatment area.

## J. Information on emergency response plans

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

Since the GMO is replication defective (requires a very limited range of tissue culture cells for growth) spread in the environment is highly unlikely. Methods for dealing with spillage of the GMO during transport or in the clinic are detailed in section J 2.

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

The following procedures for the decontamination of accidental spills will be followed.

MINOR SPILLS

(i) Clean surface with 70% IMS or 2% peroxygen compound, such as Virkon.
(ii) Decontaminate instruments by soaking in the disinfectant solution (e.g. 2% Virkon solution) for 30 minutes.

SMALL SPILLS

(a) Wipe up spill with tissue paper soaked in fresh 2% Virkon (or equivalent) solution.
(b) Leave for 10 minutes.
(c) Discard tissue into autoclave bag.
(d) Transport autoclave bag to the service lab for autoclaving

LARGE SPILLS

*(i) Large Spill outside Biological Safety Cabinet*
(a) Stop what you are doing immediately.
(b) Contain the spillage using the spill kits available.
(c) Inform the relevant authorities or hospital representative.
(d) Return to the spill.
(e) In addition to a lab coat, wear a disposable apron, eye protection, shoe protectors and water proof gloves (or equivalent).
(f) Flood the spill with a 2% Virkon (or equivalent) solution.
(g) Cover the spill with tissue or the spill kit absorbent material.
(h) Leave for a minimum of 30 minutes.
(i) Transfer spill material to an autoclave bag and transport to a suitable facility for autoclaving.
(j) Wash spill area with detergent and allow to dry.

*NB:*

- *Virkon has a wide range of microbial activity although this is reduced by organic matter. It is used for routine laboratory disinfection.*

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

In the case of a spill inside the clinic the procedure in J2 will be followed. If a spill occurs during transport, the contaminated area will be flooded with a suitable disinfectant and then any contaminated materials will be bagged for autoclaving. However since shipments will be of a small number (less than 50 2ml) vials of product and will be in approved EU shipping containers, the area likely to be contaminated will be small.

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

As the GMO TroVax may only replicate in permissive cells, it will not replicate in the environment or in patients etc. In the highly unlikely case of a replication competent vaccinia arising, vaccination of individuals in the surrounding area using MVA would be a possibility. However no incidences of this occurring when MVA or MVA recombinants have been reported even though there has been extensive use of such materials in clinical trials and research laboratories.