

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 200 1/1 8/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/08/46
- (c) Date of acknowledgement of notification: 5 July 2008
- (d) Title of the project

**A Multicenter, Double Blind, Placebo controled, Randomized study of TroVax® vs. Placebo in the First Line treatment of Patients with Metastatic Colorectal Cancer receiving chemo-based therapy – EFC 10528.**

- (a) Proposed period of release **The trial is due to commence in October 2008 and last for up to 4 years.**

2. Notifier

Name of institution or company:

**Sanofi-Aventis Recherche et Développement  
1, Avenue Pierre Brossolete  
91385 Chily-Mazarin  
France**

**Contact: Mrs Marie-Caroline Smadja  
Regulatory Development**

**Telephone: +33 (1) 01 60 49 52 85**

3. GMO characterisation

- (a) Indicate whether the GMO is a:

viroid	(.)	(.)
RNA virus	(X)	<b>Modified Vaccinia Ankara virus</b>
DNA virus	(.)	
bacterium	(.)	
fungus		
animal	(.)	
- mammals		



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 [Argentina, Xile, Xina, Hong Kong, Japan, Republic of Corea, Malasia, Philipines, Russian Federation, South Africa, Taiwán, Thailand, Turkey and United States.](#)
- Notification number .../.../...

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

GMOs. [7.1 Characteristics affecting survival, multiplication and dissemination](#)

[7.1.1 Biological features](#) [which affect survival, multiplication and dispersal](#)

The GMO (TroVax<sup>®</sup>) is based on MVA. MVA is replication defective in all mammalian cell lines tested except Baby Hamster Kidney cells (BHK-21). MVA is non-pathogenic in animals including suckling mice, rabbits and primates. It is unlikely to survive in the environment. Studies in which the wound dressing has been removed 2 hours after administration and the wound site swabbed for any remaining TroVax<sup>®</sup> have shown that the amount of vector remaining is less than 1/10000th of the original dose. This level of shedding is unlikely to pose a risk to the environment.

[7.1.2 Known or predicted environmental conditions which may affect survival, multiplication and dissemination \(wind, water, soil, temperature, pH, etc.\)](#)

Not Applicable

[7.1.3 Sensitivity to specific agents](#)

In general poxviruses (including MVA) are highly sensitive to all common approved disinfection regimens and the common sterilization procedures – thermal, chemical and/or radiation – are usually effective.

[7.2 Interactions with the environment](#) [7.2.1 Predicted habitat of the GMOs](#)

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 case of replication competent vaccinia viruses they 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'). In the case of MVA based vectors there is no replication and no scab at the inoculation site.

From previously performed clinical trials it has been found that following administration of TroVax<sup>®</sup>, the patient poses no risk to healthcare workers or to the environment. The administration site is swabbed with alcohol. An occlusive dressing is also used as an extra precaution. Vector is not detected at the injection site following swabbing with alcohol. Studies in which the dressing has been removed 2 hours after administration and the wound site swabbed for any remaining TroVax<sup>®</sup> have shown that the amount of vector remaining is

less than 1/10000th of the original dose. This level of shedding is unlikely to pose a risk to the environment.

#### 7.2.2 Genetic transfer capability

##### 7.2.2.1 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. Although MVA can infect most mammalian species, it cannot replicate in primary human cells and the cells that it infects die of a result of the infection. During this infection unless the cells are permissive for virus replication (e.g. primary chicken embryo cells or BHK-21 cells) no infectious particles are produced. Due to the short duration of this 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.

##### 7.2.2.2 Post-release transfer of genetic material from indigenous organisms to the GMOs

Since vaccinia viruses, including MVA, are considered to have no natural reservoir, no transfer is expected. MVA has been used extensively and no transfer of genetic material has been reported.

##### 7.2.3 Likelihood of postrelease selection leading to the expression of unexpected and/or undesirable traits in the modified organism

The presence of the 5T4 gene is not a selectable trait under natural conditions. It does not convey any particular function to the MVA or the human cells harboring the MVA.

##### 7.2.4 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; sequencing, PCR analysis of the final clinical lots.

MVA only replicates in permissive cells (CEFs and BHKs), thus dispersal will be limited to the first organism infected.

Sequencing and Southern blot analysis of the master viral seed stock and the final clinical lots (TroVax®) produced up to 8 passages later, using conditions comparable to regular virus manufacturing, have demonstrated the transgene to be genetically stable after multiple passages. Double immunostaining of the same p8 virus demonstrate that greater than 99% of all MVA induced plaques in CEFs expressed the 5T4 protein. Similarly, Western blot analysis also indicates no detectable change in the level of transgene protein expression between the master virus seed stock and the final clinical lots.

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

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 case of replication competent vaccinia viruses they 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'). In the case of MVA based vectors there is no replication and no scab at the inoculation site.

From previously performed clinical trials it has been found that following administration of Tro Vax<sup>®</sup>, the patient poses no risk to healthcare workers or to the environment. The administration site is swabbed with alcohol. An occlusive dressing is also used as an extra precaution. Studies in which the dressing has been removed 2 hours after administration and the wound site swabbed for any remaining TroVax<sup>®</sup> have shown that the amount of vector remaining is less than 1/10000th of the original dose. This level of shedding is unlikely to pose a risk to the environment.

Vector was detected in 6 out of the 22 patients included in the phase I/II single agent study, in 1 blood sampling each time. In all these 6 patients except 2, the vector was cleared by 24-hour post injection, and in the 2 patients where the vector was detected at 24-hour post injection, the levels were below the limit of the assay and were considered negligible. Detection of vector in the patient's circulation was not significant

Biodistribution will not be assessed in EFC 10528 study. Immune response will be assessed in a sub-group of patients.

#### 7.2.6 Description of ecosystems to which the GMOs could be disseminated

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

#### 7.2.7 Potential for excessive population increase in the environment

MVA only replicates in permissive cells (CEFs and BHKs), thus dispersal will be limited to the first organism infected. Hence it is unlikely to survive in the environment and there is no potential for population increase in the environment.

#### 7.2.8 Competitive advantage of the GMOs in relation to the unmodified recipient or parental organism(s)

The production of 5T4 protein provides no competitive advantage to the MVA. It is designed to have an effect upon therapeutic immunization in cancer patients.

#### 7.2.9 Identification and description of the target organisms if applicable

There is no formal target organism. The vector systems are designed to be transiently active in man and to function as a therapeutic cancer vaccine.

#### 7.2.10 Anticipated mechanism and result of interaction between the released GMOs and the target organism(s) if applicable

The GMO will be injected into the patients. This should result in the patients raising an immune response against the expressed 5T4 protein.

#### 7.2.11 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

MVA only replicates in permissive cells (CEFs and BHKs), thus dispersal will be limited to the first organism infected. It is possible that hospital staff may be injected by accident, but this would be unlikely to have any ill effects. No other organisms are likely to be injected. Pregnant healthcare workers should not administer the GMO. Healthcare workers of childbearing potential should be informed of the risk.

7.2.12 Likelihood of post-release shifts in biological interactions or in host range

This is extremely unlikely, MVA only replicates in permissive cells (CEFs and BHKs), thus dispersal will be limited to the first organism infected.

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

None.

7.2.14 Known or predicted involvement in biogeochemical processes

None.

7.2.15 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)
- (ii) order and/or higher taxon (for animals) [Poxviridae](#)
- (iii) genus [Orthopoxvirus](#)
- (iv) species
- (v) subspecies
- (vi) strain
- (vi) pathovar (biotype, ecotype, race, etc.)
- (vii) common name [Modified Vaccinia Ankara virus \(MVA\)](#)



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.

The quantitative PCR-based detection assay used to monitor patients is sensitive to 10 copies of virus genome in 1 µg of genomic DNA isolated from patient PBMCs.

(b) Identification techniques

MVA is detected and identified by Southern blot analysis using radiolabeled total MVA DNA probes and also by PCR using MVA-specific primers. The specificity of the genetic techniques used to detect and identify MVA is determined by the nucleotide sequence of the Southern blot probes and the PCR primers.

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

Replication competent strains of vaccinia virus (VV) are handled at a Biosafety level II environment; however, MVA, which is replication-deficient, has been assigned Biosafety level I status by the National Institutes of Health (NIH) intramural biosafety committee in the US, the biosafety authorities in France and Germany, and the Health and Safety Executive in the UK.

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 MVA vector was chosen on the basis of safety and because pre-clinical data with 5T4 and a range of other antigens have shown that it can stimulate a good immune response against expressed antigens.

MVA is a vaccinia virus that is a member of the family of DNA-containing viruses, Poxviridae and genus Orthopoxvirus that includes variola (smallpox), cowpox, monkey pox, avipox and others. These viruses replicate in the host cell cytoplasm and differ from other DNA viruses that replicate inside the cell nucleus. Vaccinia viruses elicit both humoral and cell mediated immune responses and techniques for immunization with these

viruses are well established. MVA was derived from the Ankara smallpox vaccine strain by passaging over 500 times in primary chick embryo fibroblast cells (CEFs), after which time it was found to be replication deficient in a wide spectrum of mammalian cells. Additionally, MVA has been shown to be non-virulent in a number of animal models including suckling mice and primates. More recently >10<sup>7</sup> pfu (plaque forming unit) of MVA have been inoculated into nude mice without death (Wyat, Carrol and Moss, NIH, USA, unpublished data). In contrast, 10<sup>1</sup> pfu of replication competent vaccinia virus can be lethal in nude mice.

MVA is a highly attenuated, replication-deficient poxvirus that does not replicate in any tested primary human cell type. Thus, the infection of primary human cells with TroVax<sup>®</sup> is transient, and while sufficient to allow the expression of the 5T4 protein product of the inserted transgene in an immunogenic fashion, the TroVax<sup>®</sup> infection is self-limiting due to its inability to replicate. The cells infected by TroVax<sup>®</sup> ultimately die and are eliminated from the body. The results of several biodistribution studies using polymerase chain reaction (PCR) based detection of viral nucleic acid sequences support the assertion that TroVax<sup>®</sup> does not persist in treated subjects.

MVA replicates well in avian cells, such as CEFs, but is replication deficient in most mammalian cells. MVA has been shown to replicate in baby hamster kidney cells (BHK-21), however, MVA is replication defective in all other mammalian cells tested.

During the smallpox eradication campaign, MVA was inoculated into over 120,000 humans. It was given specifically to those people that were at risk from complications with the replication competent vaccinia vaccine, i.e. the very young and old in addition to immuno-compromised recipients. There have been no reported cases of complications caused by MVA inoculation in humans when inoculated via skin scarification or intramuscularly.

## 8. Information concerning reproduction

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). However, MVA is replication defective in all other mammalian cells tested except in baby hamster kidney cells (BHK-21). 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: Sexual .. Asexual X

See above, introduction section 8.

(c) Factors affecting reproduction:

See above, introduction section 8.

9. Survivability

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

- |        |                        |     |
|--------|------------------------|-----|
| (i)    | endospores             | (.) |
| (ii)   | cysts                  | (.) |
| (iii)  | sclerotia              | (.) |
| (iv)   | asexual spores (fungi) | (.) |
| (v)    | sexual spores (funghi) | (.) |
| (vi)   | eggs                   | (.) |
| (vii)  | pupae                  | (.) |
| (viii) | larvae                 | (.) |
| (ix)   | other, specify         |     |

(b) relevant factors affecting survivability:

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® inactivation 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, p25 1-256) 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.

The main exposure routes under normal conditions are:

- contact with solution in opened vial
- contact with solution in syringe
- contact with material at injection site and
- contact with primary disposable wound dressing.

MVA only replicates in permissive cells (CEFs and BHKs), thus dispersal will be limited to the first organism infected. It is possible that hospital staff may be injected by accident, but this would be unlikely to have any ill effects. No other organisms are likely to be injected. Pregnant healthcare workers should not administer the GMO. Healthcare workers of childbearing potential should be informed of the risk.

(b) Factors affecting dissemination

MVA only replicates in permissive cells (CEFs and BHKs), thus dispersal will be limited to the first organism infected. Hence it is unlikely to survive in the environment and there is no potential for population increase in the environment.

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

Not applicable

C. Information relating to the genetic modification

1. Type of the genetic modification

- |       |                               |                                     |
|-------|-------------------------------|-------------------------------------|
| (i)   | insertion of genetic material | <input checked="" type="checkbox"/> |
| (ii)  | deletion of genetic material  | <input type="checkbox"/>            |
| (iii) | base substitution             | <input type="checkbox"/>            |
| (iv)  | cell fusion                   | <input type="checkbox"/>            |
| (v)   | others, specify               |                                     |

2. Intended outcome of the genetic modification

TroVax<sup>®</sup> is a cancer vaccine designed specifically to stimulate an anti-cancer immune response. The TroVax<sup>®</sup> vaccine active substance (the GMO) consists of a highly attenuated recombinant vaccinia virus (Modified Vaccinia Ankara – MVA) that contains a gene encoding the human 5T4 oncofoetal antigen. The 5T4 antigen is broadly expressed in a wide range of solid tumors, but at very low levels (except on trophoblast) on a limited number normal adult tissues. The 5T4 antigen is expressed in more than 80% of colorectal carcinomas and is associated with a poor prognosis. The TroVax<sup>®</sup> vaccine is administered intramuscularly and this results in the transcription and translation of the 5T4 gene to produce 5T4 protein in vivo and this induces a 5T4-specific immune response. It is theorized that tumor cells displaying the 5T4 antigen will become specific targets for the immune effector activities elicited by the TroVax<sup>®</sup> vaccine.

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

If no, go straight to question 5.

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

If no, go straight to question 5.

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

(a) Type of vector

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

(c) Identity of the vector

The vector contains: 5T4 cDNA, plasmid sequence, mH5 promoter and plasmid sequence 2. Flank 1 and Flank 2 are part of MVA

Construction of the TroVax transfer vector <sup>P</sup>TRV-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 flank 1 and 2 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 h5T4 cDNA sequence encodes for the human 5T4 protein. The 5T4 is a 72 kDa transmembrane glycoprotein that is broadly expressed in a wide range of solid tumors, but at very low levels (except on trophoblast) on a limited number normal adult tissues. The 5T4 antigen is expressed in more than 80% of colorectal carcinomas and is associated with a poor prognosis.

- Bacterial plasmid: The bacterial plasmid pNEB 193 (New England Biolabs) was used in the preparation of the transfer plasmid, propagated in E coli strain XL1-Gold (Stratagene). A plasmid DNA used in this study was prepared as follows. E coli strain XL1-Gold (Stratagene) was transformed with plasmid DNA and transformants were identified by restriction digest analysis of crude DNA preparations (mini preps). Recombinant clones were grown overnight and plasmid DNA was isolated using a QIAGEN Endofree Plasmid Maxi Kit (Cat No 12362) (Crawley, Surrey, UK) according to manufacturer's instructions. Purified DNA was assessed for integrity and contaminating forms by restriction digestion and gel electrophoresis. Plasmid configuration was confirmed by DNA sequencing.

- Promoter mH5: directs expression of the human 5T4 gene at both early and late phases of the recombinant viral replication cycle. The construct contains a regulatory promoter, H5. The H5 promoter directs expression at both early and late phases of the viral replication cycle. In the context of MVA where completion of replication is blocked at a late stage this will maximise gene expression throughout the abortive infection cycle in most cells. 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 (Wyat et al). This mH5 promoter is only 70 nucleotides long. It is not anticipated that there will be any recombination between the transfer vectors and MVA at this site.

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 (.) No (X)

There are no genes which confer resistance.

antibiotic resistance (.)

other, specify

Indication of which antibiotic resistance gene is inserted

(e) Constituent fragments of the vector

The vector contains: 5T4 cDNA, plasmid sequence, mH5 promoter and plasmid sequence 2.

Flank 1 and Flank 2 are part of MVA.

(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

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

(i) transformation (.)

(ii) microinjection (.)

(iii) microencapsulation (.)

(iv) macroinjection (.)

(v) other, specify (.)

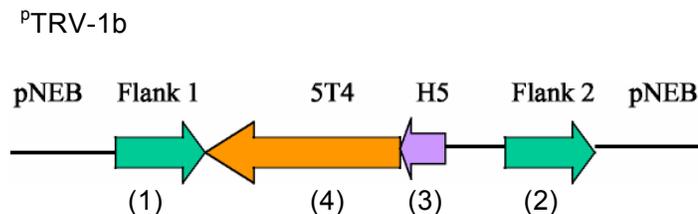
TroVax® is produced from Chicken Embryo Fibroblasts (CEFs)

After transfection and infection of CEFs, virus genomic DNA and the transfer plasmid DNA undergo recombination at sites of homology referred to as flank DNA. This results in the insertion of the 5T4 gene and associated promoter mH5 into the MVA genome to produce a 5T4 recombinant MVA. The recombinant vector forms plaques in the cell monolayer, as a result of replication in the cells and this can be identified by immuno-staining with 5T4 specific antibody. Non-recombinant virus also produces plaques that can be distinguished by immuno-staining. The recombinant is purified by repeatedly harvesting a plaque and replating it at low density until only recombinant plaques are obtained. A seed stock is then prepared by infection of cells and harvesting the lysate, the stock is tested for genetic homogeneity and expression of 5T4. The vector seed stock is then used in the production of TroVax® from infected CEFs.

## 6. Composition of the insert

### (a) Composition of the insert

Figure 1. Map of the 5T4 expression region in the transfer vector TRV-1b (TRV transfer vector) used to make TroVax®



The construction details are given below. For all the PCR primers, the upper case letters represent the bases homologous to the target DNA sequence whilst the lower case letters represent additional bases used to create restriction enzyme sites and 5' "clamps."

#### (1) Flank 1 was amplified from MVA DNA using primers

MVA F1ds

ggccaagctGATAAAGCGTGTGCGTGTATAGAG

MVA F1PXus

ccggc tgcagctcgagTACCAGCCACCGAAAGAG

digested with Pst I and Hind III and ligated to pNEB 193 (New England BioLabs), previously digested with the same enzymes, to give pNF1.

#### (2) Flank 2 was amplified from MVA DNA using primers

MVA F2ds

gcgcggtaccgctagcggcgcgccTTTGAAAGTTTTATAGGTAGTTG

MVA F2us

CcggaatcAACTAGTTTCCGGTGAATGTG

digested with Eco RI and Kpn I and ligated to pNF1, previously digested with the same enzymes, to give pNF12.

#### (3) The mH5 promoter was produced by annealing the following two oligonucleotides

MVA H5bs  
tcgacTATTTATGATTATTTCTCGCTTTCAATTTAACACAACCCTCAAGAACCTTTG  
TATTTATTTTCAATTTTaat

MVA H5ts  
tAAAAATTGAAAATAAATACAAAGGTTCTTGAGGGTTGTGTTAAATTGAAAGCG  
AGAAATAATCATAAATAg

and this was then ligated to pNF12 previously digested with Pac I and Sal I to give pNF12H5.

(4) A fragment of DNA comprising the 5T4 coding region was amplified from the cloned cDNA using the following primers

HuMo U1-Not  
taagcggccgcAACCGCGAGCCGCGATG

5T4 Sto<sup>P</sup>X

ccggctcgagTCAGACATCCGAGTTAGAAC,  
digested with Not I and Xho I and cloned into pNEBKAM (digested with the same enzymes) to give pNKh5T4.6. (pNEBKAM is pNEB 193 with additional Not I, Sfi I and Xho I sites inserted by means of a linker between the Pst I and Hind III sites of the MCS). The 5T4 cDNA was then excised with Pme I and Xho I and ligated to pNF12H5, previously digested with the same enzymes, to give pNF12\_H5\_h5T4.6, designated pTRV-1b.

After each cloning step in the construction of the plasmid transfer vector pTRV-1b, the insert was sequenced either by Oswel DNA Services Ltd or Lark Technologies Inc. The full insert (together with some of the flank regions) was sequenced from DNA extracted from the first vector (pre-MVSS) stock by Lark Technologies.

(b) Source of each constituent part of the insert

See above, section 4 c)

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

- The flank 1 and 2 regions in the plasmid vector are homologous to sequences in the MVA vector to allow the TroVax<sup>®</sup> 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. The 5T4 is a 72 kDa transmembrane glycoprotein that is broadly expressed in a wide range of solid tumors, but at very low levels (except on trophoblast) on a limited number normal adult tissues. The 5T4 antigen is expressed in more than 80% of colorectal carcinomas and is associated with a poor prognosis.

(d) Location of the insert in the host organism

- on a free plasmid (X) the pTRV-1b, TroVax<sup>®</sup> transfer plasmid
- integrated in the chromosome(.)
- other, specify

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

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

1. Indicate whether it is a:

- viroid (.)  
 RNA virus (.)  
 DNA virus (.)  
 bacterium (.)  
 fungus (.)  
 animal X  
 - mammals (X) Homo Sapiens  
 - insect (.)  
 - fish (.)  
 - other animal (.)  
 (specify phylum, class)

other, specify

2. Complete name

- (i) order and/or higher taxon (for animals) Homo  
 (ii) family name for plants ... ..  
 (iii) genus Sapiens  
 (iv) species  
 (v) subspecies  
 (vi) strain ...  
 (vii) cultivar/breeding line ...  
 (viii) pathovar ...  
 (ix) common name ...  
 Human 5T4

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

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

The 5T4 gene encodes a human oncofetal antigen, which is expressed as a 72 kDa transmembrane glycoprotein. The 5T4 glycoprotein is a tumor associated antigen (TAA), which may play a role in tumor metastasis. It is not considered to be a toxin.

If yes, specify the following:

(b) to which of the following organisms:

- humans (.)  
 animals (.)  
 plants (.)  
 other ..

(b) are the donated sequences involved in any way to the pathogenic or harmful properties of the organism

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

If yes, give the relevant information under Annex III A, point II(A)(1 1 )(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

Replication competent strains of vaccinia virus (VV) are handled at a Biosafety level II environment; however, MVA, which is replication-deficient (as described above in section 2.1.8), has been assigned Biosafety level I status by the National Institutes of Health (NIH) intramural biosafety committee in the US, the biosafety authorities in France and Germany, and the Health and Safety Executive in the UK. For Vaccinia virus 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 (.)

The 5T4 human oncofetal gene: not applicable

MVA: Recombination between closely related poxviruses may occur during virus replication under natural conditions. However, although MVA can infect most mammalian species, it cannot replicate in primary mammalian cells and furthermore, the cells that it infects die as a result of the infection. During infection, unless the cells are permissive for virus replication (e.g. CEFs or BHK-21 cells), no infectious particles are produced. Due to the short duration of this infection it is extremely unlikely that recombination will occur under natural conditions between MVA and other poxviruses.

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

MVA is replication defective in all mammalian cell lines tested except Baby Hamster Kidney cells (BHK-21). MVA is non-pathogenic in animals including suckling mice, rabbits and primates. It is unlikely to survive in the environment. Studies in which the wound dressing has been removed 2 hours after administration and the wound site swabbed for any remaining TroVax® have shown that the amount of vector remaining is less than 1/10000th of the original dose. This level of shedding is unlikely to pose a risk to the environment.

In general poxviruses (including MVA) are highly sensitive to all common approved disinfection regimens and the common sterilization procedures – thermal, chemical and/or radiation – are usually effective

(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

MVA only replicates in permissive cells (CEFs and BHKs), thus dispersal will be limited to the first organism infected. Hence is unlikely to survive in the environment and there is no potential for population increase in the environment.

(c) 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.

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

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

Specify

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 case of replication competent vaccinia viruses they 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'). In the case of MVA based vectors there is no replication and no scab at the inoculation site.

From previously performed clinical trials it has been found that following administration of TroVax<sup>®</sup>, the patient poses no risk to healthcare workers or to the environment. The administration site is swabbed with alcohol. An occlusive dressing is also used as an extra precaution. Vector is not detected at the injection site following swabbing with alcohol. Studies in which the dressing has been removed 2 hours after administration and the wound site swabbed for any remaining TroVax<sup>®</sup> have shown that the amount of vector remaining is less than 1/10000th of the original dose. This level of shedding is unlikely to pose a risk to the environment.

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

The following measures were used to assess genetic stability; sequencing, PCR analysis of the final clinical lots.

MVA only replicates in permissive cells (CEFs and BHKs), thus dispersal will be limited to the first organism infected.

Sequencing and Southern blot analysis of the master viral seed stock and the final clinical lots (TroVax<sup>®</sup>) produced up to 8 passages later, using conditions comparable to regular virus manufacturing, have demonstrated the transgene to be genetically stable after multiple passages. Double immunostaining of the same p8 virus demonstrate that greater than 99% of a I MVA induced plaques in CEFs expressed the 5T4 protein. Similarly, Western blot analysis also indicates no detectable change in the level of transgene protein expression between the master virus seed stock and the final clinical lots.

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)(1 1)(d) and II(C)(2)(i)

4. Description of identification and detection methods

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

In previous clinical trials a I patients were monitored for safety, vector persistence and biodistribution at 30 min, 1h, 3h, 6h, 24h and 2 weeks following injection and for immune response at 2 weekly intervals following each injection, for up to a maximum of 24 weeks. In addition, blood was taken for the detection and measurement of levels of circulating vector by diagnostic PCR (30 min, 1h, 3h, 6h, 24h and 2 weeks post injection).

Results of real-time quantitative PCR analysis of patient's blood showed 1 patient to be positive at 30 minutes, 2 patients positive at one hour, 1 patient positive at 6 hours, 2 patients positive at 24 hours and a I 17 patients tested were negative at 2 weeks.

Skin swabs taken of the injection site post ethanol wipe and two hours following injections were analysed using an infectivity assay. This indicated that in samples where vector was detected (10/53 of samples analysed) the levels were less than 1/10000th of the dose administered. This shows that the injection site disinfection procedure, swabbing with ethanol, worked well.

No routine tracing and monitoring of the GMO is foreseen. The clinical effects of the treatment will be monitored in terms of safety (reporting of AEs and SAEs), efficacy (radiological tumor assessment) and immunogenicity (humoral and cellular, in a subgroup of patients).

The GMO (TroVax<sup>®</sup>) will not replicate except in a limited number of cell lines in tissue culture so wide scale environment monitoring is unlikely to be required .

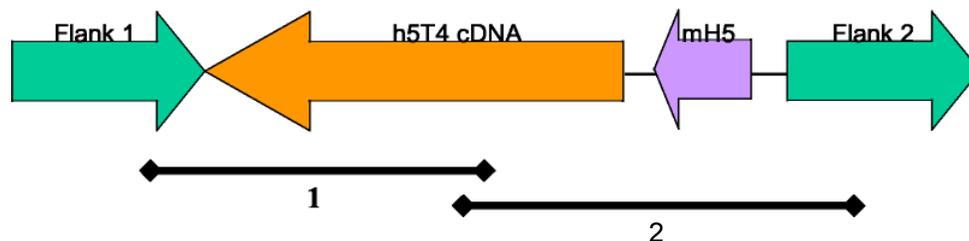
(c) Techniques used to identify the GMO

A quantitative PCR assay is available to detect TroVax<sup>®</sup> that is sensitive to 10 copies in 1ug of genomic DNA. This assay will not detect either MVA, or 5T4 sequences without the flanking MVA sequences.

A diagnostic PCR is also available to detect TroVax<sup>®</sup>. The method confirms that the full-length h5T4 cDNA and the modified H5 promoter are present in the clinical lot of TroVax<sup>®</sup>. DNA is isolated from the lot of TroVax<sup>®</sup> using SOP065 (Simple phenol chloroform extraction for isolating MVA DNA for PCR). Then two sets of primers are used to amplify sections of the region encoding the 5T4 gene (Figure 2) from the purified DNA. The first set amplifies a 851bp fragment from the 3' end of flank 1 into the 5T4 cDNA and the second set amplifies a 952 bp fragment that overlaps the first fragment, from around the middle of the 5T4 cDNA, through mH5 promoter into the 5' end of flank 2. The PCR products are then separated using gel electrophoresis and are visualized using UV illumination.

Acceptance criterion: A major band at ~850bp with primer pair 1 and ~950bp with primer pair 2 indicates correct identity. Additional minor bands do not indicate aberrant recombinant virus if they are also present in the control pTrV1b transfer plasmid sample. The 851 and 952bp products should not be amplified from the control wild type MVA DNA.

Figure 2- Position of the regions of TroVax<sup>®</sup> virus that are amplified.



The human 5T4 gene is detected and identified by Southern blot analysis using radiolabeled 5T4-specific cDNA probes, by Polymerase Chain Reaction (PCR) using 5T4-specific primers and also, by nucleotide sequence analysis. The glycoprotein product of the human 5T4 gene is detected and identified by Western blot analysis and Immunostaining techniques using a monoclonal antibody (H8) that recognizes human 5T4 in a conformationally dependent manner.

F. Information relating to the release

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

A Multicenter, Double Blind, Placebo controled, randomized study of TroVax<sup>®</sup> vs. Placebo in the First Line treatment of Patients with Metastatic Colorectal Cancer receiving chemo-based therapy – EFC 10528.

This is a randomized, double blind, placebo controled, paralel group, phase III, international study in patients with metastatic colorectal cancer designed to compare overall survival (OS) with TroVax<sup>®</sup> vs. Placebo in patients receiving chemo-based therapy in first line treatment.

Approximately 1312 patients (656 patients per arm) w i l be recruited into the study and w i l each receive one of the defined chemo-based therapy (Folfox or Folfiri, +/- bevacizumab) and intramuscular injections of TroVax<sup>®</sup> or Placebo. The two first injections of TroVax<sup>®</sup>/Placebo w i l be done alone at a one week interval (day 1, day 8). The chemo-based therapy w i l be started one week later at the time of the 3rd TroVax<sup>®</sup>/Placebo induction injection (day 15). Then, boost injections of TroVax<sup>®</sup>/Placebo w i l be done every month (or every 2 cycles of the chemo-based therapy). A l patients w i l be folowed up for survival.

A first interim analysis for futility w i l be performed based on PFS, when 152 PFS events occur. A second interim analysis of OS for efficacy is planned when approximately 512 death events occur (60% of death information).

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

Yes (.) No (.)

If yes, specify

Not applicable. Vaccinia viruses, including MVA, are considered to have no natural reservoir.

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 (BE, CZ, DE, FR, GB, HU, IT, NL, PL) and in worldwide (Argentina, Xile, Xina, Hong Kong, Japan, Republic of Corea, Malasia, Philipines, Russian Federation, South Africa, Taiwán, Thailand, Turkey and United States.

(b) Size of the site (m<sup>2</sup>):

The GMO (Tro Vax<sup>®</sup>) w i l be confined to the hospital test site, including the hospital pharmacy, clinical lab, and autoclaving/incineration area.

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 a l of the sites have been specifically identified.

(i) actual release site (m<sup>2</sup>):

(ii) wider release site (m<sup>2</sup>):

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

Not applicable

- (e) 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 dose of TroVax<sup>®</sup>/Placebo will be 1ml (1 x 10<sup>9</sup> TCID<sub>50</sub>/ml, with a range of 1.55 x 10<sup>8</sup> – 3.16 x 10<sup>9</sup> TCID<sub>50</sub>/ml) at each intramuscular injection into the deltoid muscle of the upper arm. The dose regimen will be 9 injections, administered along 29 weeks.

Induction injections will be done every week:

The first injection of TroVax<sup>®</sup>/Placebo i.m. will be done alone (D1)

The 2nd injection of TroVax<sup>®</sup>/Placebo i.m. will be done alone one week later (D8)

The 3rd injection of TroVax<sup>®</sup>/Placebo i.m. will be done 1 week later (D15), on day 1 of cycle 1 of the chemo-based therapy and 1 hour before the chemo-based therapy.

Boost injections will be done i.m. every month (or every 2 cycles of the chemo-based therapy), 1 hour before the chemo-based therapy. The first boost injection (i.e. the 4th injection of TroVax<sup>®</sup>/Placebo) will be done on day 1 of cycle 3 of the chemo-based therapy.

Detailed instructions will be provided to the pharmacist for reconstitution. TroVax<sup>®</sup>/placebo must be re-suspended by adding 1.2 mL of water for injection. The resulting solution should be mixed gently by rotation and will appear opalescent. One mL volume of the solution is then withdrawn into a syringe and injected within 5 minutes. The injection will be drawn up at the bedside by the person administering the dose and double-checked by a second responsible individual (either a registered nurse or a pharmacist).

No other release is foreseen.

- (b) Duration of the operation:

29 weeks

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

Worker protection measures taken during the release

Except for the patient, the medical staff involved in the trial are the most likely to be exposed to the vector systems although the chance for such an exposure is extremely limited.

The main exposure routes under normal conditions are:

- contact with solution in opened vial
- contact with solution in syringe
- contact with material at injection site and
- contact with primary disposable wound dressing.

In addition, accidental contact can occur via

- broken vial
- needle stick injury

Aerosol formation is highly unlikely under normal operations.

Even when exposure occurs, it is not expected to trigger a significant reaction. MVA has been used widely as a vaccinating agent against smallpox. It cannot replicate in man and would not represent a particular risk to possibly infected medical staff

Irrespective, measures will be taken to protect the involved staff. The medical staff will receive instructions on the nature of the material and the need to take special care.

Standard procedures will include

- use of disposable overcoat
- use of disposable gloves, mask, protective goggles
- standard procedures for storage, transport, preparation and administration of the product
- inactivation of GMO
- handling spillage and accidents.

#### Post-release treatment of the site

All materials potentially contaminated with TroVax<sup>®</sup>/Placebo (e.g. syringes, swabs, bandages) must be destroyed by incineration in accordance with hospital policy on genetically modified materials.

#### Techniques foreseen for elimination or inactivation of the GMOs at the end of the experiment

No specific elimination or inactivation is foreseen for material that has been injected in the patient. The presence and expression of TroVax<sup>®</sup> is transient and will remain confined to the patient.

All materials potentially contaminated with TroVax<sup>®</sup>/Placebo (e.g. syringes, swabs, bandages) must be destroyed by incineration in accordance with hospital policy on genetically modified materials.

MVA has been assigned Biosafety level I status by the National Institutes of Health intramural biosafety committee in the US, the biosafety authorities in France, Germany and the Health and Safety Executive in the UK.

All solid waste is de-activated by autoclaving and is then routinely incinerated after autoclaving.

All liquid spills on surfaces are to be treated with viral inactivation products approved locally according to the manufacturer's instructions for the vaccinia & pseudocowpox 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, gloves, is 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.

As of the cut-off date of 31 July 2007, TroVax<sup>®</sup> had been administered to over 350 patients with metastatic colorectal or renal cancer. No serious adverse events attributed to TroVax<sup>®</sup> by investigators or the sponsor have been reported except one grade 1 fever in the phase II FOLFOX study. 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<sup>®</sup> as a single agent in heavily pretreated patients or in studies combining TroVax<sup>®</sup> with chemotherapy, (5FU and leucovorin combined with either oxaliplatin or irinotecan), IL-2 (high dose intravenous regimen or low dose subcutaneous injections). TroVax<sup>®</sup> induced an immune response against the 5T4 antigen in >90% of per protocol patients in a I studies. An antibody or cellular immune response was observed after the second or third injection of TroVax<sup>®</sup>. CD8+ve cellular responses were often higher than noted with other cancer vaccines reported in the literature. (See Section 2.3.2.9 in the supporting document provided regarding non-clinic studies in animals and safety studies in humans).

Furthermore, several trials have been conducted in the EU and in the rest of the world with modified MVA used MVA as vectors. A I results confirm that MVA is a promising safe vector for medical applications.

- 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)	Homo
		... ..
(ii)	family name for plants	Sapiens
(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<sup>®</sup>) 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.

Not applicable

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 competitiveness, 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

Except for the patient, the medical staff involved in the trial are the most likely to be exposed to the vector systems although the chance for such an exposure is extremely limited.

Even when exposure occurs, it is not expected to trigger a significant reaction. MVA has been used widely as a vaccinating agent against smallpox. It cannot replicate in man and would not represent a particular risk to possibly infected medical staff. The pregnant healthcare workers will not administer the GMO. The potential childbearing healthcare workers must be informed about the risk.

- (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:

Recombination between closely related poxviruses may occur during virus replication under natural conditions. Although MVA can infect most mammalian species, it cannot replicate in primary human cells and the cells that it infects die of a result of the infection. During this infection unless the cells are permissive for virus replication (e.g. primary chicken embryo cells or BHK-2 1 cells) no infectious particles are produced. Due to the short duration of this 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.

Since vaccinia viruses, including MVA, are considered to have no natural reservoir, no transfer is expected. MVA has been used extensively and no transfer of genetic material has been reported.

(b) from other organisms to the GMO:

Unlikely

(e) likely consequences of gene transfer:

The presence of the 5T4 gene is not a selectable trait under natural conditions. It does not convey any particular function to the MVA or the human cells harboring the MVA.

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 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

As previously mentioned in section E.4.a, 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 (See section E.4.a and E.4.b)

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

Quantitative PCR (See section E.4.a and E.4.b)

4. Size of the monitoring area (m<sup>2</sup>)

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 F.4.b above.

6. Frequency of the monitoring

See section F.4.c above.

I. Information on Post-release and waste treatment

1. Post-release treatment of the site In the case of MVA based vectors there is no replication and no scab at the inoculation site.

From previously performed clinical trials it has been found that following administration of TroVax<sup>®</sup>, the patient poses no risk to healthcare workers or to the environment. The administration site is swabbed with alcohol. An occlusive dressing is also used as an extra precaution. Studies in which the dressing has been removed 2 hours after administration and the wound site swabbed for any remaining TroVax<sup>®</sup> have shown that the amount of vector remaining is less than 1/10000th of the original dose. This level of shedding is unlikely to pose a risk to the environment.

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, gloves and glasses for single use (if applicable) and cleansing material.

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

3. (b) Treatment of waste

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

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.

Three guidance procedures regarding General Spill, Accidental Exposure and Accidental Spill have been attached to this dossier.

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

A safety pack is provided to the clinical study site that details how to deal with spillages and the correct disposal of GMO, this includes the following.

### 2.1 Methods and Procedures for controlling the GMOs in case of unexpected spread

#### Procedure for cleaning up spills

- MINOR SPILLS
  - Clean surface with 70% IMS or 2% peroxygen compound.
  - Decontaminate instruments by soaking in the disinfectant solution for 30 minutes.
  
- SMALL SPILLS
  - Wipe up spill with tissue paper soaked in fresh disinfectant solution.
  - Leave for 10 minutes.
  - Discard tissue into autoclave bag.
  - Transport autoclave bag to the service lab for autoclaving
  
- LARGE SPILLS
  - Large Spill outside Biological Safety Cabinet
    - Stop what you are doing immediately.
    - Contain the spillage using the spill kits available.
    - Inform the Lab Manager.
    - Return to the spill.
    - In addition to a lab coat, wear a disposable apron, eye protection, shoe protectors and marigold gloves (or equivalent).
    - Flood the spill with a disinfectant solution.
    - Cover the spill with tissue or the spill kit absorbent material.
    - Leave for a minimum of 30 minutes.
    - Transfer spill material to an autoclave bag and transport to the Service Lab for autoclaving.
    - Wash spill area with detergent and allow to dry.
  
  - Large Spill inside Biological Safety Cabinet
    - Stop work immediately.
    - Close up the hood and label appropriately.
    - Inform the Lab Manager.
    - Fumigate the hood, as per fumigation protocol.
    - After fumigation, clear up the spill.

### 2.2 Methods for decontamination of the areas affected, for example eradication of the GMOs

As TroVax<sup>®</sup> may only replicate in permissive cells (e.g. CEFs or BHK), it will not replicate in the environment. Disinfection of the spill site is described in section 1.

### 2.3 Methods for disposal or sanitation of plants, animals, soils, etc., that were exposed during or after the spread

The disinfection procedures for use in the hospital are detailed in section 2.1.

## 2.4 Methods for the isolation of the area affected by the spread

See section 2.5 below.

## 2.5 Plans for protecting human health and the environment in case of the occurrence of an undesirable effect

As TroVax<sup>®</sup> may only replicate in permissive cells (e.g. CEFs or BHK), it will not replicate in the environment. Disinfection of the spill site is described in section 2.1.

Three guidance procedures regarding General Spill, Accidental Exposure and Accidental Spill have been attached to this dossier.

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

See section J2.

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

As the GMO TroVax<sup>®</sup> 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.