PART 2

SUMMARY NOTIFICATION INFORMATION FORMAT FOR RELEASES OF GENETICALLY MODIFIED ORGANISMS OTHER THAN HIGHER PLANTS

(in accordance with Article 9 of Directive 90/220/EEC)

Introduction

The Summary Notification Information Format has been established for the purposes and according to the procedures envisaged by Article 9 of Directive 90/220/EEC.

It is recognized that the Summary Notification Information Format is not designed to contain all the information required for carrying out an environmental risk assessment in the detail necessary for such an assessment. The information entered should, however, adequately reflect (in a condensed form) the information submitted to the competent authority according to Articles 5 and 6 of Directive 90/220/EEC under the conditions specified in the preface to Annex II. The space provided after each question is not indicative of the depth of the information required for the purposes of the Summary Notification Information Format.

GENERAL INFORMATION

1. Details of notification

Member State of notification: ..*BELGIUM*.....

Notification number: .....

Date of acknowledgment of notification: .....

Title of the project(s):
Study TG4010.04: "Randomized, multicenter, phase II study evaluating two doses of TG4010 (MVA-MUC-1-IL-2) in patients with metastatic breast cancer"
Study TG4010.05: " Randomized Multicenter PHASE II study Evaluating he Clinical Efficacy of TG4010 (MVA-MUC-1-IL-2) in association with Chemotherapy in Patients with Non Small Cell Lung Cancer"

Proposed period of release: *TG4010.04: Q4 2001-2003*
TG4010.05 : Q 42001-Q3 2003

2. Notifier

Name of institution or company: ...*TRANSGENE S.A*.....

3. GMO characterization

(a) Indicate whether the GMO is a :
~~viroid~~
~~RNA virus~~
* **DNA virus** *
~~bacterium~~
~~fungus~~
~~animal~~
~~other, please specify~~ .....

(b) Identity of the GMO: ***the recombinant vaccinia virus MVATG9931***

4. Is the same GMO release planned elsewhere in the Community (in conformity with Article 5 (1))?

Yes ~~No Not known~~

If yes, insert the country code(s) .....***France, (and Switzerland).***.....

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

~~Yes~~ ***No***

If yes:
- Member State of notification: .....
- Notification number: .....

INFORMATION RELATING TO ANNEX II

A. Information relating to the recipient or parental organisms from which the GMO is derived

1. Indicate whether the recipient or parental organism is a:
~~viroid~~
~~RNA virus~~
* **DNA virus** *
~~bacterium~~
~~fungus~~
~~animal~~
~~other, please specify~~ .....

2. Complete name :

(i) order and/or higher taxon (for animals)	***Poxviridae (family)***
(ii) genus	***Orthopoxvirus***
(iii) species	***vaccinia virus***
(iv) subspecies	-
(v) strain	***Modified Virus Ankara***
(vi) pathovar (biotype, ecotype, race, etc.)	-
(vii) common name	***MVA***

A simplified filiation between the attenuated strain originating from an hypothetical ancestor, and the recombinant vector is the following :

Dermovaccinia virus of Ankara (CVA) strain obtained by several passages donkey –calf-donkey => MVA strain => MVATG9931 vector

Note that all the above strains until the MVA included were isolated following natural selection in vivo (cells or animals). Therefore they are not GMO according to 90/219/CEE.

3. Geographical distribution of the organism

(a) Indigenous to the country where the notification is made:

~~Yes~~ * **No** * ~~Not known~~

(b) Indigenous to other EC countries:
The MVA was specially developped in 1970 in Germany to immunize patients at high risk for complications from vaccination against smallpox.

(i) Yes

If yes, indicate the type of ecosystem in which it is found:
None, the MVA is a laboratory strain of the vaccinia virus. This virus is itself not part of any known ecosystem (beyond the context of the smallpox vaccination, at present discontinued)

Atlantic Mediterranean	***Not applicable***
Arctic Continental	***Not applicable***

(ii) No Not known

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

~~Yes~~ * **No** *

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

~~Yes~~ * **No** *

4. Natural habitat of the organism

M (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~~
~~in association with animals~~
other (specify) :

the natural habitat of the recombinant vector, or of its parental virus (the MVA), is a laboratory setting. Neither one has been found in a natural habitat. (See also 3b above)

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

.....

5. (a) Detection techniques

Detection by Assessment of the Infectious Titer: vaccinia virus titration is performed on BHK-21 cells. Cells are infected by dilutions of the test article to be titrated and by dilutions of an internal reference. Viruses are adsorbed onto cells then cultures are incubated until obtention of plaques. Detection of plaques is performed by immunodetection using a peroxidase reaction.

(b) Identification techniques

b-1. Identity of MVA strain: this test is done to confirm by PCR the identity of the vector. It is based on the presence of MVA deletion III, a characteristic encountered only in the MVA strain of vaccinia virus. For this purpose, viral DNA is extracted from the test article, and from two positive control samples: another recombinant vaccinia virus encoding for MUC-1 antigen and IL-2 and an isolate of a non-recombined MVA strain. Using oligonucleotides flanking the MVA-specific deletion III region, an amplicon of a defined size is amplified by PCR. This amplicon cannot be revealed if the target backbone is the parental genome of the MVA.

b-2. Characterization of the genetic insert: This test is performed to confirm that the genome region carrying both passenger genes of the vector (MUC-1 and IL-2) is present. This assessment is performed by the demonstration that a DNA fragment, corresponding to its intended size, is present at the selected locus inside the vector. The PCR reaction is done with three couples of oligonucleotides binding inside and outside this region. The test is carried out on vector DNA extracted either from a purified sample of particles, or from an infected cell lysate. Amplification of a region encompassing the genes reveals its presence – or absence - through the size of the amplified fragments (expected: 2177, 1965 and 1462 bp), as determined on gel electrophoresis, and compared to a positive control obtained with MVATG9931 DNA extracted from a reference research stock, and to a negative control obtained with parental vector DNA (no passenger genes) extracted from a reference stock.

6. Is the recipient organism classified under existing Community rules relating to the protection of human health and/or the environment?

~~Yes~~ * ***No*** *

Note that vaccinia virus are classified as Class 2. No pathogenicity related to MVA has been able to be demonstrated in laboratory conditions in the following animals : mouse, rat, monkey, pig, calf, rabbit, dog, horse and sheep. Only two strains of cells are known to be permissive for the MVA strain propagation : primary chicken cells, and the continuous cell line BHK-21 (hamster). Consequently, according to 90/219/CEE, the MVA has been classified by the French Genetic Engineering Committe as Class 2 Group II confinement L1 for quantities inferior or equal to 10E9 pfu /sample or L2 for quantities superior to 10E9 pfu /sample.

If yes, specify: ***not applicable***

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

~~Yes~~ * ***No*** *

If yes:

(a) to which of the following organisms:
humans
animals
plants

(b) give the relevant information specified under Annex II, point II. (A).(11).(d)

.....

8. Information concerning reproduction:

(a) Generation time in natural ecosystems: .....***not applicable***
No natural ecosystem ; in permissive chicken embyonic cells and in optimized laboratory conditions, the generation time is about 1-3 days.

(b) Generation time in the ecosystem where the release will take place: ***not applicable***
The vector does not propagate in humans.

(c) Way of reproduction:
Sexual Asexual ***not applicable***

(d) Factors affecting reproduction:
In laboratory conditions, and in permissive cells : temperature, growth medium, namely.

9. Survivability

(a) Ability to form structures enhancing survival or dormancy:
not applicable

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

(b) Relevant factors affecting survivability:

.....

10. (a) Ways of dissemination : *Contact*

.....

(b) Factors affecting dissemination : ***Contaminated instruments***

.....

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

None

B. Information relating to the genetic modification

1. Type of the genetic modification
* (i) ***Insertion of genetic material*** *
~~(ii) Deletion of genetic material~~
~~(iii) Base substitution~~
~~(iv) Cell fusion~~
~~(v) Other, please specify~~

2. Intended result of the genetic modification

Vectorization of the two inserted coding sequence into human patients

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
bacteriophage
virus
cosmid
phasmid
transposable element
other, please specify
.....

(b) Identity of the vector :
.....

(c) Host range of the vector
.....

(d) Presence in the vector of sequences giving a selectable or identifiable phenotype
yes No

Antibiotic resistance
Heavy metal resistance
Other, specify :
.....

(e) Constituent fragments of the vector
.....

(f) Method for introducing the vector into the recipient organism
(i) transformation
(ii) electroporation
(iii) macroinjection
(iv) microinjection
(v) infection
(vi) other, please specify
.....

5. If the answer to question B.3 (a) and (b) is no, what was the method used to introduce the insert into the recipient/parental cell?

not applicable (the insert is introduced into the parental viral genome)

(i) ~~transformation~~
~~(ii) microinjection~~
~~(iii) microencapsulation~~
~~(iv) macroinjection~~

(v) other, please specify :
in vitro homologous recombination between a pBR322-derived plasmid carrying both passenger genes flanked by MVA sequences and the parental virus (MVA) genome.

6. Information on the insert

(a) Composition of the insert

- A MUC-1 expression cassette (Human Mucin), composed of the pH5R vaccinia virus promoter and a cDNA coding for MUC-1.
- A human interleukin-2 (IL-2) expression cassette, composed of the p7.5k vaccinia virus promoter and a cDNA coding for IL-2.
- 2 synthetic polylinkers, of 23 and 82 bases pairs

(b) Source of each constituent part of the insert

- for both coding sequences : human messenger RNA
- linkers have been isolated from commercial pBR322-derived plasmids

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

- To direct in patients the synthesis of IL-2 and MUC-1 at the site of administration
- to physically link the genes and the vector together

(d) Location of the insert in the host organism

- ~~on a free plasmid~~
- integrated in the chromosome : integrated in the vector's genome
- ~~other, please specify~~ .....

(e) Does the insert contain parts whose product or function are not known?
~~Yes~~ * ***No*** *
If yes, please specify: .....

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

1. Indicate whether it is a:
~~viroid~~
~~RNA virus~~
~~DNA virus~~
~~bacterium~~
~~fungus~~
~~plant~~
~~animal~~
other, please specify ***human***

2. **Complete name :** ***Homo sapiens***
   (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

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

not applicable
Yes, No, Not known
If, yes, specify the following:

(a) to which of the following organisms?

not applicable
humans
animals
plants

(b) are the donated sequences involved in any way to the pathogenic or harmful properties of the organism?
not applicable
~~Yes, No, Not known~~
If yes, give the relevant information under Annex II, II A, 11 d:
.....

4. **Is the donor organism classified under existing Community rules relating to the protection of human health and the environment?**

not applicable
Yes No
If yes, please specify:
.....

5. **Do the donor and recipient organism exchange genetic material naturally?**
~~Yes~~ * ***No*** * ~~Not known~~

D. Information relating to the genetically modified organism

1. Genetic traits and phenotypic characteristics of the recipient or parental organism which have been changed as a result of the genetic modification.

(a) Is the GMO different from the recipient as far as survivability is concerned?
~~Yes No~~ * ***Not known*** * ***(unlikely)***
If yes, please specify
.....

(b) Is the GMO in any way different from the recipient as far as mode and/or rate of reproduction is concerned?
~~Yes~~ * ***No*** * ~~Not known~~
If yes, please specify:
.....

(c) Is the GMO in any way different from the recipient as far as dissemination is concerned?
~~Yes~~ * ***No*** * ~~Not known~~
If yes, please specify:
.....

2. Genetic stability of the genetically modified organism :

The genetic stability of the recombinant vector (MVATG9931) is monitored throughout the process of production and several passages beyond. Release specifications regarding its genetic stability are set for the clinical material in order to ensure that it is conform to what it is intended to be. Once administered, the vector cannot propagate further from the infected cells, and thus there is no more opportunity for the genome to be rearranged.

3. Is the GMO pathogenic or harmful in any other way (including its extracellular products) either living or dead?
~~Yes~~ * ***No*** * ~~Not known~~

Note that, according to 90/219/CEE, the GMO has been classified by the French Genetic Engineering Committe as Class 2 Group II, confinement L1 for quantities inferior or equal to 10E9 pfu /sample or L2 for quantities superior to 10E9 pfu /sample

If yes,

(a) to which of the following organisms?:
humans
animals
plants

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

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment *: see above, § A.6.(a)*

.....

(b) Techniques used to identify the GMO *: see above, § A.6.(b)*

.....

E. Information relating to the release

1. Purpose of the release

Treatment of cancer by stimulation of the host's anti-tumor immunity

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

Not applicable : the vector is injected subcutaneously, and does not disseminate outside the body of the patients. Namely, no excretion of vaccinia vectors can be detected in body fluids.

Yes No
If yes, please specify: .....

3. Information concerning the release and the surrounding area

(a) Geographical location (administrative region and where appropriate grid reference):
.....***Erasmus Hospital, Brussels***.....

(b) Size of the site (m2): ***not applicable***
(c) (i) actual release site (m2): ***not applicable***

.....

(d) (ii) wider release area (m2): ***not applicable***

.....

(e) Proximity to internationally recognized biotopes or protected areas (including drinking water reservoirs), which could be affected: ***None***

.....

(f) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO: ***None***

.....

4. Method and amount of release

(a) Quantities of GMOs to be released: ***10E8 pfu per administration***

.....

(b) Duration of the operation: *< 5 minutes*

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

Previous preclinical studies in laboratory animals with pox vectors have shown that no dissemination from the site of administration to other part of the body can be detected, as well as no subsequent excretion of the vector. This has been subsequently confirmed during clinical trials in humans with the previous generation of the vector when monitoring of the excretats did not reveal any particle of vector. The planned route of administration – subcutaneous – is considered by the clinicians to be as leak-proof as the intramuscular route that has been used previously.

Moreover, the biological confinement of the vector is built into its attenuation since it cannot propagate in patients, and a fortiori not in people of their family circle who, hypothetically, would have been contaminated by traces of the vector.

It should be also noted that all contaminated instruments, and/or clothes will be decontaminated according to current hospital practices for infectious material.

F. Interactions of the GMO with the environment and potential impact on the environment

1. Complete name of target organisms :

No target organism is known since all animals tested so far have proven negative (see above § A.7.).

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

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism

Not applicable

3. Other potentially significant interactions with other organisms in the environment :

Not known.

4. Is post-release selection for the GMO likely to occur?

~~Yes~~ * ***No*** *

As indicated above, reproduction of the vector requires a specific laboratory environment (specialized cell lines and growth medium, namely).

If yes, give details:

.....

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

The site of release being an hospital room, « ecosystems » that could be affected are limited in availability. Moreover, ecosystems encompassing animals that have been tested (all negatives) for the capacity of the vector to replicate should not be able to maintain it.

6. Complete name of non-target organisms which may be effected unwittingly

Not applicable

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

7. Likelihood of genetic exchange in vivo

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

In addition to being unlikely, a dissemination of the vector to a given ecosystem should not lead to a genetic exchange with another related microorganism : indeed, no human pox virus is known to be endemic in humans. In animals susceptible to infection by the virus (even without being permissive for its propagation), few opportunity for genetic recombination with animal poxviruses could occur, since the level of replication that the vector DNA undergoes in vivo is low, and limited to cells infected by the inoculum (no generation of infectious particles).

.....

(b) from other organisms to the GMO: ***same answer as above.***

.....

8. Give references to relevant results from studies of the behaviour and characteristic of the GMO and its ecological impact carried out in simulated natural environments (e.g. microcosms, etc.):

No such references are available, unless the natural ecosystem of this laboratory strain is considered to be the human body. In that case, references relating to human vaccination carried out with the parental virus (MVA) of the vector on about 150000 children and adults are the following :

Hochstein-Mintzel, V., Hänichen, H. C., & Stickl, H., (1975) Vaccinia und variolaprotektive Wirkung des modifizierten Vaccinia-Stammes, MVA bei intramuskulärer Immunisierung. Zentralblatt für Bakteriologie, Mikrobiologie und Hygiene.1. Abt. Originale. A: Medical, Microbiology, Infectious Diseases, Virology, Parasitology, 230 , 283-.

Hochstein-Mintzel, V., Huber, H. C., & Stickl, H., (1972) Virulenz und Immunogenität eines modifizierten Vaccinia-Virus (Stamm MVA). Zeitschrift für immunitatsforschung, 144 , 140-156.

Holzner, A., (1976) Zur Beurteilung der MVA-Stufenimpfung bei der pockenerstimpfungen. Med Diss München, ,
Mahnel, H., & Mayr, A., (1994) Erfahrung bei der Schutzimpfung gegen Orthopocken von Mensch und Tier mit dem Impstamm MVA. Berliner und Münchener Tierärztliche Wochenschrift, 107 , 253-256.

Mayr, A., & Danner, K., (1979) Bedeutung von Tierpocken für den Menschen nach Aufhebung der Pflichtimpfung gegen Pocken. Berliner und Munchener Tierarztliche Wochenschrift, 92 , 251-256.

Mayr, A., Hochstein-Mintzel, V., & Stickl, H., (1975) Abstammung, Eigenschaften und Verwendung des attenuirten Vaccinia-Stammes MVA. Infection, 3 (1), 6-14.

Mayr, A., Stickl, H., Müller, H. K., Danner, K., & Singer, H., (1978) Der Pockenimpfstamm MVA: Marker, genetische Struktur, Erfahrung mit der parenteralen Schutzimpfung und Verhalten im abwehrgeschwächten Organismus. Zentralblatt für Bakteriologie, Mikrobiologie und Hygiene.1. Abt. Originale. B: Umwelthygiene. Krankenhaushygiene. Arbeitshygiene. Praventive Medizin., 167 , 375-390.

Stickl, H., & Hochstein-Mintzel, V., (1971) Die intrakutane Pockenimpfung mit einem schwach virulenten Vakzine-Viruz ("MVA-Virus"). Deutsche Medizinische Wochenschrift, 99 , 2386.

Stickl, H., Hochstein-Mintzel, V., Mayr, A., Huber, H. C., Schäfer, H., & Holzner, A., (1974) MVA-Stufenimpfung gegen Pocken. Klinische Erprobung des attenuierten Pocken-Lebendimpfstoffes, Stammes MVA. Deutsche Medizinische Wochenschrift, 99 , 2386-2392.

G. Information relating to monitoring

1. **Methods for monitoring the GMOs :** ***see above, § A.6.a and A.6.b***

.....

2. **Methods for monitoring ecosystem effects :** ***none, see above § F.5.***

.....

3. **Methods for detecting transfer of the donated genetic material from the GMO to other organisms :** ***none, see above § F.7.***

.....

4. **Spatial extent of the monitoring area (m2)** ***not applicable***

.....

5. **Duration of the monitoring** ***not applicable***

.....

6. **Frequency of the monitoring** ***not applicable***

.....

H. Information on post-release and waste treatment

1. **Post-release treatment of the site :**

Remaining and wastes from product use (syringes, needles, etc.) will be kept in a specific garbage and will be decontaminated following the standard hospital procedures for contaminated wastes.

2. **Post-release treatment of the GMOs :** ***none, beyond treatment of wastes (see above)***

.....

3. **(a) Type and amount of waste generated :**

The maximal dose administered to patients will be 10E8 pfu of the vector. Thus, the amount of waste generated at each administration will be equivalent to an amount much smaller than this dose, and will remain so since the vector cannot propagate itself independantly.

(b) Treatment of waste : ***see above § H.1.***

.....

I. Information on emergency response plans

1. Methods and procedures for controlling GMOs in case of unexpected spread :

During product manipulations, goggles will be worn, labcoat and gloves will be recommended. All transfers of the preparation will be done using a closed container. Prior to the administration of the product, the product will be prepared under conditions compliant with injectable preparations. In case of accidental shedding of the product (cracked or broken ampoules), every contaminated surface area will be treated according to applicable procedures at the hospital. In case of needle injury, the injection site will be immediately treated locally with hydrogen peroxide (3%) and cover with a sterile gauze dressing, which will be discarded when removed. The injured person will receive counselling from the investigator and will then be closely followed for a period of at least 2 weeks. In case of skin contamination: a local disinfection will be performed with hydrogen peroxide and the contaminated skin will be washed thoroughly with water and soap. In case of eye contamination, the contaminated area will be washed with water. An examination by an ophthalmologist will take place as soon as possible. In case of ingestion, it is recommended not to induce vomiting and to call the investigator or a doctor immediately. The person will be closely followed for a period of at least 2 weeks.

2. Methods for decontamination of the areas affected :

Bleach, or any other anti-viral product used routinely as a viral disinfectant in the hospital.

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

They will be kept in a specific garbage and decontaminated following the standard hospital procedures for contaminated wastes.

4. Plans for protecting human health and the environment in case of the occurence of an undesirable effect :

Patients will be monitored for the occurrence of serious adverse events (SAE) according to the clinical protocol : Each SAE will be recorded and evaluated by the hospital staff, and the sponsor of the clinical trial and the relevant health agencies will be notified.