PART 1 (COUNCIL DECISION 2002/813/EC)

SUMMARY NOTIFICATION INFORMATION FORMAT FOR THE RELEASE OF GENETICALLY MODIFIED ORGANISMS OTHER THAN HIGHER PLANTS IN ACCORDANCE WITH ARTICLE 11 OF DIRECTIVE 2001/18/EC

In order to tick one or several possibilities, please use crosses (meaning x or X) into the space provided as (.)

A. General information

1. Details of notification

(a) Member State of notification Germany
(b) Notification number: **B/DE/11/PEI1393**
(c) Date of acknowledgement of notification 27/06/2011
(d) Title of the project

VXM01 phase I dose escalation study in patients with locally advanced, inoperable and stage IV pancreatic cancer to examine safety, tolerability, and immune response to the investigational VEGFR-2 DNA vaccine VXM01

…

(e) Proposed period of release From . 01/12/2011 until 31/10/2012

2. Notifier

Name of institution or company: **VAXIMM GmbH**
MAFINEX-Technologiezentrum
Julius-Hatry-Straße 1
68163 Mannheim

3. GMO characterisation

VXM01 is an investigational oral vaccine consisting of attenuated *Salmonella typhi* strain Ty21a carrying multiple copies of plasmid DNA encoding a eukaryotic expression cassette for the human vascular endothelial growth factor receptor 2. VXM01 is developed for the treatment of solid tumor patients with or without metastases.

It is not intended to release VXM01 into the environment. It is planned to administer VXM01 orally, within a clinical study, to pancreatic cancer patients. The shedding of the GMO by the patients will be monitored in body fluids and excreta during the study. Patients will only be discharged from the study unit after demonstration of absence of VXM01 from all body fluids and excreta, after the last vaccination.

(a) Indicate whether the GMO is a:

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

fungus (.)
animal
- mammals (.)

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

specify phylum, class Proteobacteria, Gammaproteobacteria

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

Salmonella enterica serovar Typhi Ty21a (carrying a plasmid with an eukaryotic expression cassette encoding human vascular endothelial growth factor receptor 2)

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

Genetic stability of known genomic mutations of attenuated Ty21a (*Kopecko et al, 2009*) was confirmed for the VXM01 Master Cell Bank. Plasmid stability was demonstrated due to presence of selective marker located on plasmid.

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

Yes (.) No (X)

If yes, insert the country code(s) …

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

Yes (.) No (X)

If yes:
- Member State of notification …
- Notification number B/../../…

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

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

Yes (.) No (X)

If yes:
- Member State of notification …
- Notification number B/../../…

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

The overall impact on the environment of the release of VXM01 within the study VXM01-01-DE is considered to be low as all possible measures have been taken to prevent an uncontrolled spread of the GMO into the environment as patients are confined within the clinical site during the administration period of the study. Excretion of VXM01 or another bacterium carrying the plasmid after the unlikely event of a horizontal gene transfer can be monitored. Patients identified for positive excretion can be treated with antibiotics and monitored for success of treatment. Appropriate exclusion criteria have been defined and the

in-house confinement ascertains that study patients are under control during the sensitive period of the trial.

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		(.)
bacterium		(X)
fungus		(.)
animal		
-	mammals	(.)
-	insect	(.)
-	fish	(.)
-	other animal	(.)
	(specify phylum, class)	(Proteobacteria, Gammaproteobacteria)

other, specify …

2. Name

(i)	order and/or higher taxon (for animals)	Bacteria
(ii)	genus	*Enterobacteriaceae*
(iii)	species	*Salmonella*
(iv)	subspecies	*Enterica*
(v)	strain	Ty21a
(vi)	pathovar (biotype, ecotype, race, etc.)	Serotype Typhi
(vii)	common name	*S.typhi* Ty21a

3. Geographical distribution of the organism

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

(b) Indigenous to, or otherwise established in, other EC countries:
(i) Yes (X)

If yes, indicate the type of ecosystem in which it is found:

Atlantic	..
Mediteranean	..
Boreal	..

Alpine ..
Continental ..
Macaronesian ..
Licensed typhoid fever vaccine (Vivotif™, Typhoral™)

(ii) No (.)
(iii) Not known (.)

(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: Licensed typhoid fever vaccine (Vivotif™, Typhoral™)

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

N/A

5. (a) Detection techniques

Culture

(b) Identification techniques

Culture on selective agar plates + serum agglutination
(Ph. Eur. monograph for Ty21a)

6. Is the recipient organism classified under existing Community rules relating to the protection of human health and/or the environment?
Yes (.) No (X)
If yes, specify

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

…

8. Information concerning reproduction

(a) Generation time in natural ecosystems:
S. typhi Ty21a has very limited viability due to its attenuation

(b) Generation time in the ecosystem where the release will take place:
S. typhi Ty21a has very limited viability due to its attenuation

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

(c) Factors affecting reproduction:
Temperature, specific culture medium

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	…	None

(b) relevant factors affecting survivability:
Temperature, *S. typhi Ty21a* has very limited viability due to its attenuation

10. (a) Ways of dissemination

Shedding with feces or other excreta

(b) Factors affecting dissemination

Ingested dose (Shedding of Ty21a has only been observed at a dose of > 10^9 CFU; *Gilman et al.,1977, and Wahdan et al., 1982*)

11. Previous genetic modifications of the recipient or parental organism already notified for release in the country where the notification is made (give notification numbers)
…, B/../../…
Not known

C. **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)	others, specify	Transformation with plasmid DNA

2. Intended outcome of the genetic modification

Usability as anti-angiogenic vaccine for the treatment of cancer and other diseases

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	…

(c) Identity of the vector

Bacterial plasmid containing a selectable marker gene, and an origin of replication, in addition to the eukaryotic expression cassette encoding hVEGFR-2.

(c) Host range of the vector

Bacteria (capable of multiplying plasmid by control of origin of replication).

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

	Yes	No
	(X)	(.)

antibiotic resistance	(X)
other, specify	…

Indication of which antibiotic resistance gene is inserted

Kanamycin

(e) Constituent fragments of the vector

Vector backbone, origin of replication, selectable marker, eukaryotic expression cassette

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

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

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

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

6. Composition of the insert

(a) Composition of the insert

Eukaryotic expression cassette encoding humanVEGFR-2

(b) Source of each constituent part of the insert

Synthetic DNA sequence, derived from gene banks

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

Expression cassette: Silent, no transcription/translation within GMO. Becomes translated in the patients gut cells upon vaccination.

(d) Location of the insert in the host organism

- on a free plasmid (X)
- 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
- mammals (X)
- insect (.)
- fish (.)
- other animal (.)
(specify phylum, class) (Chordate, Mammalia)

other, specify

2. Complete name
N/A

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

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

Yes (.) No (.) Not known (.)
If yes, specify the following:

(b) to which of the following organisms:

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

(b) are the donated sequences involved in any way to the pathogenic or harmful properties of the organism
Yes (.) No (.) Not known (.)

If yes, give the relevant information under Annex III A, point II(A)(11)(d):
…

4. Is the donor organism classified under existing Community rules relating to the protection of human health and the environment, such as Directive 90/679/EEC on the protection of workers from risks to exposure to biological agents at work?
N/A

Yes (.) No (.)
If yes, specify …

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

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

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 …

(b) is the GMO in any way different from the recipient as far as mode and/or rate of reproduction is concerned?
Yes (.) No (X) Unknown (.)
Specify …

(c) is the GMO in any way different from the recipient as far as dissemination is concerned?
Yes (.) No (X) Not known (.)
Specify …

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

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

Specify …

2. Genetic stability of the genetically modified organism

The plasmid contains a selection marker, leading to plasmid stability under selective conditions.

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

Cultivation on selective agar plates, followed by serum agglutination and identification of plasmid by PCR methods.

(c) Techniques used to identify the GMO

Selective culture, serology, PCR

F. Information relating to the release

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

Oral administration to treat pancreatic cancer patients enrolled in the clinical study VXM01-01-DE.

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 (X)

If yes, specify

3. Information concerning the release and the surrounding area

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

(b) Size of the site (m^2): 200 m^2
(i) actual release site (m^2): … m^2
(ii) wider release site (m^2): … m^2
N/A

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

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

4. Method and amount of release

(a) Quantities of GMOs to be released:

Ca. $3x10^{12}$ CFUs will be given in total, to 24-30 patients.

(b) Duration of the operation:

12 months. Four oral administrations over a period of seven days per study patient.

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

The shedding of the GMO by the patients will be monitored in body fluids and excreta during the study. Patients will only be discharged from the study unit after demonstration of absence of VXM01 from all body fluids and excreta, after the last vaccination. Until then all excretions will be collected and incinerated.

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

N/A

6. Relevant data regarding previous releases carried out with the same GMO, if any, specially related to the potential environmental and human health impacts from the release.

N/A

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) …
   (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)

   N/A

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

   N/A

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

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

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

   N/A

6. Complete name of non-target organisms which (taking into account the nature of the receiving environment) may be unintentionally significantly harmed by the release of the GMO

   N/A

   (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:

Very low

(b) from other organisms to the GMO:

Very low

(d) likely consequences of gene transfer:

None

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

N/A

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

N/A

H. Information relating to monitoring

1. Methods for monitoring the GMOs

Sampling of body fluids and excreta, cultivation on selective agar plates and identification of plasmid or bacteria by PCR methods via validated methods.

2. Methods for monitoring ecosystem effects

N/A

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

PCR

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

200 m^2 (patients' excreta)

5. Duration of the monitoring

One year for the study. Per patient, from the first dose to last dose (7 days) and further up to negative results for presence of VXM01 in patients'excreta.

6. Frequency of the monitoring

Every few days.

I. Information on post-release and waste treatment

1. Post-release treatment of the site

All Patients excreta and waste material that has been in contact with the GMO is collected and autoclaved or incinerated. Adequate hygienic precautions are taken.

2. Post-release treatment of the GMOs

All Patients excreta and waste material that has been in contact with the GMO is collected and autoclaved or incinerated. Adequate hygienic precautions are taken.

3. (a) Type and amount of waste generated
- VXM01 remnants and empty vials
- Plastic laboratory material for VXM01 reconstitution
- Patient excreta

3. (b) Treatment of waste
Autoclaving and/or incineration

J. Information on emergency response plans

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

Treatment of patients with antibiotics in case of (prolonged) shedding. Cleaning with biocide solution or wipes for spills.

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

Biocide solutions or wipes

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

N/A

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

Treatment of patients with antibiotics to sanitize the gut ecosystem and adequate hygienic precautions are taken.