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	Netherlands
(b)	Notification number	B/NL/19/003
(c)	Date of acknowledgement of notification	19/02/2019
(d)	Title of the project An open-label, Phase I/II multicenter clinical trial of VXM01 in combination with avelumab in patients with progressive glioblastoma following standard treatment, with or without second surgery. Study Code VXM01-AVE-04-INT	
(e)	Proposed period of release	From 01/08/2018 until 01/08/2021

2. Notifier

Name of institution or company: VAXIMM GmbH

3. GMO characterisation

The GMO named VXM01 is an orally administered cancer immunotherapeutic gene transfer medicinal product (investigational cancer vaccine). It consists of a live attenuated bacterium (*Salmonella enterica* subsp. enterica Serovar Typhi Strain Ty21a) transfected with a plasmid DNA carrying a eukaryotic expression cassette encoding the human vascular endothelial growth factor (VEGF)-Receptor 2 gene.

(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)
Order of the parental organism: Enterobacteriales
Genus of the parental organism: Salmonella
Species and Subspecies of the parental organism: *Salmonella enterica* subsp. enterica
Serovar Typhi Strain Ty21a

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

For the parental organism *Salmonella* Typhi Strain Ty21a it was demonstrated that the genetic mutations which determine the attenuation remained stable over the past 25 years of manufacturing of the typhoid vaccine Typhoral®/Vivotif®. A reversion of *Salmonella* Typhi Strain Ty21a to wild type has not been reported so far. The presence of the same attenuating mutations in the GMO was confirmed by sequencing of the bacterial genome of the VXM01 Master Cell Bank. It is concluded that the genetic mutations rendering the GMO attenuated are genetically stable. The genetic stability of the genetic modification i.e. the presence of the expression plasmid in the GMO is confirmed for each batch of the GMO at release testing by determining the presence of the selective marker. Long term stability studies conducted with the GMO have demonstrated that this parameter remains stable over the entire shelf-life assigned to the GMO.

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

Yes	(x)	No	(.)

If yes, insert the country code(s) FR, DE

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

Yes	(x)	No	(.)

If yes:

-	Member State of notification	DE
-	Notification number	B/DE/11/PEI1393 B/DE/16/PEI/2516 B/DE/15/PEI/2509

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 GMO VXM01 is transformed bacterium (an attenuated strain of *Salmonella enterica* serovar Typhi Strain Ty21a) carrying multiple copies of a plasmid DNA encoding for the human vascular endothelial growth factor-2 (VEGFR-2). The parental organism strain was derived from a licensed live oral vaccine against typhoid fever. During the clinical study low doses (compared to the approved dose of the typhoid vaccine Typhoral L®) of the GMO will be orally administered to patients suffering from progressive glioblastoma.
The overall impact on the environment of the release of VXM01 within the planned study is considered to be low. Clinical experience is available with the GMO from three clinical studies in cancer patients that have been conducted in the past. No unexpected safety findings were observed. Appropriate exclusion criteria for patients have been defined to minimize the risk for patients. Appropriate measures have been taken to minimize spread of the GMO into the environment or transmission to third parties. Strict procedures are in place to prevent the release of the GMO at the clinical site up to the administration of the GMO. Furthermore, hygienic measures are implemented to prevent the transmission of the GMO to third parties. 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. The parental organism is used for decades as an approved live bacterial typhoid vaccine. Its safety profile is very well documented and no impact on the environment can be envisaged. The parental organism cannot survive in the natural environment. In the absence of antibiotic selection, the genetic modification does not confer any survival advantage to the GMO compared to the parental organism. The highly attenuated bacterium is not able to proliferate or survive in the environment.

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/ Enterobacteriaceae
(ii)	genus	*Salmonella*
(iii)	species	*Enterica*
(iv)	subspecies	*Enterica*
(v)	strain	Ty21a
(vi)	pathovar (biotype, ecotype, race, etc.)	Serovar Typhi
(vii)	common name	S. Typhi Ty21a

The recipient organism from which the GMO is derived is the attenuated *Salmonella enterica* subsp. enterica serovar Typhi Strain Ty21a. The *Salmonella* Typhi Ty21a strain is the active component of the live oral typhoid vaccine Typhoral L®/Vivotif®.

3. Geographical distribution of the organism

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

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

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

Atlantic	..
Mediteranean	..
Boreal	..
Alpine	..
Continental	
Macaronesian	..

The vaccine strain does not exist in the natural environment. It is a licensed typhoid fever vaccine (Vivotif®, Typhoral®)

(ii) No (x)
(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 The licensed typhoid fever vaccine (Vivotif®, Typhoral®) does not exist in the natural ecosystem.

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

Not applicable

5. (a) Detection techniques

The parental organism can be detected by culture on media selective for the growth of *Salmonellae*.

(b) Identification techniques

Identification methods employ the specific biochemical characteristics of the attenuated parental organism in accordance with the monograph of the European Pharmacopoeia <1055> *Typhoid Vaccine (Live, Oral, Strain Ty21a)*. Strain Ty2la does not produce hydrogen sulphide on Kligler iron agar. When grown on an agar medium containing 1% of galactose and bromothymol blue light blue, concave and transparent colonies are formed. Further identification is achieved by serological testing using antisera specific to the O5 and O9 antigens in accordance with Ph. Eur. <1055>.

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 (.)

The recipient/parental organism is the highly attenuated vaccine strain Salmonella enterica subsp. enterica serovar Typhi Strain Ty21a being the active component of the approved live oral typhoid vaccine Typhoral L®/Vivotif®. This strain is non-pathogenic. Extensive safety information is available from large clinical studies as well as due to its widespread and longstanding use as a live oral vaccine against typhoid fever. Wild-type *Salmonella* Typhi is a human obligatory pathogen and does not infect animals. Consequently, any harmful effect of *Salmonella* Typhi Strain 21a on animals can be excluded.

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:

Due to its attenuation S. Typhi Ty21a has very limited viability in natural ecosystems. Survival studies have shown that the GMO does not proliferate in water of different qualities or in sewage. No other information on the generation time in any other natural ecosystem is available.

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

Due to its attenuation S. Typhi Ty21a has very limited viability in natural ecosystems. Survival studies have shown that the GMO does not proliferate in water. No other information on the generation time in any other natural ecosystem is available.

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

(c) Factors affecting reproduction:
(d)

For growth of *Salmonella* Typhi Ty21a suitable culture media are required as stated in Ph. Eur. <1055>. Proliferation depends on the culture conditions such as the composition of the culture medium, temperature, pH, oxygen content. Strain Ty21a has been shown in different studies to be highly susceptible to various environmental stresses such as elevated temperature, solutions with high osmolality, acidic or alkaline pH, solutions with peroxides or starvation.

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:

Due to its attenuation *S.* Typhi T21a is very sensitive towards unfavourable environmental conditions. Relevant factors are temperature, pH and specific nutrients in the medium. Strain Ty21a has been shown in different studies to be highly susceptible to various environmental stresses such as elevated temperature, solutions with high osmolality, acidic or alkaline pH, solutions with peroxides or starvation. The strain was shown to be unable to survive in human tissues, blood or stool. The sensitivity of the strain to adverse environmental conditions is thought to be due to multiple attenuating mutations, most notably the ropS gene, affecting a range of metabolic and structural elements. Survival studies have shown that the GMO does not proliferate in water of different qualities or in sewage.

10. (a) Ways of dissemination

Dissemination of *Salmonella* Typhi Strain Ty21a has been investigated in the past for its use as a typhoid vaccine. Since *Salmonella* Typhi Strain Ty21a is orally administered in viable form, studies were undertaken to determine the frequency and level at which it is excreted in the faeces. Clinical trials have shown either a limited and transient level of shedding or a complete lack of shedding in the stools of volunteers depending on the administered dose of the vaccine. With a 3-10 x 10^{10} CFU dosage (ten times higher than commercial formulations of Vivotif®) a low rate of excretion, mainly on day one post-vaccination, was observed. Vaccine organisms could be isolated from the stool of vaccinated subjects only on the first day following vaccination.

(b) Factors affecting dissemination

Shedding appears to depend on the ingested dose. Shedding of *S.* Typhi Ty21a has been observed at a dose of > 10^9 CFU.

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)

To the knowledge of the sponsor no notifications of release of any genetic modification of the parental organism *Salmonella* Typhi Strain Ty21a have been made in the past.
Two releases of genetically modified *Salmonella* Typhi Strain Ty2 (not Ty21a) have been notified:

B/BG/03/R35/02
B/GB/10/R40/01

Two clinical trials performed in Germany assessed *Salmonella* Typhi Ty21a as a carrier for antigens of *Helicobacter pylori*. In these trials, experimental formulations of a Ty21a strain expressing urease A and B subunits of *H. pylori* were administered orally after mixing either freshly harvested cultures or frozen aliquots with bicarbonate buffer. The strain was found to be safe and immunogenic in both studies.

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	transfection with plasmid DNA

2. Intended outcome of the genetic modification

VXM01 is supposed to exert its activity by using the attenuated *Salmonella* Typhi strain Ty21a as a carrier to deliver the plasmid to intestinal antigen presenting cells of cancer patients with aim to elicit a T-cell dependent immune response against VEGFR-2. The series of events that lead to the biological activity of VXM01 can be briefly summarized as follows: Orally administered *Salmonellae* carrying the expression plasmid encoding for human VEGFR-2 enter the host via M cells in the intestine. After transcytosis the bacteria are taken up by phagocytic cells such as macrophages and dendritic cells. The expression plasmids are released followed by a transfer of the plasmids into the cytosol either via a specific transport system or by endosomal leakage. The expression plasmid enters the nucleus and is transcribed, leading to antigen expression in the cytosol of the host cell. Infected macrophages go into apoptosis and are taken up by dendritic bystander cells which present antigens from apoptotic material on MHC-I. These activated antigen presenting cells induce VEGFR-2-specific cytotoxic $CD8^+$ T cells which subsequently target VERGFR-2 expressing cells of tumour vasculature or of the tumour itself.

3. (a) Has a vector been used in the process of modification?

Yes (x) No (.)

If no, go straight to question 5.

(b) If yes, is the vector wholly or partially present in the modified organism?

Yes (x) No (.)

If no, go straight to question 5.

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

(a) Type of vector

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

(b) Identity of the vector

pVAX10.VR2-1

(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 (x) No (.)

antibiotic resistance (x)
other, specify …

Indication of which antibiotic resistance gene is inserted
The Kanamycin resistance gene was inserted as a selection marker

(e) Constituent fragments of the vector
The expression plasmid used in VXM01 contains the human VEGFR-2 cDNA coding sequence. The vector backbone contains

- the human cytomegalovirus immediate-early (CMV) promoter
- the bovine growth hormone (BGH) polyadenylation signal
- a Kanamycin resistance gene.
- an origin of replication derived from plasmid pBR322.

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

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

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

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

6. Composition of the insert

(a) Composition of the insert

The insert consist of the cDNA sequence the human Vascular Endothelial Growth Factor Receptor-2 cloned downstream of the human cytomegalovirus immediate-early (CMV) promoter and is followed by the bovine growth hormone (BGH) polyadenylation signal.

(b) Source of each constituent part of the insert

The full-length cDNA coding sequence for human VEGFR-2 as well as the other constituents of the plasmid were produced by chemical synthesis (synthetic DNA sequences derived from gene data bases).

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

The insert i.e. the expression cassette for the VEGFR-2 cDNA is silent (no transcription/translation) within GMO as the CMV promoter exerts its function only in mammalian cells. The VEGFR-2 cDNA sequence is expressed when the plasmid enters antigen presenting cells in the intestinal tract of the patient after oral administration of the GMO (See C.2).

(e) Location of the insert in the host organism

- on a free plasmid (x)
- integrated in the chromosome (.)
- other, specify …

(f) 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) Chordata, Mammalia

other, specify …

2. Complete name

(i)	order and/or higher taxon (for animals)	…
(ii)	family name for plants	…
(iii)	genus	*Homo*
(iv)	species	*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?

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

(c) to which of the following organisms:

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

Not applicable

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

In vivo, the Vascular Endothelial Growth Factor Receptor binds the Vascular Endothelial Growth Factor that regulates the growth and differentiation of multiple component of the vascular system, especially blood and lymph vessels. However, as noted above, when administered to patients the GMO is taken up by antigen presenting cells located in the intestinal tract of the patients, the plasmid is released and the protein is transiently expressed. The protein is then degraded and fragments thereof are presented on the surface of the antigen presenting cells leading to the induction of an antigen specific T-cell mediated immune response in treated patients.

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

4. Is the donor organism classified under existing Community rules relating to the protection of human health and the environment, such as Directive 90/679/EEC on the protection of workers from risks to exposure to biological agents at work?
Yes (.) No (X)
If yes, specify …

5. Do the donor and recipient organism exchange genetic material naturally?
Yes (.) No (x) Not known (.)

E. Information relating to the genetically modified organism

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

(a) is the GMO different from the recipient as far as survivability is concerned?
Yes (.) 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 …

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

(e) 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

As stated in Section A.3.c genetic stability of the parental organism *Salmonella* Typhi Strain Ty21a has been demonstrated over the past 25 years of manufacturing of the typhoid vaccine Typhoral®/Vivotif®. The genetic stability of the genetic modification i.e. the presence of the expression plasmid in the GMO is confirmed for each batch of the GMO at release testing by determining the presence of the selective marker. Long term stability studies conducted with the GMO have demonstrated that this parameter remains stable over the entire shelf-life assigned to the GMO.

3. Is the GMO significantly pathogenic or harmful in any way (including its extracellular products), either living or dead?
Yes (.) No (x) Unknown (.)

The GMO is currently in the clinical stage of development. The first-in-human study with VXM01-01-DE in patients with pancreatic cancer and the clinical study VXM01-03-DE in colorectal cancer patients have been completed. A further study VXM01-02-DE in patients suffering from glioblastoma is ongoing. Based on the available safety data from all studies it can be concluded that the safety and tolerability profile of VXM01 was acceptable. No unexpected toxicities became apparent.
Toxicity was evaluated by one GLP-compliant single dose toxicity study with VXM01 and two GLP-compliant repeated-dose toxicity studies in mice with VXM01m (the murine

homologue to VXM01 i.e. an expression plasmid containing the murine VEGFR-2 receptor sequence transfected into *Salmonella Typhimurium* which infects mice).

(a) to which of the following organisms?

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

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

4. Description of identification and detection methods

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

The GMO can be detected by culture on media selective for the growth of *Salmonellae*. In addition, Polymerase Chain Reaction (PCR) can be used applying primers specific for sequences on the expression plasmid to detect the presence of the expression plasmid.

(b) Techniques used to identify the GMO

Identification methods for the carries bacterium, *Salmonella* Typhi Strain Ty21a, employ the specific biochemical characteristics of the attenuated parental organism in accordance with the monograph of the European Pharmacopoeia <1055> *Typhoid Vaccine (Live, Oral, Strain Ty21a)*. Strain Ty2la does not produce hydrogen sulphide on Kligler iron agar. When grown on an agar medium containing 1% of galactose and bromothymol blue light blue, concave and transparent colonies are formed. Further identification is achieved by serological testing using antisera specific to the O5 and O9 antigens in accordance with Ph. Eur. <1055>. The expression plasmid is identified by restriction analysis using appropriate restriction enzymes for release testing. It can further be identified by PCR using primer pairs specific for sequences in the expression plasmid and nucleotide sequencing.

F. Information relating to the release

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

The company intends to conduct clinical study to evaluate the safety and efficacy of the GMO in combination with an anti-PD-L1 monoclonal antibody in patients with recurrent glioblastoma. The rationale for this study is the assumption that the GMO in combination with the anti-PD-L1 antibody will induce an immune response against VEGFR-2 and the resulting anti-angiogenic effect will lead to an anti-tumoural efficacy in the patients.

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)

The parental organism *Salmonella* Typhi Strain Ty21a has no natural habitat.

If yes, specify …

3. Information concerning the release and the surrounding area

(a) Geographical location (administrative region and where appropriate grid reference): The releases will take place at two clinical centres in the Netherlands.

Erasmus Medical Center Kanker Instituut, Rotterdam, Netherlands
University Medical Center, Utrecht, Netherlands

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

No specific site size is required for the release. The release of the GMO will take place in a designated standard-sized hospital or clinic examination room within each of the designated clinical institutions. No wider release is expected.

(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:

A cumulative dose of not more than $6.88x10^{10}$ CFU of the GMO will be orally administered to 30 study patients assuming that all patients receive treatment for the maximal study duration. Patients will be enrolled in several centres participating in the study. Consequently, less the $6.88x10^{10}$ CFU of the GMO will be released per site.

(b) Duration of the operation:

The entire trial including follow-up will last approximately 5 years (recruitment phase of approximately 12 months, treatment phase of up to 24 months, follow-up phase of 24 months). Release takes place from the beginning of the recruitment up to the end of treatment. Therefore, the duration of the release will be approximately 36 months.

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

Handling of the GMO will be conducted only by qualified health care professionals. All involved study personnel, care givers and patients will be trained regarding hygienic precautionary measures to prevent spreading of the GMO as stipulated in the study protocol and site-specific study procedures. All surfaces potentially contaminated with the GMO will be carefully disinfected according to documented local procedures using locally approved disinfectants. All waste materials will be destroyed in accordance with local and national regulations applicable to the GMO.

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

All GMO administrations are to be performed in conventional hospital/clinical rooms at the clinical institutions listed above.

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.

The GMO was released in the past in the course of the three clinical studies VXM01-01-DE, VXM01-02-DE and VXM01-03-DE. No impact of the release on the environment or human health was discernible. Based on the available safety data from all studies it can be concluded that the safety and tolerability profile of VXM01 was acceptable. No unexpected toxicities became apparent. Transient faecal shedding of viable bacteria was detected in some patients after administration of VXM01.

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	Homo
(iv)	species	sapiens
(v)	subspecies	…
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	Human

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

The anticipated mechanism of action of the GMO is presented in Section C.2. Briefly, orally administered *Salmonellae* carrying the expression plasmid encoding for human VEGFR-2 enter the host via M cells in the intestine. The bacteria are taken up by phagocytic cells such as macrophages and dendritic cells and the target antigen is expressed after release of the expression plasmid. Antigen fragments are presented and specific cytotoxic $CD8^+$ are induced which subsequently target VERGFR-2 expressing cells of tumour vasculature or the tumour itself.

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

Potentially, the expression plasmid delivered by the bacteria could be transferred to other bacteria present in the intestinal tract of treated patients or integrated into the genome of host cells. Based on a risk analysis the likelihood of such events is considered to be low or negligible.

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

Yes (.) No (x) Not known (.)

Give details

Survival studies demonstrated that the GMO VXM01 does not proliferate or survive in the environment. The genetic alteration (i.e. transfection with the VEGFR-2 expression plasmid) does not confer any survival advantage to VXM01 compared to other bacteria in the natural environment.

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

Due to the context of the proposed GMO release, where the GMO is administered to subjects in an enclosed hospital or clinical examination room, it is unlikely the GMO will come into contact with any non-target organisms in the ecosystem. If the GMO is disseminated after faecal shedding into the sewage system persistence of the GMO is excluded as the GMO cannot survive in natural environments due to its attenuation.

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

(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 …

Not applicable

7. Likelihood of genetic exchange in vivo

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

Events of genetic transfer of the plasmid from VXM01 to other bacteria in the intestinal tract of patients have never been observed so far in faeces samples taken from patients in the clinical studies conducted. Based on current data it is concluded that such events are rare.

(b) from other organisms to the GMO:

The likelihood is estimated to be negligible.

(d) likely consequences of gene transfer:

An event of genetic transfer would render the receiving bacteria resistant to the antibiotic kanamycin but would not confer any other survival benefit in the absence of kanamycin. Alternative antibiotics will still be effective. The risk is therefore qualified as low. The target antigen encoded by the expression plasmid is not expressed in bacteria as the promoter is specific for eukaryotic cells.

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.):

Survival studies have demonstrated that the GMO has no capability of proliferation in the environment and rapidly dies off after accidental spill onto solid surfaces or release into water or sewage.

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

   For monitoring of the GMO in biological specimen such as blood, urine or faeces with high sensitivity liquid enrichment cultures of the samples are analysed by quantitative PCR specific for sequences of the expression plasmid. In parallel streak plating of the enrichment cultures on selective agar plates is conducted. If the plasmid is detected by qPCR in any liquid culture, visible colonies grown on the selective plates are serologically analysed whether they are of Ty21a origin. This method allows enriching the GMO against other bacteria, which may be necessary for samples showing a high bio-burden naturally resistant to kanamycin.

2. Methods for monitoring ecosystem effects

   Not applicable

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

   For detection of horizontal gene transfer samples are plated onto medium containing kanamycin but is otherwise rich and unselective. Any colonies which are macroscopically different from the GMO are picked, subcultured and analysed by PCR for the presence of the expression plasmid.

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

   Not applicable. The GMO is orally administered only to patients.

5. Duration of the monitoring

   Extensive safety monitoring of the patients will be conducted throughout the study period and well as after treatment in the two years of safety follow-up. As the shedding profile of the GMO has been well characterised in the previous studies VXM01-01-DE, VXM01-02-DE and VXM01-03-DE no further monitoring for the GMO will be conducted.

6. Frequency of the monitoring

   During the treatment phase of the study, visits are schedules every two weeks. During the safety follow-up period visits will be performed 1, 3, 6, 12, and 24 months. As the shedding profile of the GMO has been well characterised in previous studies no further monitoring for the GMO will be conducted.

I. Information on post-release and waste treatment

1. Post-release treatment of the site
All surfaces will be disinfected according to documented local procedures using locally approved disinfectants.

2. Post-release treatment of the GMOs
All waste material that has been in contact with the GMO is collected, stored in a tightly closed unbreakable container and finally destroyed.

3. (a) Type and amount of waste generated

For each administration the following waste materials are generated

- primary packaging components (vial, stopper, residual volume of the GMO in the empty vial)
- disposable materials used for reconstitution and administration (plastic beaker, plastic lid, vial connector, syringe, tubings, plastic spoon, secondary plastic container)
- personal protective equipment (such as disposable protective gowns, gloves, masks, goggles).

3. (b) Treatment of waste
All waste materials will be destroyed in accordance with local and national regulations applicable to the GMO.

J. Information on emergency response plans

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

In case of contamination (such as skin or eye contamination) the personnel involved in the preparation, packaging or product management will notify the responsible person/entity. A written procedure for emergency measures in case of accidently spillage of the GMO is available at each study site. All persons involved in the study will be trained on the procedures to act in case of accidental release.

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

Cleaning with biocide solution or wipes for spills.

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

Not applicable

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

Patients included in the clinical trial will be monitored for the occurrence of adverse events and serious adverse events (SAE) according to the clinical protocol. Each SAE will be recorded and assessed by the hospital staff and the study sponsor, and Health Authorities will

be notified when applicable. Adverse events will be registered and reported according to detailed procedures in the clinical study protocol.
In particular, treatment of patients with antibiotics to sanitize the gut ecosystem may be considered.
Due to the extensive procedural controls in place for the transport, storage, administration, disposal and monitoring of the administration of the GMO, the risk of an accidental environmental release, or a resulting undesirable effect from such an accidental release, is considered negligible.