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

A. General information

1. Details of notification

(a)	Member State of notification	Germany
(b)	Notification number	B/DE/12/PEI1713
(c)	Date of acknowledgement of notification	07.09.2012
(d)	Title of the project	

- **"A Phase I-II Gene Therapy trial for X-CGD with a SIN gamma retroviral vector"**

(e)	Proposed period of release	From 2013 until 2015

2. Notifier

Name of institution or company:

Dept of Internal Medicine II
Hematology/Oncology
Medical School
JW Goethe University
Theodor-Stern-Kai 7
60596 Frankfurt
Germany

3. GMO characterisation

(a) Indicate whether the GMO is a:

viroid		(.)
RNA virus		(.)
DNA virus		(.)
bacterium		(.)
fungus		(.)
animal		
-	mammals	(.)*
-	insect	(.)
-	fish	(.)
-	other animal	(.)

specify phylum, class

* **The GMO consists of gene modified primary human CD34+ cells.**

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

- **The GMO consists of human CD34+ cells from an X-CGD patient. The cells are genetically modified with a replication-defective retroviral vector. The cells will be used only for therapeutic purposes in the same patient from whom the cells were obtained (autologous application). Homo sapiens.**

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

- **After infection, the vector sequences remain integrated and genetically stable as provirus in the genome of the transduced cell.**

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 GMOs (gene modified CD34+ cells) are intended for a single application. After the genetic modification with a replication defective retroviral vector (SINfes.gp91s) the GMOs are directly applied intravenously into the patient. There is no further *in vitro* expansion of the GMOs nor will the GMOs kept frozen. The vector expressing the therapeutic protein gp91^{phox} lacks viral enhancer and promoter elements within the long terminal repeats (LTR) and consequently cannot be mobilized by endogenous retroviruses due to the absence of vector transcripts containing packaging signals.
There are no specific data concerning potential environmental impact of CD34+ cells transduced with SINfes.gp91s. However, gene modified CD34+ cells can only survive *in vitro* under special cell culture conditions in a CO_2 incubator at 37°C. Outside of the incubator or the individual patient´s body the GMOs are

not viable. Thus the environmental risk conferred by inappropriate disposal of waste or unused product or the accidental dissemination during product handling is considered to be negligible. Also an excretion of live product or its progeny ("shedding") by the patient is not possible. Vector sequences cannot be mobilized.
Donation of blood or an organ is prohibited for X-CGD patients after gene therapy.

B. **Information relating to the recipient or parental organism from which the GMO is derived**

The GMO is derived from the peripheral blood of humans. The recipient organism is the patient's autologous CD34+ hematopoietic stem cells obtained after apheresis from peripheral blood. The CD34+ cells are to be transduced *ex vivo* with the retroviral vector encoding the codon-optimized cDNA for the human gp91^{phox} encoding gene. The transduced CD34+ cells constitute the final GMO to be administered by intravenous infusion into patients conditioned by a cyto-reductive chemotherapy treatment.

1. Recipient or parental organism characterization:

(a) Indicate whether the recipient or parental organism is a:

(select one only)

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

other, specify **<u>Homo sapiens</u> (human primary cells)**

2. Name

(i)	order and/or higher taxon (for animals)	Primates
(ii)	genus	Homo
(iii)	species	Homo sapiens
(iv)	subspecies	…
(v)	strain	…
(vi)	pathovar (biotype, ecotype, race, etc.)	…
(vii)	common name	…

3. Geographical distribution of the organism

Not applicable: cells are derived from individual patients only.

(a) Indigenous to, or otherwise established in, the country where the notification is made:
Yes (.) No (.) 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 .

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

(c) Is it frequently used in the country where the notification is made?
Yes (.) No (.)

(d) Is it frequently kept in the country where the notification is made?
Yes (.) No (.)

4. Natural habitat of the organism

Inside the individual patient´s body

(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

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

5. (a) Detection techniques
- Not applicable

(b) Identification techniques
CD34+ cells are harvested from the peripheral blood of the patients and isolated by a magnetic separation procedure. Cells are identified by FACS (fluorescence assisted cell sorting) using commercially available CD34+ specific antibodies.

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

- **Not applicable.**

(a) Generation time in natural ecosystems:

…

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

…

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

(c) Factors affecting reproduction:

…

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:
…

10. (a) Ways of dissemination
…

(b) Factors affecting dissemination
…

11. Previous genetic modifications of the recipient or parental organism already notified for release in the country where the notification is made (give notification numbers)
None

C. **Information relating to the genetic modification**

1. Type of the genetic modification

(i)	insertion of genetic material	(X)
(ii)	deletion of genetic material	(.)
(iii)	base substitution	(.)
(iv)	cell fusion	(.)
(v)	others, specify …	

2. Intended outcome of the genetic modification
Expression of a functional gp91^{phox} protein in hematopoietic cells obtained from patients with functional defects in gp91^{phox}. The genetic modified cells will be reinfused into the patients from whom the cells were isolated for therapeutic purposes. The gene modified cells will persist in the recipient for a limited time or life-long, depending on their engraftment capacity.

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

(b) Identity of the vector

SINfes.gp91s is a MLV-based self-inactivating gammaretroviral vector (cf. Moreno-Carranza et al., Gene Therapy 2009, 16:111-118).

(c) Host range of the vector

SINfes.gp91s is pseudotyped using a GALV (gippon ape leukemia virus) envelope, which mediates the transduction of human cells.

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

Yes (**X**) No (.)

antibiotic resistance: (.)

- **other, specify:**
  The GMO can be identified by the expression of the therapeutic protein $Gp91^{phox}$. Expression of $Gp91^{phox}$ can be measured by FACS analysis using monoclonal antibodies against the $gp91^{phox}$ protein, which is associated with the membrane of genetically modified cells.

Indication of which antibiotic resistance gene is inserted

- **None**

(e) Constituent fragments of the vector

- **Self-inactivating retroviral vector including an expression cassette for the expression of the therapeutic transgene $gp91^{phox}$.**

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

(i)	transformation	(.)
(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?

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

6. Composition of the insert

(a) Composition of the insert

SIN-LTRs: Long terminal repeats without Enhancer/Promoter-sequences derived from Spleen Focus Forming Virus (SFFV). The LTRs are necessary to integrate the therapeutic gene into the genome of the target cell.

PolyA addition site: transcriptional termination sequence within the LTR at the boundary between R and U5 region (Kraunus et al., Gene Therapy, 2004, 11:1568-1578).

Leader Sequence: derived from Murine Embryonic Stem Cell Virus (MESV) vector. The leader region contains the Psi sequence, which acts as a signal sequence and is necessary for packaging RNA with the therapeutic gene into virions.

c-fes promoter: 507 bp fragment from promoter region of the human *c-FES* gene for expression of the transgene (NM_002005; Heydemann et al., Blood 2000, 96:3040-3048).

Gp91s: codon-optimized, synthetic $gp91^{phox}$ cDNA sequence. The transgene is the entire human CYBB cDNA (GenBank reference NM_000397) encoding full length $GP91^{phox}$ protein (570 AA). The clinical vector will contain the transgene encoded by the ORF from start to end of translation (1506 nt) as this produces the expected full-length therapeutic protein which has a molecular weight of about 65 KD by SDS-PAGE analysis.

WPRE: woodchuck hepatitis virus posttranscriptional regulatory element
A 583 bp safety optimized DNA sequence to enhance transgene expression. Promoter and X-protein coding region have been mutated as well as all ATG sequences, to suppress the initiation of peptide sequences longer than 25 amino acids without compromising transgene expression levels (Schambach et al., Gene Therapy 2006, 13:641-645).

(b) Source of each constituent part of the insert
- See above

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

(d) Location of the insert in the host organism

This retroviral vector leads to the integration of a 3.8 kb proviral sequence into the cellular genome.

- on a free plasmid (.)
- integrated in the chromosome (**X**)

- other, specify …

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

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

The insert contains sequences derived from the murine leukemia virus (vector backbone), the Woodchuck Hepatitis Virus (WPRE) as well as sequences derived from the human genome (c-fes promoter). The $gp91^{phox}$ sequence is a synthetic, codon-optimized copy of the human $gp91^{phox}$ cDNA.

I. Sequences from Moloney Murine Leukemia Virus

1. Indicate whether it is a:

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

2. Complete name

(i)	order and/or higher taxon (for animals)	…
(ii)	family name for plants	Retroviridae
(iii)	genus	Gammaretrovirus
(iv)	species	Murine Leukemia Virus
(v)	subspecies	…
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	…

3. Is the organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?
Yes (**X**) No (.) Not known (.)
If yes, specify the following:

(b) to which of the following organisms:

humans (.)
animals (**X**)
plants (.)
other ..

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

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

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

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

Yes (.) No (**X**)

If yes, specify

- **Please note: The donor organism is a replication defective retrovirus vector. The vector cannot be propagated in nature, nor can survive outside the laboratory environment**.

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

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

II. Sequence from Woodchuck Hepatitis Virus

1. Indicate whether it is a:

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

other, specify

2. Complete name

(j) order and/or higher taxon (for animals) …
(ii) family name for plants Hepadnaviridae
(iii) genus Orthohepadnavirus
(iv) species Woodchuck Hepatitis Virus
(v) subspecies …

(vi) strain …
(vii) cultivar/breeding line …
(viii) pathovar …
(ix) common name …

3. Is the organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?
Yes (**X**) No (.) Not known (.)
If yes, specify the following:

(c) to which of the following organisms:

humans (.)
animals (**X**)
plants (.)
other ..

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

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

- **Please note: The sequence is derived from the Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element (WPRE) which is a DNA sequence that, when transcribed creates a tertiary structure enhancing expression. It is commonly used to increase expression of genes delivered by viral vectors. However the sequence used for the present vector is devoid of any open reading frame longer than 25 amino acids and lacks promoter activity or transcriptional enhancer structures.**

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

- **The GMO consists of genetically modified CD34+ cells. No influence on cell survival is expected from the vector or the insert contained in the vector. The only phenotypic modification is the expression of the transgene by the corrected hematopoietic cell.**

(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

- **The GMO consists of genetically modified CD34+ cells. No influence on reproduction is expected from the vector or the insert contained in the vector**

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

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

Specify

- **The GMO consists of genetically modified CD34+ cells. No influence on dissemination is expected from the vector or the insert contained in the vector.**

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

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

Specify

- **The GMO consists of genetic modified CD34+ cells. The genetic modification of hematopoietic stem cells with this vector will not result in any changes in the pathogenicity of the genetic modified cells for the environment, as determined by the experimental assays currently available.**

2. Genetic stability of the genetically modified organism
   - **After integration of the SIN vector the gene modified CD34+ cells are genetically stable.**

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)

- **Gene modified CD34+ cells can only survive *ex vivo* under special cell culture conditions in a CO_2 incubator at 37°C. Outside of the incubator the GMOs are**

not viable. Thus the environmental risk conferred by inappropriate disposal of waste or unused product or the accidental dissemination during product handling is considered to be negligible. Also an excretion of live product or its progeny ("shedding") by the patient is not possible as the vector has a SIN configuration. Donation of blood or an organ is prohibited for X-CGD patients after gene therapy.

4. Description of identification and detection methods

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

- **To identify proviral genomes in transduced cells genomic DNA is extracted and submitted to several PCR amplifications using oligonucleotide sets specific of the proviral vector sequence. Confirmation of the vector sequence identity is given according to the fidelity of amplicon sizes followed by sequencing.**

(b) Techniques used to identify the GMO

- **A specific quantitative PCR method for detection of gene modified cells is established. Primers used in the qPCR are specific for the cDNA encoded by the SINfes.gp91 vector.**

F. Information relating to the release

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

- **Purpose of the release is a therapeutic application of the GMO for patients with X-linked chronic granulomatous disease in the context of a gene therapy clinical application. Thus patients will receive their own CD34+ hematopoietic stem and progenitor cells. The only difference between this protocol and conventional hematopoietic stem cell transplantation is that the cells are genetically modified for therapeutic purposes.**
- **The medicinal product is patient-specific and corresponds to autologous CD34+ cells transduced *ex vivo* with the SINfes.gp91s retroviral vector containing the human gp91^{phox} gene in final formulation and container closure system, ready for intended medical use. The autologous CD34+ cells transduced ex vivo with the SINfes.gp91s vector containing the human gp91^{phox} gene in autologous serum will be transferred into a transfer bag before being infused into the patient.**

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):

- **Isolation room at the bone marrow transplantation unit, Department of Internal Medicine II, University Medical School Frankfurt, Germany.**

(b) Size of the site (m^2):
(i) actual release site (m^2): **hospital room of less than 30 m^2**
(ii) wider release site (m^2):

(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 GMO, gene modified CD34+ cells, are intended for a single application in the patients from whom the cells were isolated. For a therapeutic effect more than 2 x 10^6 CD34+ cells per kg body weight have to be applied. From these about 20-40% are genetically modified, the rest of the material is not modified.**

(b) Duration of the operation:
- **The infusion of cells for transplantation usually lasts 30 - 45 minutes.**

(c) Methods and procedures to avoid and/or minimise the spread of the GMOs beyond the site of the release
- **The GMO will be prepared at the EUFETS GmbH in Idar-Oberstein and transported to Frankfurt by a dedicated carrier in a sealed S1 container. The actual release will occur within a closed area (the isolation room at the bone marrow transplantation unit of the University Hospital Frankfurt) with restricted personnel access. The staff involved in the release of the GMO has wide experience in stem cell transplantation and will follow Good Clinical Practice rules as in any stem cell transplantation routine.**
The GMO is only intended for clinical use and will be administrated immediately after preparation by experienced personnel. Personnel responsible for the administration of the GMO will wear disposable gloves and masks. Standard rules for spraying, wiping, gowning up and entry of personnel/goods into the room are followed. Remaining waste will be transported in sealed containers labeled with appropriate stickers to the Georg-Speyer-Haus for inactivation.

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.

- **No data is available for this particular GMO. However the GMO cannot survive outside of the laboratory space.**

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)	Primates
(ii)	family name for plants	Hominidae
(iii)	genus	Homo
(iv)	species	Homo sapiens
(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 was obtained from the target organism (human patient) and after genetic modification will be reintroduced into the same target organism. The purpose of this is to provide a therapeutic advantage to patients suffering from CGD. The GMO will reconstitute hematopoiesis in the patients after homing and engrafting in the bone marrow of patients. The GMO will then contribute to the generation of all hematopoietic lineages including granulocytes which now will have the ability to protect the patients from severe and life-threatening infections. Thus, the reconstitution of function in cells derived from the GMO should reconstitute immune responses towards infections in treated patients.**

3. Any other potentially significant interactions with other organisms in the environment
   - **No.**

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

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

   Give details
   - **The GMO consists of genetic modified CD34+ cell. No increased competitiveness or increased invasiveness is expected from the vector, the genetic modification or the insert contained in the vector.**

5. Types of ecosystems to which the GMO could be disseminated from the site of release and in which it could become established
   - **None.**

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
   - **Not applicable**

7. Likelihood of genetic exchange in vivo

(a) from the GMO to other organisms in the release ecosystem:
- **Not applicable**

(b) from other organisms to the GMO:
- **Not applicable**

(c) likely consequences of gene transfer:
- **Not applicable**

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)
- **Not applicable**

H. Information relating to monitoring

1. Methods for monitoring the GMOs
- **The GMO will be applied into patients suffering from X-CGD. No other application is intended. Thus the monitoring of the GMO will occur in the patients at regular intervals as defined in the treatment protocol. Essentially PCR and FACS analysis will be used to detect the presence of the GMO in the patient organism.**
  The follow-up of the patient during a period of 3 to 6 weeks after transplantation in confined environment would allow detecting any problem and adapting the control measures of such situation, should it arise. In the absence of RCR, there is no possibility of propagation within the environment.

2. Methods for monitoring ecosystem effects
- **As there is no risk for the ecosystem outside the patient's body there is no need to assess for ecosystem effects.**

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms
- **No transfer of genetic material to other organisms is expected. Not applicable**

4. Size of the monitoring area (m^2)
- **30 m^2. Only applicable during infusion of the GMO to the patient.**

5. Duration of the monitoring
- **2013-2020**

6. Frequency of the monitoring
- **In the first 6 months once per month. Thereafter every 3 months for 5 years.**

I. Information on post-release and waste treatment

1. Post-release treatment of the site
   - **The site will be cleaned with a decontaminating solution containing 0.5% incidine plus, according to local directives ruling the cleaning and decontamination of isolation rooms at the University Hospital Frankfurt.**

2. Post-release treatment of the GMOs
   - **GMO containing wastes are collected in special containers labeled as "S1 waste" and will be autoclaved at the Georg-Speyer-Haus. Transportation of waste will be performed in special, sealed containers or bags identified with stickers indicating "S1 waste"**

3. (a) Type and amount of waste generated
   - **Emptied bags, tubing and other solids. Sharps (needles etc.) will be stored in different specific containers appropriately labeled. The estimated total amount of waste is expected to be minimal (less than 1000 ml).**

3. (b) Treatment of waste
   - **Waste will be collected by trained staff in containers labeled "S1 waste" Waste will be transported to the Georg-Speyer-Haus for inactivation by autoclaving.**

J. Information on emergency response plans

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread
   - **The GMO will be released in an isolation ward at the bone marrow transplantation unit of the University Hospital Frankfurt. Thus any accidental spread will be limited to this space (30 m^2) or the personnel involved in the production or administration of the GMO. The GMO is fragile and cannot survive outside the body of the patient therefore limiting the risk of dissemination. Accidental stick injury with a needle contaminated with the GMO will not lead to a spread of the GMO as the immune system of the affected person will destroy the GMO as soon as this comes in contact with human serum. All of the people involved in the trial will be trained about the procedures and measures to be taken in case of accidental release.**

2. Methods for removal of the GMO(s) of the areas potentially affected
   - **The site will be cleaned with a decontaminating solution containing 0.5% incidine plus, according to the directives ruling the cleaning and decontamination of isolation rooms at the University Hospital Frankfurt**

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

- **The application will take place in the isolation ward for bone marrow transplantation. Here only experienced and specifically trained personnel are employed. Also weekly meetings between physicians and caretakers for discussion of organizational issues and training are held. Within these conferences the personnel will be trained about the GMO and specific issues concerning patients treated with gene-modified cells. In brief, staff handling the drug product will wear area-specific clothing (for the BMT-ward), gloves and a surgical mask. Standard rules for spraying, wiping, gowning up and entry of personnel/goods into the room are followed.**

  Due to the administration modalities the risk of accidental release is considered negligible.

K. Literature

Anliker, B., Longhurst, S., Buchholz, C.J. (2010). Environmental risk assessment for medicinal products containing genetically modified organisms. Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz *53*(1), 52-57.

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