Part 1

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 The Netherlands
(b) Notification Number B/NL/08/001
(c) Date of acknowledgement of notification April 17th, 2008
(d) Title of the project A randomized double-blind placebo-controlled parallel group study of the efficacy and safety of 4 administrations of XRP0038/NV1FGF 4mg at 2-weeks intervals on amputation or any death in Critical Limb Ischemia patients with skin lesions
(e) Proposed period of release Global study-start and end dates: 10 December 2007. End date when 490 patients are reached. Netherlands start and end dates: May 2008 and 30th December 2011.

2 Notifier

Name of institution or company Erasmus MC

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
Other, specify (kingdom, hylum and class			recombinant DNA plasmid

(b) Identity of the GMO (genus and species) XRP0038 / NV1FGF is a recombinant DNA plasmid, constructed by inserting a gene encoding fibroblast growth factor type 1 (FGF1) into a pCOR plasmid backbone.

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

Production process is not part of the permit., but tests on the genetic stability of the production banks were performed on the parental End of Production Cells and show that the sequence of the produced plasmid did not change.

4 Is the same GMO release planed elsewhere in the Community (in conformity with Article 6(1)), b the same notifier?

Yes ☒	No ☐
If yes, insert the country code(s): AU, BE, CZ, DK, FI, FR, DE, EL, HU, IT, NL, PL, ES, SE, UK.	

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	

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

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

IM administration was shown in animals and CLI subjects to induce FGF1 expression in a restricted area of the injected muscle only, not in distant organs.

Preclinical data indicate that IM administration of NV1FGF leads to a transient tissue distribution of NV1FGF sequences, mostly product of plasmid degradation with no capability to express FGF1 transgene. Indeed, NV1FGF –derived mRNA was restricted to the injected muscle only. NV1FGF is rapidly degraded when present in blood: the half-life of NV1FGF sequences in blood was shown to be 3.00 min and 6.00 min in rats and CLI patients, respectively. Clinical data indicate no dissemination of quantifiable levels of NV1FGF sequences in the urine samples collected at various time points post administration.

NV1FGF is rapidly degraded after IM administration when present in blood. Even though NV1FGF plasmid would be disseminated in its intact sequence, it could not be replicated in a bacteria present in subjects and in the environment. NV1FGF is a pCOR plasmid carrying the sp.FGF1 expression cassette. pCOR are small size plasmids carrying a Conditional Origin of Replication (R6K) which requires a trans-acting replication factor (π) meaning that pCOR can only replicate in a specifically engineered E. coli host strain producing π, greatly reducing the potential for propagation in treated patients and in the environment; There is no significant possibility that NV1FGF will spread from the patient to other persons or to the environment. It is a product classified as biosafety level I. Institutional biosafety policies and procedures will be followed.

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

1 recipient or parental organism characterisation:

(a) Indicate whether the recipient or parental organism is a :		
viroid		☐
RNA virus		☐
bacterium		☒
fungus		☐
animal		☐
- mammals	☐	
- insect	☐	
- fish	☐	
- other animal	☐	(specify phylum, class)
other, specify		The parental organism from which the GMO is derived is a specifically engineered host: E. Coli XAC-1 pir-116

2 Name

(i) Order and/or higher taxon (for animals)
(ii) genus
(iii) species
(iv) subspecies
(v) strain Escherichia Coli
(vi) pathovar (biotype, ecotype, race,etc.)
(vii) common name Escherichia Coli

3 Geographical distribution of the organism

(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 ☐
Mediterranean ☐
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

(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 in laboratory (specifically engineered host E. Coli XAC-1 pir-116).

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

5(a) Detection techniques

The drug substance is tested for appearance, identity, purity, potency quantity and general safety tests. Each release test has defined acceptance criteria. All release tests should be compliant to allow DS batch release.

For identity DNA plasmid size is measured using agarose gel electrophoresis before and after digestion with site specific endonucleases.

For identity, product related impurities as well as process related impurities are measured. The percentage of multimeric forms of plasmid is measured using capillary electrophoresis, the percentage of supercoiled and depurinated forms using ion exchange HPLC. Remaining

contaminants from the host are tested: host cell proteins using a specific ELISA assay, host cell DNA using Q-PCR and host cell RNA using Q-RT-PCR. The impurities from the process are tested using ion exchange HPLC: the oligonucleotide (leachable from the resin of chromatographic column) and ammonium sulfate (last buffer used before final diafiltration).

The dose is measured using ion exchange HPLC.

For potency a specific in vitro bioassay is used to compare each batch to a reference standard.

As this drug substance is dedicated to manufacture an injectable, bioburden, endotoxine level and pH are also measured.

The drug product is tested for appearance, purity, potency, identity, quantity and general safety tests. Each release test has defined acceptance criteria. All release tests should be compliant to allow DP batch release.

For purity the percentage of supercoiled and depurinated forms are measured using ion exchange HPLC. The potency and the identity are controled using a specific bioassay and the dose using HPLC.

As this drug product is an injectable product, the following standard tests are also performed: appearance, sterility assessment and measurement of endotoxin level, pH, and extractable volume.

5(b) Identification techniques

See 5a.

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

Yes ☐	No ☒
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 ☒	Not Know ☐
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:

Technically there is no "natural" ecosystem: The pCOR plasmid is produced using the E. coli strain XAC-1pir116, a derivative of XAC-1. A specific E. coli host, XAC-1 pir-116, was engineered supporting plasmid replication and selection. Its genome contains: i) a copy-up mutant of pir (gene encoding π), pir-116 and ii) the argEam gene for plasmid selection. The chromosomal argE and lacZ genes of XAC-1pir116 contain amber mutations that lead to truncated and inactive proteins. The argE gene encodes an N-acetylornithinase enzyme essential for arginine biosynthesis and the amber–mutation in this gene results in the bacteria being unable to grow in minimal medium. The amber mutation in the lacZ gene that encodes the β-galactosidase enzyme allows for the visual selection of plasmid containing bacteria.
The suppressor tRNA sup Phe gene encoded by the pCOR plasmid allows the correct translation of both the N-acetylornithinase and β-galactosidase enzymes. Therefore only XAC-1pir116 bacteria containing the pCOR plasmid can grow on minimal medium allowing for the selection of the pCOR containing bacteria.

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

(c) way of reproduction: Sexual ☐ Asexual ☒

(d) factors affecting reproduction: see above

9 Survivability

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

(i)	endospores	☐
(ii)	cysts	☐
(iii)	sclerotia	☐
(iv)	asexual spores (fungi)	☐
(v)	sexual spores (fungi)	☐
(vi)	eggs	☐
(vii)	pupae	☐
(viii)	larvae	☐
(ix)	other, specify Not applicable	

(b) relevant factors affecting survivability

The argE gene encodes an N-acetylornithinase enzyme essential for arginine biosynthesis and the amber –mutation in this gene results in the bacteria being unable to grow in minimal medium.

10(a) ways of dissemination

See 8(a)

10(b) Factors affecting dissemination

See 8(a)

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

see 8(a)

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) other, specify recombinant DNA plasmid, constructed by inserting a gene encoding fibroblast growth factor type 1 (FGF1) into a pCOR plasmid backbone.

2 Intended outcome of the genetic modification

The purpose of NV1FGF treatment is to provide therapeutic angiogenesis via intra muscular injection of a non-viral vector, allowing subsequent local expression of FGF1 for the treatment of Critical Limb Ischemia (CLI). This investigational medicinal product will be used in a Phase 3 multinational Clinical Study to confirm the positive results of a phase II clinical trial performed in CLI patients.

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

Yes ☒	No ☐

If no, go straight to question 5.

3(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 ☐
transposable element ☐

other, specify

(b) Identity of the vector pCOR plasmid

(c) Host range of the vector specifically engineered E.Coli XAC-1 pir-16

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

Yes ☒ No ☐

Antibiotic resistance ☐
Other, specify
Indication of which antibiotic resistance gene is inserted

(e) constituents fragments of the vector

pCOR plasmids are very narrow-host range plasmid vectors for non viral gene therapy and DNA vaccination. The pCOR conditional origin of replication does not come from widely-used ColE1- derived plasmids, but from another natural E. coli plasmid, R6K. The pCOR backbone is significantly smaller than standard ColE1-derived plasmids. It contains three key elements: the R6K γconditional origin of replication (ori γ), which requires the R6K π initiator protein to be functional; a selectable tRNA suppressor gene, and a cer (ColE1 resolution) fragment to resolve pCOR oligomers. Such plasmids can only replicate in π-producing enterobacteria, considerably limiting their host range. pCOR selection requires expression of a synthetic amber suppressor tRNA gene specific for phenylalanine (sup Phe). This corrects an E. coli argE amber mutation, making it possible for the recombinant host strain to grow on a minimal medium lacking arginine on which bacteria without the plasmid cannot grow. We engineered a specific E. coli host, (XAC- 1 pir-116), supporting pCOR replication and selection. Its genome contains: i) a copy-up mutant of pir (gene encoding π), pir-116 and ii) the argEam gene for plasmid selection.

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

(i) transformation ☒
(ii) electroporation ☐
(iii) macroinjection ☐
(iv) microinjection ☐
(v) infection ☐

(vi) other, specify

5 If the answer to question B3 (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 construction strategy used to create the plasmid pXL3179 containing sp.FGF-1 expression cassette led to the following features. sp.FGF-1 is a fusion gene between sequences encoding the secretion signal peptide (sp) from human fibroblast interferon β and the naturally occurring truncated form of human FGF-1 from amino acids 21 to 154. Expression of sp.FGF-1 is driven by the human cytomegalovirus (CMV) immediate early enhancer / promoter module. The late polyadenylation signal from Simian Virus 40 is inserted downstream of sp.FGF-1 to ensure proper and efficient transcription termination and subsequent polyadenylation of sp.FGF-1 transcript. The sp.FGF-1 expression cassette is inserted into a pCOR backbone creating a closed circular plasmid.

(b) source of each constituents part of the insert

Plasmid features are presented in Jouanneau et al., 1991. It is a pBR322-derived backbone and carries the sp.FGF1 gene under the control of the SV40 early promoter and the CMV (cytomegalovirus) enhancer with downstream SV40 regulatory sequences. sp.FGF1 corresponds to heterologous human fibroblast interferon signal peptide (Taniguchi et al., 1980) fused to truncated human FGF1 (FGF1 21-154) (Jaye et al., 1988).

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

See 6(a)

(d) Location of the insert in the host organism

- on a free plasmid ☒
- Integrated in the chromosome ☐
- other, specify

(e) Does the insert contain parts whose product or function are not known?

Yes ☐ No ☒

If yes, specify

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

1 Indicate whether it is a:

viroid		☐
RNA virus		☐
bacterium		☐
fungus		☐
animal		☐
	- mammals	☐
	- insect	☐
	- fish	☐
	- other animal	☐ (specify phylum, class)
other, specify		See C.6(b)

2 Complete name

(i) Order and/or higher taxon (for animals) Homo sapiens sapiens
(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 significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?

Yes ☐	No ☒	Not Known ☐
If yes: (a) 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 IIIA, point II(A) (11) (d):		

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

Yes ☐	No ☒
If yes, specify	

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

Yes ☐	No ☒	Not Know ☐

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 result of the genetic modification

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

Yes ☒ No ☐ Not known ☐

Specify survivability does not apply to DNA plasmid

(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 ☐

pCOR are small size plasmids carrying a Conditional Origin of Replication (R6K) which requires a trans-acting replication factor (π) meaning that pCOR can only replicate in a specifically engineered E. coli host strain producing π. Sanofi-aventis engineered a specific E.

coli host (XAC-1 pir-116), supporting pCOR replication and selection.

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

Yes ☒ No ☐ Not known ☐

NV1FGF is a non conjugative, non infectious plasmid that can only replicate in specific bacterial strain (limited to E. Coli and parented) that were in addition specifically designed in laboratory to express the replication factor π of R6K. The accidental uptake of this plasmid by E. Coli family bacteria would not allowed plasmid dissemination. No replication outside a bacterial host is possible. There is no significant possibility that NV1FGF will spread from the patient to other persons or to the environment.

E.coli XAC-1pir116 strain is used to produce the therapeutic plasmid. Due to the contained facilities, the genetic material of the strain cannot be exchanged with any other genetic material from other organisms.

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

Yes ☐ No ☒ Not known ☐

Specify None of the element is pathogene

2 Genetic stability of the genetically modified organism

Production process is not part of the permit., but tests on the genetic stability of the production banks were performed on the parental End of Production Cells and show that the sequence of the produced plasmid did not change.

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

Yes ☐	No ☒	Unknown ☐

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) and II(C)(2)(i)

4 Description of identification and detection methods

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

Not Applicable- IM administration was shown in animals and CLI subjects to induce FGF1 expression in a restricted are of the injected muscle only, not in distant organs.

Preclinical data indicate that IM administration of NV1FGF leads to a transient tissue distribution of NV1FGF sequences, mostly product of plasmid degradation with no capability to express FGF1 transgene. Indeed, NV1FGF –derived mRNA was restricted to the injected muscle only. NV1FGF is rapidly degraded when present in blood: the half-life of NV1FGF sequences in blood was shown to be 3.00 min and 6.00 min in rats and CLI patients, respectively. Clinical data indicate no dissemination of quantifiable levels of NV1FGF sequences in the urine samples collected at various time points post administration.

Based on these data there is no evidence that support an environmental risk. NV1FGF is rapidly degraded after IM administration when present in blood. Even though NV1FGF plasmid would be disseminated in its intact sequence, it could not be replicated in a bacteria present in subjects and in the environment.

(b) techniques used to identify the GMO

Not applicable, based on previous results, see above.

F Information relating to the release

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

XRP0038 / NV1FGF is a recombinant DNA plasmid, constructed by inserting a gene encoding fibroblast growth factor type 1 (FGF1) into a pCOR plasmid backbone. The purpose of NV1FGF treatment is to provide therapeutic angiogenesis via intra muscular injection of a non-viral vector, allowing subsequent local expression of FGF1 for the treatment of Critical Limb Ischemia (CLI). This investigational medicinal product will be used in a Phase 3 multinational Clinical Study to confirm the positive results of a phase II clinical trial performed in CLI 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 ☒

If yes, specify Not Applicable

3 Information concerning the release and the surrounding area

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

Hospital sites

(b) Size of the site (m2): Not applicable
(i) actual release site (m2):
(ii) wider release area (m2):

(c) Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs), which could be affected: Not applicable

(d) 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:

XRP0038/NV1FGF 4mg will be administrated at 2-week intervals for a total of 4 administrations (16mg). At each administration of XRP0038/NV1FGF, 8 intra muscular injections of 0.5mg (in 2.5ml) each will be performed in the leg to be treated. 490 patients are planned to be enrolled worldwide in the study.

(b) Duration of the operation

The treatment period for each patient is 6 weeks. Global study-start and end dates: 10 December 2007. End date when 490 patients are reached.

Netherlands start and end dates: May 2008 and 30th December 2011.

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

NV1FGF is a non conjugative, non infectious plasmid that can only replicate in specific bacterial strain (limited to E. Coli and parented) that were in addition specifically designed in laboratory to express the replication factor π of R6K. The accidental uptake of this plasmid by E. Coli family bacteria would not allowed plasmid dissemination. No replication outside a bacterial host is possible. There is no significant possibility that NV1FGF will spread from the patient to other persons or to the environment.

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

Clinical Study in biosafety level 1 environment

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

Expression of FGF-1 protein or/and FGF-1 mRNAs in the injected muscle was demonstrated after NV1FGF I.M. administration in various animal species (mice, rats and dogs) and in CLI patients. Expression of NV1FGF-derived FGF-1 protein was detected by immunohistochemistry in the myofibers of the injected muscle only. Expression of NV1FGF-derived mRNA was shown to be local and restricted to a few cm around the injection site as shown in animals and CLI patients.

Our studies conducted in rats with NV1FGF show that IM NV1FGF leads to transient dissemination of NV1FGF sequences into blood and distal organs. The NV1FGF sequences were mostly products of plasmid degradation with no capability to express FGF1 protein. There was no evidence of NV1FGF-derived mRNA expression in the assessed organs suggesting that the transient distribution of NV1FGF sequences did not led to expression in organs other than the injected muscle.

Identically, Pharmacokinetics of NV1FGF was determined in patients in 4 (Phase 1 or 2) clinical studies. The results revealed that NV1FGF was rapidly cleared from the circulating blood at all dose regimens and at all dose levels (plasma half-life: 5 to 6 minutes). There was no evidence of accumulation in the circulating blood after repeated IM administration. There was no finding showing the presence of FGF1 protein in the circulating blood for any dose regimen or dose level. Furthermore, data from 2 clinical studies (PM202, PM211) indicate no evidence of humoral immune response to FGF1 after repeated IM administrations. Clinical data indicate no dissemination of quantifiable levels of NV1FGF sequences in the urine samples collected at various time points post administration.

Based on these data there is no evidence that support an environmental risk. NV1FGF is rapidly degraded after IM administration when present in blood. Even though NV1FGF plasmid would be disseminated in its intact sequence, it could not be replicated in a bacteria present in subjects and in the environment.

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 organisms (if applicable)

(i) order and/or higher taxon (for animals) Homo sapiens sapiens
(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)

The NV1FGF DNA plasmid has no biological activity on its own. After its transfer into the muscle cells following IM injection, NV1FGF is taken up into nuclei of muscle cells near the injection site and results in localized production and release of NV1FGF derived mRNA and FGF1 protein.

The purpose of NV1FGF treatment is to provide therapeutic angiogenesis via intra muscular injection of a non-viral vector, allowing subsequent local expression of FGF1 for the treatment of Critical Limb Ischemia (CLI).

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

Not applicable

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

Yes ☐ No ☒ 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

Not Applicable- NV1FGF is a non conjugative, non infectious plasmid that can only replicate in specific bacterial strain (limited to E. Coli and parented) that were in addition specifically designed in laboratory to express the replication factor π of R6K. The accidental uptake of this plasmid by E. Coli family bacteria would not allowed plasmid dissemination. No replication outside a bacterial host is possible. There is no significant possibility that NV1FGF will spread from the patient to other persons or to the environment.

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) cultival/breeding line
(viii) pathovar
(xi) common name

7 Likelihood of genetic exchange in vivo

(a) from the GMO to other organisms in the release ecosystem: None
(b) from other organisms to the GMO: None

(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 simulated natural environments (e.g. microcosms, etc.):

Expression of FGF-1 protein or/and FGF-1 mRNAs in the injected muscle was demonstrated after NV1FGF I.M. administration in various animal species (mice, rats and dogs) and in CLI patients. Expression of NV1FGF-derived FGF-1 protein was detected by immunohistochemistry in the myofibers of the injected muscle only. Expression of NV1FGF-derived mRNA was shown to be local and restricted to a few cm around the injection site as shown in animals and CLI patients.

Our studies conducted in rats with NV1FGF show that IM NV1FGF leads to transient dissemination of NV1FGF sequences into blood and distal organs. The NV1FGF sequences were mostly products of plasmid degradation with no capability to express FGF1 protein. There was no evidence of NV1FGF-derived mRNA expression in the assessed organs suggesting that the transient distribution of NV1FGF sequences did not led to expression in organs other than the injected muscle.

Identically, Pharmacokinetics of NV1FGF was determined in patients in 4 clinical studies. The results revealed that NV1FGF was rapidly cleared from the circulating blood at all dose regimens and at all dose levels (plasma half-life: 5 to 6 minutes). There was no evidence of accumulation in the circulating blood after repeated IM administration. There was no finding showing the presence of FGF1 protein in the circulating blood for any dose regimen or dose level. Furthermore, data from 2 clinical studies (PM202, PM211) indicate no evidence of humoral immune response to FGF1 after repeated IM administrations. These data provide evidence that NV1FGF is a local treatment, with no particular risk of exposure to functional sequences at distance of injection site.

In conclusion, these data indicated that the NV1FGF sequences transiently disseminated are mostly product of plasmid degradation with no capability to drive FGF1 expression. The transient presence of NV1FGF sequences is not associated with NV1FGF-derived mRNA expression in organs other than the injected muscle.

Based on these data there is no evidence that support an environmental risk. NV1FGF is rapidly degraded after IM administration when present in blood. Even though NV1FGF plasmid would be disseminated in its intact sequence, it could not be replicated in a bacteria present in subjects and in the environment. NV1FGF is a pCOR plasmid carrying the sp.FGF1 expression cassette. pCOR are small size plasmids carrying a Conditional Origin of Replication (R6K) which requires a trans-acting replication factor (π) meaning that pCOR can only replicate in a specific E. coli host strain producing π, greatly reducing the potential for propagation in treated patients and in the environment; There a no significant possibility that NV1FGF will spread from the patient to other persons or to the environment. It is a product classified as biosafety level I. Institutional biosafety policies and procedures will be followed.

NV1FGF is a non conjugative, non infectious plasmid that can only replicate in specific bacterial strain (limited to E. Coli and parented) that were in addition specifically designed in laboratory to express the replication factor π of R6K. The accidental uptake of this plasmid by E. Coli family bacteria would not allowed plasmid dissemination. No replication outside a bacterial host is possible. There is no significant possibility that NV1FGF will spread from the patient to other persons or to the environment.

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

As per Phase 2 trial (in EU including France): all patients will be followed during the whole duration of the study (12 months) with full clinical evaluation and laboratory monitoring.

2 Methods for monitoring ecosystem effects

Not Applicable- NV1FGF is a non conjugative, non infectious plasmid that can only replicate in specific bacterial strain (limited to E. Coli and parented) that were in addition specifically designed in laboratory to express the replication factor π of R6K. The accidental uptake of this plasmid by E. Coli family bacteria would not allowed plasmid dissemination. No replication outside a bacterial host is possible. There is no significant possibility that NV1FGF will spread from the patient to other persons or to the environment.

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

Not Applicable- NV1FGF is a non conjugative, non infectious plasmid that can only replicate in specific bacterial strain (limited to E. Coli and parented) that were in addition specifically designed in laboratory to express the replication factor π of R6K. The accidental uptake of this plasmid by E. Coli family bacteria would not allowed plasmid dissemination. No replication outside a bacterial host is possible. There is no significant possibility that NV1FGF will spread from the patient to other persons or to the environment.

4 Size of the monitoring area (m2)

Study site

5 Duration of the monitoring

Patients will be monitored for 12 months plus an additional safety follow-up (visit or phone call) at 18 months

6 Frequency of the monitoring

Patients will be monitored by full clinical evaluation and laboratory assessment.

I Information on post-release and waste treatment

1 Post-release treatment of the site

Surfaces should be disinfected with a 5% bleach solution or "DNA away" solution

2 Post-release treatment of the GMOs

NV1FGF is a product classified as biosafety level 1.

3(a) Type of amount of waste generated

XRP0038 / NV1FGF product is glass vials at hospital/site level and will be administered to the patients via Intramuscular injection. The waste on site would consist of partially used/ unused Clinical supply (including syringes/needles).

3(b) Treatment of waste

A detailed treatment log of the returned to sponsor/destroyed on site IP will be established with the investigator (or the pharmacist) and counter signed by the investigator and the monitoring team. The investigator or a designated person will not destroy the unused IP unless the sponsor provides written authorization. In case the hospital pharmacy is not able to destroy the unused IP, the sponsor will take this in charge. NV1FGF is a product classified as biosafety level I and will be destroyed as such.

J Information on emergency response plans

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

Not Applicable- NV1FGF is a non conjugative, non infectious plasmid that can only replicate in specific bacterial strain (limited to E. Coli and parented) that were in addition specifically designed in laboratory to express the replication factor π of R6K. The accidental uptake of this plasmid by E. Coli family bacteria would not allowed plasmid dissemination. No replication outside a bacterial host is possible. There is no significant possibility that NV1FGF will spread from the patient to other persons or to the environment.

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

Surfaces should be disinfected with a 5% bleach solution or "DNA away" solution

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

NV1FGF is a product classified as biosafety level I. Institutional biosafety policies and procedures will be followed. Sterile condition will be ensured as the product is injected in IM and biosafety level 1 procedure will be followed.

All patients in the study will be carefully monitored during the whole duration of the study. For the reasons exposed before, no emergency plan response is necessary, similarly to previous Phase 2 trial.