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 NL
(b) Notification number B/NL/15/013
(c) Date of acknowledgement of notification 16/12/2015
(d) Title of the project
Gene therapy Clinical development program comprising of clinical trials using SAR422459, a non-replicating, recombinant lentiviral vector derived from Equine Infectious Anemia Virus (EIAV) to express ATP Binding Cassette A4 (ABCA4) transporter and correct its defective expression or function in photoreceptors of patients with Stargardt macular dystrophy and other ABCA4 retinopathies.
(e) Proposed period of release From 1st November 2015 until 1st November 2050

2. Notifier

Name of institution or company: sanofi-aventis Groupe

3. GMO characterisation

(a) Indicate whether the GMO is a:

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

specify phylum, class …

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

Genus: Lentivirus
Species: Equine Infectious Anemia Virus (EIAV)
SAR422459, replication -defective vector based on a lentivirus, Equine Infectious Anemia Virus (EIAV), pseudotyped with the vesicular stomatitis virus glycoprotein (VSV-G: rhabdovirus) envelope

(c) Genetic stability – according to Annex IIIa, II, A(10)
Since this is an Investigational Medicinal Product, stability testing programs are in place to monitor the stability (strength, potency etc.) of the SAR422459.

SAR422459 vector is produced by the transient transfection of HEK293T cells with three plasmids: (1) the recombinant EIAV SAR422459 vector genome (pONYKABCA4, BSG474), (2) the synthetic EIAV Gag/Pol expression vector (pESGPK) and (3) the VSV-G envelope expression vector (pHGK) (cf section C.4.(e)). All genetic units encoding EIAV peptides have been removed. This is an irreversible and stable modification. The full construct is controlled by sequencing and there are no deviations in the vector construct. The GMO is not replication competent and therefore is not infectious, pathogenic, virulent or able to colonise other organisms either directly or via a vector.

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) IT

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

Yes (.) No (x)

(The same GMO has only previously been used under contained conditions in the UK, France and Belgium by another notifier.)
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)

(The same GMO has only previously been used under contained conditions in the USA.)
If yes:
- Member State of notification …
- Notification number B/../../…

7. Summary of the potential environmental impact of the release of the GMOs.
As SAR422459 is built with deletion of the majority of the viral genome and replacement with the cDNA of interest (ABCA4 gene), it renders the vector unable to replicate. Therefore

SAR422459 is unlikely to become persistent and invasive in natural habitats, or to persist for a significant length of time outside the patient (it will be affected by the temperature and cleaning procedures), or to interact with non-target organisms in the environment and induce adverse pathogenic effects due to viral replication or presence of viral particles.
In patients, transduced photoreceptors and retinal pigment epithelial cells have biological characteristics that prevent multiplication and/or spreading outside the eye of the treated patient.
Real-time RT-PCR assays will be used to monitor potential shedding of the vector in urine from treated patients. Blood samples (plasma and buffy coat) will also be analysed using real-time PCR. The total amount of the GMO present at the hospital site at any one time will be very limited. Overall the risk to the environment is considered to be negligible.

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

other, specify …

2. Name

(i)	order and/or higher taxon (for animals)	Retroviridae…
(ii)	genus	Lentiviridae…
(iii)	species	Equine Infectious Anemia Virus
(iv)	subspecies	…
(v)	strain	…
(vi)	pathovar (biotype, ecotype, race, etc.)	…
(vii)	common name	EIAV…

3. Geographical distribution of the organism

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

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

(i) Yes (x)

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

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

(ii) No (.)

(iii) Not known (.)

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

Yes (.) No (x)

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

Yes (.) No (x)

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 natural habitat of wild-type EIAV is equidae, primarily, horses but also ponies, mules, donkeys and zebras. In contrast, the GMO is an artificial laboratory produced replication defective viral vector and is therefore not considered to have a natural habitat.

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

5. (a) Detection techniques

Real-time reverse transcriptase PCR assays have been developed to detect the EIAV packaging signal RNA sequence in blood and urine samples as an indirect highly sensitive measure for the detection of SAR422459 vector particles. These are complemented by real-time PCR assays to detect the DNA form of the vector following entry to target cells (as a measure of transfer of the vector, including transgene sequences). These techniques could also be used to detect the parental organism EIAV.

ELISA and Western blot techniques are available to detect antibodies raised against EIAV (and the GMO) in *in vivo* models.

(b) Identification techniques

The techniques outlined in section 5(a) can also be identified EIAV and/or the GMO

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 (x) No ( ) Not known (.)

If yes, specify: The pathology caused by EIAV, equine infectious anemia (EIA), is limited to equidae (including horses, ponies, donkeys, mules and zebras). GMO is not replication competent and therefore is not infectious, pathogenic, virulent or able to colonise other organisms either directly or via a vector.

(a) to which of the following organisms:

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

(b) give the relevant information specified under Annex III A, point II. (A)(11)(d) of Directive 2001/18/EC

The SAR422459 vector is a replication-defective vector and as such is not considered to pose a significant risk in terms of pathogenicity (infectivity, toxicogenicity, virulence…). Whilst the wild type EIAV is pathogenic to equidae, the EIAV vector (SAR422459) is not replication competent and therefore is not infectious, pathogenic, virulent or able to colonise other organisms either directly or via a vector. Since the GMO is pseudotyped with the VSV glycoprotein it will enter a wider range of animal cells than the wild type EIAV. However, once a cell that has come in contact with the vector has been transduced, there is no potential for dissemination.

8. Information concerning reproduction

(a) Generation time in natural ecosystems:
The therapeutic lentiviral vector cannot reproduce by itself and there is no production of replicative competent virus as the vector has been designed to avoid the expression of viral genes. Tranduced photoreceptor and retinal pigment epithelial cells have biological characteristics that prevent multiplication and/or spreading outside the eye of the treated patient.

(b) Generation time in the ecosystem where the release will take place:
Not applicable

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

(c) Factors affecting reproduction:
Not applicable

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:
It is not expected that the vector will survive anywhere in the natural environment.

10. (a) Ways of dissemination
EIAV is primarily transmitted by bloodsucking insects. SAR422459 vector can only transduce animal cells and is replication-defective, and therefore dispersal will be limited to the first organism infected. The route of infection will usually be by injection, though other possible exposure routes are:
- contact with solution in opened vial
- contact with solution in syringe
- contact with material at injection site
- accidental exposure through injury (e.g. 'needle stick' injury)

However, these are considered unlikely and all personnel handling the product are required to wear appropriate personal protective equipment to minimize the risk of exposure.

(b) Factors affecting dissemination
None.

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, since no deliberate releases have occurred. However, another EIAV-based minimal lentiviral vector (UshStat), that is produced using the same manufacturing method, is currently undergoing clinical evaluation in France.

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

1. Type of the genetic modification

(i)	insertion of genetic material	(x)
(ii)	deletion of genetic material	(x)

(iii)	base substitution	(x)
(iv)	cell fusion	(.)
(v)	others, specify …	

2. Intended outcome of the genetic modification
Deletions from the original EIAV virus genome are intended to abrogate the possibility of the GMO to replicate, and to largely limit or even abolish the risk of interaction with neighboring organisms upon insertion in target cell genome.
The GMO is also intended to allow the expression in the target cell (photoreceptors of the retina) of the ABCA4 transporter in order to correct its defective expression due to the disease. An expression cassette allowing this expression is inserted in the GMO.

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 (.)

The plasmid DNA is extensively degraded by Benzonase® treatment during manufacture. This has the effect of degrading all DNA, including plasmids, into small fragments (>99% of DNA is degraded to fragments of less than 120bp) and will be present at extremely low levels.

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
The EIAV vector system is comprised of three plasmid constructs: the vector genome, the Gag/Pol packaging component and the envelope packaging component. The plasmid used, which are fully described in section C.4(e) below, are based on pUC. Only the sequences from the insert in the genome plasmid (an RNA transcript that encodes a vector genome) are present in the final product. Any remaining plasmid DNA is degraded into small fragments (>99%, less than 120bp) by Benzonase® treatment at two stages of the production process and residual DNA is largely removed by vector purification processes.

(c) Host range of the vector
Plasmid vector
E. coli and closely related species.

(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.
Functional Kanamycin resistance genes are present on the plasmids for the SAR422459 vector genome, the synthetic EIAV Gag/Pol and the VSV-G envelope. The coding sequence for the neomycin phosphotransferase II gene is present in the vector genome; however a promoter is not associated with this gene and hence expression is unlikely.

(e) Constituent fragments of the vector

The majority of the EIAV sequences have been removed (~90%) to produce a replication defective minimal vector system. The development of the SAR422459 vector from the wild type EIAV is described below and illustrated schematically in Figure 1.

Wild Type EIAV (Figure 1a) has six distinct genetic units encoding; Gag (structural polyprotein), Pol (polyprotein comprising the enzymes protease, reverse transcriptase, RNaseH, dUTPase, integrase), Env (envelope glycoprotein), tat (transcriptional transactivator specific for viral enhancers contained within the LTR), Rev (an RNA binding protein involved in RNA export to the cytoplasm), and S2 (unknown function, which may be required for disease in horses).
There are also sequences on the viral nucleic acid that are involved in viral replication and gene expression. The long terminal repeats (LTR) contain R regions (repeated sequences at the extreme ends of the RNA required for reverse transcription and which also interact with Tat and contribute to gene expression); the U3 region (region located at the 3' end of the viral RNA preceding R that contains an attachment [att] target recognition site for integration, viral enhancers and promoters); and, the U5 region (short region at the 5' end of the viral RNA just downstream of R that contains a partial polyadenylation (pA) site that only functions in the integrated DNA copy, and an att site for integration). The primer binding site (PBS) is located immediately downstream of U5, and is required to initiate the process of reverse transcription via binding to a cellular tRNA. The packaging signal (Ψ) is located from the extreme 5' end of the RNA transcript to the first 300 nucleotides of gag; it interacts with the nucleocapsid (NC) region of Gag in a process referred to as packaging. The polypurine tract (3' PPT), is a region towards the 3' end of the genome that is required for second strand DNA synthesis. The central polypurine tract (cPPT) is conserved among lentiviruses but its precise function is unknown. The Rev Response Element (RRE) is recognized by Rev protein and exports unspliced or partially spliced RNAs to the cytoplasm from the nucleus.

The EIAV vector system, shown in Figure 1b, is comprised of three plasmid constructs: the vector genome, the Gag/Pol packaging component and the envelope packaging component.

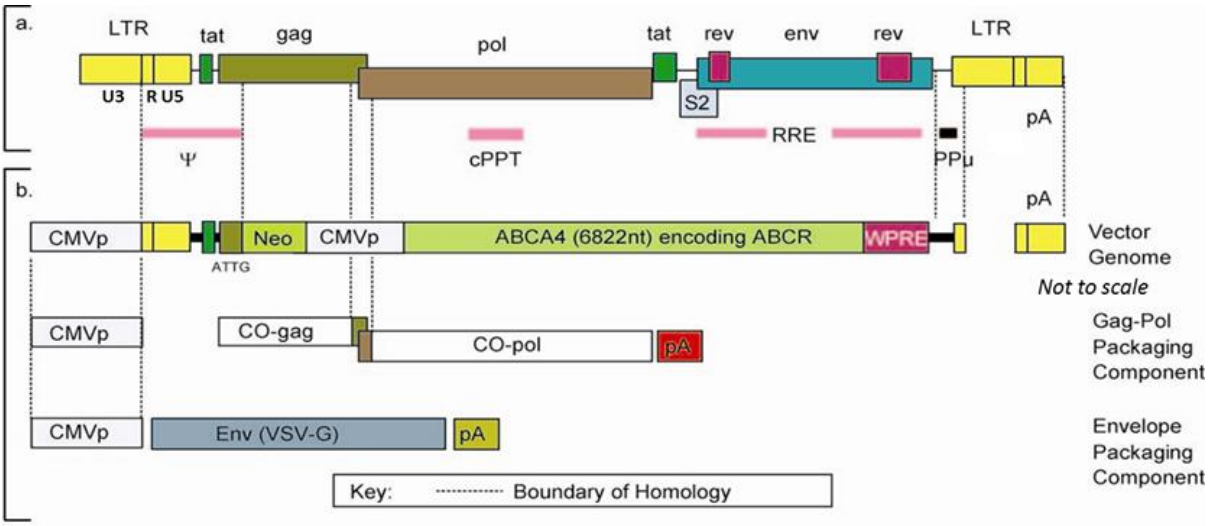
- The Gag/Pol packaging component contains (in order from 5' to 3') a Cytomegalovirus promoter (CMVp) and a codon optimized ORF for Gag/Pol,

linked by a non-codon optimized ribosome frame shift region required to maintain the correct balance of Gag and Gag/Pol proteins, followed by a heterologous pA site.

- The envelope packaging component contains (in order from 5' to 3') a CMVp, an ORF for vesicular stomatitis virus glycoprotein (VSV-G), and a heterologous pA site.
- The SAR422459 vector genome, pONYKABCA4 (BSG474) illustrated in Figure 1b contains (in order from 5' to 3') an CMVp-R-U5 region, a Ψ region (in which all the initiation codon [ATG] sequences have been mutated to ATTG), Neo (an open reading frame that enables high titre manufacture in the absence of Rev), an internal CMVp, an open reading frame (ORF) for the photoreceptor-specific ATP-binding cassette (ABCA4) transporter, a modified woodchuck hepatitis virus post-transcriptional regulatory element (WPRE), and a self-inactivating LTR (SIN LTR). Regions of the packaging components that share sequence homology with wild-type EIAV or each other are indicated with dotted lines.

The minimal vector system design, in combination with the use of a codon-optimised Gag/Pol construct and a heterologous envelope glycoprotein, means that sequence homology between vector components has been minimized. This strategy was employed to reduce the chance of replication competent lentivirus generation. SAR422459 contains only 861 nt of EIAV sequence. No functional viral proteins can be expressed from this construct.

Figure 1: Schematic diagram showing the genetic structure of a. EIAV wild-type virus and b. The EIAV vector system including SAR422459 (pONYKABCA4)



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

(i) transformation (.)
(ii) electroporation (.)
(iii) macroinjection (.)
(iv) microinjection (.)

(v) infection (.)
(vi) other, specify …

Transfection.
Specifically, the three plasmids are transfected into the human cell line HEK293T using lipofectamineTM 2000 CD. This results in the production of the EIAV gag/pol proteins, the VSV-G envelope protein and the RNA genome. SAR422459 particles are then assembled from these components.

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
See section C.6(c) and C.4(e)

•

(b) Source of each constituent part of the insert
See section C.6(c) and C.4(e)

• (c) Intended function of each constituent part of the insert in the GMOAn open reading frame (ORF) coding for neomycin phosphotransferase II (NeoR) from Escherichia coli transposon Tn5. The presence of this ORF enables manufacturing of high titre batches of lentiviral vectors in the absence of Rev gene.
• The Human Cytomegalovirus (CMV) immediate-early enhancer/promoter (CMVp). The function of this sequence is to transcribe a messenger RNA coding for the therapeutic human ABCA4 after insertion in the target cell genome.
• An ORF coding for the human photoreceptor-specific ATP-binding cassette (ABCA4) transporter.
• The modified woodchuck hepatitis virus post-transcriptional regulatory element (WPRE). This sequence has been modified to remove the wild type WPRE ORF. The function of this sequence is to provide a polyadenylation signal and to enhance ABCA4 expression through stabilization of the transcript.

(d) Location of the insert in the host organism

- on a free plasmid (.)

- integrated in the chromosome (x)
- other, specify Integrated in the RNA genome of the vector

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

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

1. Indicate whether it is a:

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

other, specify …

2. Complete name

(i) order and/or higher taxon (for animals)
- Herpesvirales
- Not applicable
- Primates
- Hepadnaviridae (family)
- Rhabdoviridae (family)

(ii) family name for plants
(iii) genus
- Herpesviridae
- Not applicable
- Homo sapiens
- Orthohepadnavirus
- Vesiculovirus

(iv) species
- Human Cytomegalovirus (CMVp)
- Escherichia coli transposon Tn5 (Neo)
- Homo sapiens (ABCA4 cDNA)
- Woodchuck Hepatitis Virus (WPRE)
- Vesicular Stomatitis virus (VSV-G)

(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 (x) Not known (.)
If yes, specify the following:

(b) to which of the following organisms:

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

(b) are the donated sequences involved in any way to the pathogenic or harmful properties of the organism
Yes (.) No (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 (x) No (.)
If yes, specify …
Human cytomegalovirus (CMV) is classified as risk Class 2
Escherichia coli is classified as risk Class 2
Homo sapiens are classified as risk Class 1
Woodchuck Hepatitis Virus (WHV) is classified as risk Class 2
Vesicular Stomatitis Virus (VSV) is classified as risk Class 2

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 (x) No (.) Not known (.)
Specify …
SAR422459 is a non-replicative vector and therefore not infective/virulent/pathogenic or able to colonise other organisms either directly or via a vector. This means that

survival would be limited to the initial number of particles released. The vector is not expected to survive anywhere in the natural environment.

(b) is the GMO in any way different from the recipient as far as mode and/or rate of reproduction is concerned?

Yes (x) No (.) Unknown (.)

Specify …

SAR422459 is a non-replicative vector and therefore not infective/virulent/pathogenic or able to colonise other organisms either directly or via a vector. However, since the vector is pseudotyped with the VSV glycoprotein (VSV-G), it will enter a wider range of animal cells than the wild type EIAV.

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

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

Specify …

The modifications to the vector system have made the vector particles unable to replicate in any cells. Therefore, once a cell that has come into contact with the vector has been transduced, there is no potential for dissemination.

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

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

Specify

Whilst the wild type EIAV is pathogenic to equidae, SAR422459 is a non-replicative vector and therefore not infective/virulent/pathogenic or able to colonize other organisms either directly or via a vector. SAR422459 does not encode for any of the accessory genes known to be associated with pathogenicity of the wild type parental virus, and no indications of vector pathogenicity have been observed during nonclinical testing.

2. Genetic stability of the genetically modified organism

The vector is using a transient co-transfection system. Once the particles have been made, they are replication defective and hence the sequence of the genome will not change until integration into the host genome occurs. The lentivirus integration process, which initiates with reverse transcription, may introduce errors. These occur because reverse transcriptase does not possess 3’ exonuclease activity capable of excising mispaired nucleotides and thus lacks the proof-reading function of cellular DNA polymerases. The fidelity of HIV-1 reverse transcriptase has been reported to be in the range of 1 misincorporation per 2,000 to 7,000 bases. The EIAV reverse transcriptase has been found to be similar to HIV-1 reverse transcriptase in vitro. Based on these data, integrated copies of the SAR422459 vector would be expected to contain up to 5 base substitutions. Unpublished data, from analysis of EIAV proviral vector sequences in transgenic chickens, indicate that the mutation rate is lower than predicted from in vitro studies and is less than 1 in 19,332 bp. Therefore, approximately 1 in every 2 integrated SAR422459 vectors would be expected to contain a single base substitution. This number of base substitutions is unlikely to result in any phenotypic changes. Further, as SAR422459 vector particles are only capable of a single transduction event, any mutations that occur are not cumulative.

Since SAR422459 is an Investigational Medicinal Product, stability testing programs are in place to monitor the stability of SAR422459.

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)

Point II(A)(11)(d):

SAR422459 is a non-replicative vector and therefore not infective/virulent/pathogenic or able to colonise other organisms either directly or via a vector. Testing of the vector in vivo in nonclinical studies has indicated no toxigenicity or allergenicity, or activation of latent viruses would be expected. Since the GMO is pseudotyped with the VSV glycoprotein it will enter a wider range of animal cells than the wild type EIAV. However, once a cell that has come in contact with the vector has been transduced, there is no potential for dissemination; in order for the GMO to replicate, it would require the cell to already contain a retrovirus. However, in this event, the SAR422459 vector is unlikely to be packaged into chimeric vector particles as it is a self-inactivating vector, and any RNA produced from the integrated genome is unlikely to contain a packaging signal and therefore would not be actively packaged into viral particles. If the RNA were to be packaged, the efficiency of reverse transcription and integration would be severely compromised and therefore the likelihood of genetic exchange is negligible. Long term nonclinical safety studies in rabbits and NHPs demonstrated that following subretinal administration SAR422459 remains localized within the ocular compartment. The vector will not transduce plant cells.

Point II(C)(2)(i)

The potential risks associated with the use of SAR422459 as a treatment for Stargardt disease have been evaluated in formal toxicology studies in rabbits and NHPs, in which SAR422459 was shown to be safe and well tolerated.

Following subretinal injection, the severity of cell-like vitreous, retinal or vitreous opacities and retina/choroid pigmentation in the eye was higher in SAR422459 treated group when compared to the vehicle; however these ophthalmic changes did not resolve into any unexpected treatment-related histopathological findings at the level of the retina. There were no SAR422459-related histopathological findings.

The potential for SAR422459 to induce an immune response has been evaluated by monitoring serum from rabbits and NHPs for antibodies to the vector and to the therapeutic gene products and by immunohistochemistry at the target site. Antibodies against the envelope protein (VSV-G) were detected in 21 of 38 rabbits and against production cell (HEK293T) antigens in 17 of 38 rabbits, by 3 months post

administration. No antibodies to any of SAR422459 vector components were detected in the NHP samples.
Wild type EIAV is pathogenic in equidae and cannot replicate in human cells. The GMO (SAR422459) is a nonreplicant vector, which does not encode for any of the EIAV genes and is therefore not pathogenic in both humans and equidae. No pathogenic effects have been recorded in any nonclinical study performed to date.
The system has been designed so that no viral genes (whether from EIAV or VSV) are carried into the target cells in the patient, and tested to support the claim that there are no replication competent lentiviruses (RCL) present in preparations. To date, GLP compliant testing has been completed on three batches of post-production cells and one vector lot of SAR422459. All were reported to be free of replication competent lentivirus.
In addition to SAR422459, other lentiviral vector products based on the Oxford BioMedica platform manufactured in compliance with cGMP have undergone RCL testing at the same manufacturing stages. Common to all of these products are the production cells, manufacturing methods and two of the three plasmids (specifically those encoding Gag/Pol and VSV-G envelope used at transfection; the third plasmid component is the vector plasmid encoding the vector genomic RNA. The vector backbone is identical for these lentiviral vector products, but the internal expression cassette containing the therapeutic transgene(s) is product specific). These additional batch tests comprised 15 batches of post-production cells and 6 vector lots. Vector and post-production cell materials have also been tested from vector development lots for the purposes of assay qualification and/or validation. All results obtained to date have been reported as 'No RCL detected'.
In summary, neither the parental organism (EIAV) nor the GMO are pathogenic in humans. The vector production system has been designed to minimize the risk of a recombinant lentivirus being produced and several GMP scale vector batches have tested negative for RCL to support this. It is unlikely that any pathogenicity could result.

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment
Sensitive real-time reverse transcriptase PCR assays are available to detect the EIAV packaging signal RNA in test samples as a measure of vector particles. These assays are complemented by real-time PCR assays to detect the DNA form of the vector. ELISA and Western blot techniques are available to detect antibodies raised against the vector in in vivo models.
These could be used to detect the presence of the GMO in animals and the environment.

(b) Techniques used to identify the GMO
The techniques outlined in section E.4(a) also identify the product.
In the event of positive detection of the EIAV vector sequence in environmental or patient samples, additional PCR-based methods could be employed to further characterise and identify the sequences (e.g. the conventional RT-PCR based SAR422459 identity assay that is performed as part of the QC testing strategy for the SAR422459 IMP).

F. Information relating to the release

1. Purpose of the release (including any significant potential environmental benefits that may be expected)
   The main purpose is the treatment of patients with Stargardt disease, which should halt the disease progression and thereby prevent further deterioration of the sight of the patients. The product is to be used to conduct a clinical trial. No specific environmental benefits are expected.

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 (x) No (.)
   If yes, specify
   SAR422459 is to be administered via subretinal injection to patients in an operating theatre in a hospital.

3. Information concerning the release and the surrounding area

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

   The release will occur in the context of a clinical study conducted at the Department of Ophthalmology, Radboud university medical center, Philips van Leydenlaan 15, 6525 EX, Nijmegen, The Netherlands

   (b) Size of the site (m^2):
   (i) actual release site (m^2): Not applicable
   (ii) wider release site (m^2): Not applicable

   (c) Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs), which could be affected:
   Not applicable considering that shed material, if any at all, is non-infectious.

   (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:
   The maximum tolerated dose of SAR422459 will be provided, packaged as 0.1 mL aliquots in 0.3 mL vials. In order to deliver 300µL of SAR422459, 6-7 vials are necessary to treat one patient. It is predicted that there will be no more than 8 vials containing a maximum of 6.0×10^5 TU per vial in the hospital at any one point in time. Up to 500 patients will take part in the studies.

   (b) Duration of the operation:
   Following administration of SAR422459 (the duration of the operation is around 30 minutes), the patients will be followed up for 48 weeks. After this period, they will

enter an open-label safety study and long-term follow-up visits, including ophthalmological examinations and recording of adverse events, will continue for 5 years. In addition, the investigator will follow the patient for a subsequent 10 years, at a minimum interval of once a year, to monitor delayed adverse events.

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

The amount of SAR422459 supplied to the site at any one time is limited, and access to the product is restricted. The clinical protocol requires the use of appropriate protective equipment by personnel, and there are written procedures in place to be used in the event of a spillage, or when disposing of the product or contaminated materials.

The subretinal route of administration will minimize potential shedding from the patient, and samples are taken to monitor this following treatment.

The first dressings removed from the patient's eye following surgery will be disposed of by incineration.

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

Not applicable as SAR422459 is to be administered to subjects in an operating theatre in a hospital.

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.

SAR422459 has previously been released and used in France and the United States for clinical trial purposes. To date, no environmental and/or human health impacts have been reported from the release/use.

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)	Primate…
(ii)	family name for plants	…
(iii)	genus	Homo…
(iv)	species	Homo sapiens …
(v)	subspecies	Homo sapiens sapiens …
(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)

SAR422459 is a gene therapy product designed to introduce the correct ABCA4 cDNA to photoreceptors, and thereby attenuate and possibly reverse the pathophysiology which leads to the development of Stargardts disease.

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

The likelihood of the GMO spreading outside of the hospital is considered to be negligible, as the vector is not expected to survive in a natural environment and the route of administration poses a negligible risk of shedding from patients. Data from nonclinical studies indicates that the vast majority of the subretinally injected SAR422459 remains within the eye after ocular administration; therefore shedding into the environment via body fluids (tears, urine) is extremely unlikely. Further, the entry of the GMO into non-target cells is considered to have a very low likelihood of occurrence, as the GMO is inactivated by the human immune system and its spread within the body will be limited to the area in which it is administered.

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?
Yes (.) No (x) Not known (.)
Give details
SAR422459 is a non-replicative vector.

5. Types of ecosystems to which the GMO could be disseminated from the site of release and in which it could become established
No dissemination is expected from the site of release. In addition, it is not expected that the vector will survive anywhere in the natural environment and so cannot become established.

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

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

7. Likelihood of genetic exchange in vivo

(a) from the GMO to other organisms in the release ecosystem:
Not likely, as SAR422459 is a non-replicative vector and therefore not infective/virulent/pathogenic or able to colonise other organisms either directly or via a vector.

(b) from other organisms to the GMO:
Not likely, as SAR422459 is a non-replicative vector, and is produced under GMP conditions where the cells used for production, excipients, drug substance and drug product are tested for the presence of adventitious agents.

(c) likely consequences of gene transfer:

The transfer of the normal functional human ABCA4 gene to the photoreceptor cells is intended to restore normal cellular functions. Expression of the ABCA4 protein is not thought to constitute a risk based on the findings from nonclinical toxicology studies.

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.):
To date, no specific studies on the potential ecological impact of SAR422459 have been performed.
However, as SAR422459 can only transduce animal cells and is non-replicative, dispersal will be limited to the first organism infected and therefore there is no potential for population increase within the environment. The vector is not expected to survive anywhere in the natural 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
Real-time reverse transcriptase PCR assays, to detect the EIAV packaging signal RNA sequence in blood and urine samples of patients as an indirect highly sensitive measure for the detection of vector particles, have been developed. These are complemented by real-time PCR assays to detect the DNA form of the vector following entry into target cells (as a measure of transfer of the vector, including transgene sequences). In the event of positive detection of the EIAV vector sequence in environmental or patient samples, additional PCR-based methods could be employed to further characterize and identify the sequences (e.g. the conventional RT-PCR based SAR422459 identity assay that is performed as part of the QC testing strategy for the SAR422459 IMP).

2. Methods for monitoring ecosystem effects
Not relevant as there will be no impact on the ecosystem.

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms
A modified version of the real-time qualitative PCR assay mentioned in section H.1 above may be used to detect integrated vector genomes in a range of organisms.

4. Size of the monitoring area (m^2)
There are no plans to directly monitor the site of administration or the operating theatre in which the patient is injected. However, samples are taken from treated patients in order to monitor potential shedding.

5. Duration of the monitoring
Blood samples are taken for PCR at screening 28 days prior to Day 0, and at 60 minutes after surgery, at Day 1 and in Weeks 1, 2, 4, 12, 24, 36 and 48.
Urine samples will be collected for PCR at screening, at Day 0/surgery, Day 1, Week 1 and Week 2, to assess biodistribution.

Samples for determination of anti-drug antibodies (ADA) will be collected at baseline visit (Day -1),) and in Weeks 4, 12 and 24. In the event of a positive antibody response in Week 24, additional samples will be collected (at approximately 12 week intervals) to monitor the return of the antibody response to baseline levels.

6. Frequency of the monitoring
Blood samples are taken for PCR at screening 28 days prior to Day 0, and at 60 minutes after surgery, at Day 1 and in Weeks 1, 2, 4, 12, 24, 36 and 48.
Urine samples will be collected for PCR at screening, at Day 0/surgery, Day 1, Week 1 and Week 2, to assess biodistribution.
Samples for determination of anti-drug antibodies (ADA) will be collected at baseline visit (Day -1),) and in Weeks 4, 12 and 24. In the event of a positive antibody response in Week 24, additional samples will be collected (at approximately 12 week intervals) to monitor the return of the antibody response to baseline levels.

I. Information on post-release and waste treatment

1. Post-release treatment of the site
All disposable surgical supplies, including primary IMP containers, gloves, masks, gowns, dressings and swabs used during the surgical procedure, will be destroyed by incineration according to hospital policy on genetically modified materials at the end of the procedure.
In addition, the first dressings removed from the patient's eye following surgery will also be disposed of by incineration.
The biosafety cabinet class 2, the operating theatre floor, surfaces and suite will be cleaned with a suitable disinfectant which is bactericide, fungicide and active against HIV-1, HVB and BK.
Any unused IMP remaining at the hospital site will be destroyed by incineration according to hospital policy at the end of the operation.

2. Post-release treatment of the GMOs
No special procedures to prevent the spread of GMSs outside the release site, because transduced retinal cells cannot spread beyond the patient's eye and the vector genome integrates into the host cell chromosome.

3. (a) Type and amount of waste generated
The types of waste expected to be generated include general disposable surgical supplies (e.g. primary IMP containers, gloves, masks, gowns, dressings and swabs), the injection device and remaining parts of patient samples which are not used for analysis.
The amount of waste generated will be limited due to the small number of patients taking part in this study. Each patient is expected to generate a minimum amount of waste according to the list above.

3. (b) Treatment of waste
All disposable surgical supplies, including gloves, masks, gowns, dressings and swabs used during the surgical procedure, will be destroyed by incineration according to hospital policy at the end of the operation. The first dressings removed from the patient's eye following surgery will also be disposed of by incineration.
All vials of IMP at the hospital (used or unused) will be destroyed by incineration according to hospital policy.

J. Information on emergency response plans

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread
   Standard hospital procedures will be used for the containment of the GMO in the event of a spill. The likelihood of the GMO spreading outside of the hospital is considered to be negligible as the vector is non-replicative, and not expected to survive in a natural environment. Further, the subretinal route of administration will permit only minimal shedding from patients. The vector is readily inactivated by hospital disinfectants.

2. Methods for removal of the GMO(s) of the areas potentially affected
   Spillages will be cleaned with Terralin 0,5% (method qualified with EIAV vectors), produced by Schulke & Mayr. All contaminated materials will be disposed of locally by incineration.

3. Methods for disposal or sanitation of plants, animals, soils, etc. that could be exposed during or after the spread
   Not applicable as there is no exposure of plants, animals, soils.

4. Plans for protecting human health and the environment in the event of an undesirable effect
   The clinical trial includes safety monitoring of the patients in order to detect any unexpected adverse events. Following administration of the GMO, each patient will be followed for 48 weeks and, after this period, they will enter a 15-year follow-up study to assess safety and biological activity. During the first 5 years, 2 visits are planned each year, followed by an annual visit for the subsequent 10 years.
   No specific environmental plans are necessary as the GMO is non-replicative and is not expected to survive in the natural environment.