

# Application form

## Assessment of clinical research involving gene therapeutics in the Netherlands

-

### *Viral vectors*

July 2015



**Gene  
Therapy  
Office**

Streamlining submission  
procedures of gene therapy  
clinical trials  
in the Netherlands

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# **Application form Assessment of clinical study involving genetically modified viruses**

## Part A: Bio-safety aspects

- A1. General information (Public section)
- A2. Construction and composition (Public section)
- A3. Production (Public section)
- A4. Research description (Public section)
- A5. Environmental risk assessment (Public section)
- A6. Conclusions about possible environmental effects (Public section)
- A7. General (personal) information (Confidential section)

## Part B: Patient-related aspects (*not included in this form*)

If you have any questions, please get in touch with the Gene Therapy Office (E-mail: rik.bleijs@rivm.nl, phone: +31-30-2747569).

July 2015

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## Consent form

The undersigned:

Hereby gives his/her consent to the gene therapy office to send on the application forms and the accompanying file to the relevant assessment authorities.

Gives his/her consent to the assessment authorities to use the fully completed file in making its assessment.

Gives his/her consent to the assessment authorities to inform each other and the gene therapy office about the status of the submitted application.

Signed:

Name

Date

## Part A. Bio-safety aspects

This part of the application form provides the information needed for the Ministry of Infrastructure and Environment (IenM) to grant the necessary licenses.

All information provided in this form and the accompanying documentation constitutes part of the decision to be made and for this reason is in principle open to inspection by the public; the information will also be available for such public inspection during the procedure.

The applicant may ask for parts of the information provided to be kept confidential. In that case, the applicant must give reasons why the information is of a confidential nature as well as a convincing explanation that the lifting of confidentiality will adversely affect the applicant's competitive position. A publicly available summary of confidential information must be given, containing enough information for a clear general understanding of the application and in order to assess the risk analysis as described in the application and the decision. Confidential information must be included in a separate attachment marked 'confidential'.

An application does not need to be limited to the specific clinical protocol that the applicant wishes to perform. If there are no consequences for the risk analysis, the application can be drawn up with a wider scope, such as for a larger number of patients.

The aim is to draw up the final decision in such a way that several clinical protocols can be performed under it, using the information described in this application. Naturally these activities must be covered by the description of the experiment and the risk analysis provided. Before submitting such a broader application, you are advised to contact the GMO office for an informal discussion of the options.

The term 'test subjects' as used in this form means patients or volunteers taking part in the study.

Specific personal details of the contact person and the environmental safety officer are to be submitted by entering the required information under Section A7, General (personal) information. The submitted data in this appendix will be kept confidential and will thus not be made publicly available in accordance with the Personal Data Protection Act.

This Notification Form may contain some questions that are not relevant to your case. We would ask you NOT to answer in your notification any questions that are irrelevant to the activities for which you are applying.

Specific issues:

- Literature that is referred to has to be sent in together with the application form.
- Confidential information has to be marked as such and has to be sent in separately.
- A SNIF B (other GMO) form has to be completed and to be sent in as an electronic file in Word format.

## A1. General application details

### General information

#### A1.1. Application title:

*Please give a descriptive title that provides sufficient information on the objectives and aspects relating to the genetic modification. The title should state the type of GMO(s) and insertion(s) used and the nature of the application(s).*

Gene therapy Clinical development program comprising of clinical trials using SAR422459, a non-replicating, recombinant lentivirus 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 degeneration (SMD) and other ABCA4-associated retinopathies.

#### A1.2. The objective of the study for which the application is submitted:

*Provide a short description of the study's objective. For example: 'The objective of this Phase I clinical trial is to study the safety of the oncolytic adenovirus for the purpose of developing a new therapy to treat skin cancer.'*

The purpose of the work is to develop a new therapy for the treatment of SMD. The specific purpose of the ongoing Phase I/IIa clinical study is to assess the safety and tolerability of ascending doses of SAR422459 in patients with SMD. Additionally, the study will assess the biological activity of SAR422459.

#### A1.3. Describe briefly the contents of the application, the objective of the study being aimed for and the intended application of the results

*Provide a short description – of no more than half a page – of the genetically modified organisms (GMOs) applied, or of the GMOs that may thus be created, such as via recombination of genetic information between organisms or by integration of genetic material into a genome. Describe the expected action of the gene products of the transgenes and give an explanation of the biological mechanism. Also describe the scientific and public importance of the study, and state how the results of the study may be used in this context.*

*The information provided will be used as the basis for a brief description of the study in the decision.*

The study is primarily intended to assess the safety and tolerability of ascending doses of SAR422459 in patients with SMD and secondly to evaluate the possible biological activity of SAR422459 as a treatment for SMD. Patients from this study will also enter a long term follow-up study to determine the long term safety, tolerability, and biological activity of SAR422459 in patients with SMD.

SAR422459 is a non-replicating, recombinant lentiviral vector derived from the genome of the non-primate lentivirus, Equine Infectious Anemia Virus (EIAV). SAR422459 contains approximately 10% (861 nucleotides) of the wild type EIAV genome and there are no functional viral proteins or viral coding regions in the recombinant EIAV vector.

SMD is a devastating ocular disease that produces a severe form of macular dystrophy, similar to advanced age-related macular degeneration (AMD) in the elderly, but which begins in childhood. SMD is the most common inherited retinal dystrophy affecting approximately one in 10,000 individuals worldwide. The disease is nearly always inherited as an autosomal recessive trait and has been linked to mutations in a retina-specific

adenosine triphosphate (ATP)-Binding Cassette (ABC) transporter gene (ABCA4, also known as ABCR). SMD typically presents with the onset of bilateral central vision loss in children and young adults and is associated with atrophic macular lesions with or without the presence of yellow fleck like lesions at the level of the retinal pigment epithelium. Patients invariably progress to legal blindness consequent to the death of photoreceptors and retinal pigment epithelial cells in the macula. Histopathologic examination of eyes from SMD patients has demonstrated RPE cells densely packed with lipofuscin.

The prevalence of Stargardt disease in the Netherlands is estimated to be approximately 1000 patients for 16 million inhabitants. In a recent natural history cohort of 51 Stargardt patients from the Netherlands, the mean (standard deviation) age at onset of disease was determined to be 7.2 (2.2) years (median, 8; range, 1-10)<sup>1</sup>. Currently, there are no effective treatments to reverse or slow the progression of SMD in affected patients most of whom will live the majority of their lives severely visually impaired or blind in both eyes.

SAR422459 is a gene therapy product designed to introduce the correct ABCA4 cDNA into photoreceptors (PR) and thereby attenuate and possibly reverse the pathophysiology which leads to SMD. The presence of the wild type ABCA4 is intended to compensate for the defective transport of retinal compounds and reduce the formation of dirretinal compounds (such as pyridinium bis-retinoid [A2E], a major component of lipofuscin), which accumulates in and leads to the degeneration of the light sensitive photoreceptors (PR) and their supporting retinal pigment epithelial (RPE) cells.

The efficacy of SAR422459 vector has been demonstrated in a murine model of SMD, the *Abca4*<sup>-/-</sup> mice, in which the defect of ABCA4-mediated transport leads to the age-dependent accumulation of A2E and subsequent visual defects. The reduction in lipofuscin accumulation following the subretinal administration of the SAR422459 vector in this model established the rationale for gene therapy in patients with SMD<sup>2</sup>. Subretinal delivery of SAR422459 in patients with SMD aims to introduce an ABCA4 transgene permanently into the host chromosome by a process referred to as integration and to induce the expression of the ABCA4 protein and restore its function in PR.

The studies in this clinical program are therefore intended, in addition to evaluating the safety and tolerability of SAR422459, to show any evidence of possible biological activity that could serve as proof of concept to support subsequent registration trials of SAR422459 as a treatment for patients with SMD.

#### **A1.4. Describe briefly the intended work.**

*State in chronological order which types of procedures will be carried out, and for which a license is being applied for (e.g. production, transport, storage and administration of the vector, observation of patients, sampling, transport, storage and processing of samples, waste treatment). Where applicable, also indicate for which part of the study a Contained Use (IG) permit applies, and provide the number of the GMO permit concerned.*

The license is being applied for storage of the vector at the investigational clinical center, administration of the investigational medicinal product by subretinal injection, observation and sampling of the patients, processing and transport of patient samples, and waste treatment.

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<sup>1</sup> Lambertus S, , Van Huet RAC, Bax NM, Hoefsloot LH, Cremers FPM, Boon CJF, Klevering BJ, Hoyng CB, Early-Onset Stargardt Disease : Phenotypic and Genotypic Characteristics, *Ophthalmology* 2015; 122(2):335–344

<sup>2</sup> Kong J, Kim SR, Binley K, Pata I, Doi K, Mannik J, et al.: “Correction of the disease phenotype in the mouse model of Stargardt disease by lentiviral gene therapy”. *Gene Ther.* 2008;15:1311-20

Production and transport of the gene therapy product to the clinical site are the responsibility of Sanofi and its contracted vendors.

Storage of the gene therapy product (SAR422459) is done in a secured area of the Radboudumc (Radboud University Nijmegen Medical Center). This storage room is approved for storage of biohazardous material.

The injection will be prepared in the biological safety cabinet (level II) in a MLII laboratory of Radboudumc, which is close to the storage room of the gene therapy product. This is an area approved for preparation of GMO per the hospital policy. SAR422459 will be used, following preparation, in the operating theatre of the department of Ophthalmology. Preparation will be done by an employee with ample experience in MLII activities under the supervision of an employee who is authorized to prepare medical samples. The preparation consists of aspiration of SAR422459 from several vials into an injection device. The injection device will be transported to the operation theatre conforming the hospital's policy (I4 "werkprotocollen GGO"): packed in a closed, unbreakable and leakfree container that is indicated with a biohazard sign. The container will be disinfected using 70% ethanol before transport to the operating room.

The study eye will be marked prior to the patient entering the operating room of the Ophthalmology department on the premises of Radboudumc and once in the operating room, the study eye will be confirmed. Surgery, including the administration of SAR422459, will be performed under sterile conditions in accordance with hospital operating room procedures. The operation theatre complies with measures for prevention of infections in a operating theatres class I (G4 "WIP:Omstandigheden (kleine) chirurgische en invasieve ingrepen"). This includes the use of only hard, smooth materials for floor, walls and ceilings that can easily be disinfected, as well as measures for ventilation and temperature control and the use overpressure in the operating room.

Surgery includes vitrectomy and subretinal injection. The surgeon first makes very small incisions in the sclera (white part of the eye) and removes the vitreous (gel-like fluid in the eye) and exchanges this by a balanced salt solution. The surgeon injects a solution of SAR422459 subretinally using a very thin needle. The needle will be removed and the incisions will be closed with stitches. The stitches will dissolve on their own after several weeks. At the completion of the subretinal injection procedure, the eye will be patched and the patient will be taken into the post-operative observation area within the Ophthalmology department. After 60 minutes, if the patient is feeling well and able to tolerate food and drink, he/she will be discharged. Before discharge, blood and urine samples will be obtained for PCR assay for viral particles. After one day, the patient will come to the clinic to remove the eye bandage, which will be destroyed by incineration.

Should the eye bandage loosen outside the clinic, it should be reattached using the bandage material that is supplied by Radboudumc (together with protective gloves) and removed the next day in the clinic. A procedure will be supplied on how the bandage should be reattached; in short, the patient will wear protective gloves, take a piece of medical tape and reattach the already supplied bandage material. After this procedure, the patient will disinfect his/her hands using the hand disinfection material ("handalcohol") supplied. All materials during this procedure will be saved in a plastic sealable bag that is marked with a biohazard sign and brought to the clinic for incineration.

To decontaminate areas affected (e.g. eradication of the GMOs), spillages in the operation room will be cleaned up with Terralin 0,5% (method qualified with EIAV vectors), produced by Schulke & Mayr. All contaminated materials will be disposed of locally by incineration or autoclaving. All other places will be cleaned using 70% ethanol, according to normal decontamination procedures (I5 "Decontaminatie procedures").

All health care staff handling SAR422459 will work according to the Working Group Infection Prevention guideline Gene Therapy (G1 "WIP richtlijn genterapie"). The guideline has been attached to this application. In the operating theatre, health care staff will work according to the guideline Basic measures infection prevention in an operating room (OR class 1 and 2 (G2"Basismaatregelen infectiepreventie in een operatiekamercomplex" of the Radboudumc. This guideline has been attached to this application.

Storage and processing of samples: Samples taken include blood and urine samples, which are collected before the administration of SAR422459 and at study visits after SAR422459 administration. Blood samples for routine clinical laboratory safety tests will be performed in the clinical laboratory of Radboudumc. Urine pregnancy tests will be performed at Radboudumc: in case of positive pregnancy test at screening, patients will be considered as screen failed and will not be included in the study. Patients or partners of a study patient who become pregnant after administration of SAR422459 will be followed for safety as well as the results of the pregnancy. The processing of blood samples for PCR analysis and immunology analysis will be performed at Radboudumc, either in the Ophthalmology department or at the clinical laboratory. Samples will be stored in the freezer prior to the shipment to Oxford Biomedica, the company performing the testing of the samples. SAR422459 is not expected to be present in the samples (based on analyses of samples of previous treated patients, see section A2.17) and therefore the samples test will be performed according the standard procedures (I4 "Werkprotocollen GGO").

**A1.5. Intended start and end date:**

*The decision must state a time period within which the procedures will be carried out, and so an end date must be given. The chosen end date will be included in the decision. It is possible to obtain an extension to the decision; please note, however, that any extension procedure must be completed before the decision end date has passed.*

The start and end date in the Netherlands are Q2 2016 – Q4 2050.

**A.1.6. Do you want to keep other information confidential? If so, please specify in concrete terms how the release of the information would harm your competitive position.**

*Unless marked "Confidential", all the information contained in the notification and its appendices may enter the public domain when the notification is publicly processed and the decision is published.*

*For the sections marked "Confidential", you are requested to give a publishable summary that contains enough information to ensure a good general understanding of the notification. Furthermore, give a reason why certain information is marked "Confidential".*

In Section A.4.4., the precise dose actually used for treating patients is confidential. Indeed, this dose could be one of the elements of a potential future patent application, and therefore, should be kept unavailable to the public to not jeopardize the validity of a future patent. However, a dose range will be provided.

In section A.3.2 and A.3.3., quality control taken place at specific steps of production process and criteria used for batch release are detailed and should not be disclosed to public to not jeopardize the validity of a future patent.

In section A.2.8, full card and backbone give information of construct of the vectors and should be kept confidential to not jeopardize the validity of a future patent.

In A2.10 and A2.12 more detailed information is given about the production and molecular characterization of the vector, which should be kept confidential not to jeopardize the validity of a future patent.

In section A2.13, full tests performed on HEK293T cells are confidential. However, HEK293T cells are cleared of HIV-1, HIV-2, HTLV-1 and -2, SIV and other non-human lentiviruses.

## Details of applicant

*Only the legal entity that has final responsibility for the work to be carried out may act as the applicant. This means that the applicant will normally be the Board (management) of the hospital (institution) where the treatment will be given. The license holder must be able to enforce compliance with the license regulations when carrying out the work. In order to do so, it is necessary for the employees involved in the clinical procedures to come under the authority of the license holder. For this reason, employees must be directly employed by the license holder. In those cases where an employee does not come under the authority of the license holder, such as where a treating doctor is part of a partnership that is independent of the license holder, an employment contract must be arranged for carrying out work under the license, such as through a zero-hours contract with the license holder. A contract must be concluded with the party or parties carrying out this work for non-clinical procedures that are not carried out in the institution in question, in such a way that final responsibility continues to rest with the license holder.*

**A1.7. Name of legal entity:**

Radboudumc (Radboud University Medical Center)

**A1.8. Address of legal entity:**

Board of Directors Radboudumc  
P.O. Box 9101

**A1.9. Postal code and town/city of location of legal entity:**

6500 HB Nijmegen, The Netherlands

**A1.10. At which locations will the intended work take place?**

*Since the work applied for may only be carried out under the direct control of the license holder, it is only possible to carry out work at several locations if the license holder has full control of the way in which the work being applied for is carried out at all locations, in such a way that the license conditions are complied with. In that case you must state for each location what work will be carried out at which address and in which building. To clarify: you must state for all activities at which location they will be carried out. Apart from the location for the clinical activities with the GMO, you must also state the location or locations of laboratories in which activities with the GMO are carried out under the terms of this license application, such as procedures with patient samples.*

*In cases where central control is not possible, such as with a multi-center study, a separate application must be submitted for each location.*

The work is intended to take place in Radboud University Nijmegen Medical Centre in Nijmegen, the Netherlands.

Activities that will be performed at this site are storage of the vector at the investigational clinical center, administration of the investigational medicinal product by subretinal injection, observation and sampling of the patients, processing and transport of patient samples, and waste treatment.

## A2. Construction and composition of the GMO

### Virus from which the genetically modified vector was derived

#### A2.1. Which virus was used as the original virus in the construction of the GMO?

*Where applicable, supply the full scientific name, a trivial name (e.g. the commercial name), the strain and the isolate used.*

SAR422459 is based on the Equine Infectious Anemia Virus (EIAV), which belongs to Retroviridae's family. The original EIAV genome has been extensively modified. It no longer carries any of the original viral genes and has had all extraneous viral nucleic acids removed to leave less than 10% of the original virus genome.

The pathology caused by EIAV, equine infectious anemia (EIA), is limited to equidae (including horses, ponies, donkeys, mules and zebras). Equine infectious anemia is characterized by an acute clinical phase following infection with associated viremia, fever and thrombocytopenia, a chronic phase with also recurring viremia and anemia, weight loss and edema. Chronic phases can be separated by subclinical asymptomatic phases associated with low viremia which may last the full life of the animals<sup>3,4</sup>. Although some horses may die during the acute phase of the disease, death is a very uncommon feature of the EIA and is linked to intense virus replication. Most horses typically control the infection through an immune response to the virus and enter the chronic phase of the disease<sup>4</sup>.

During symptomatic phases, the immune response towards the heavily replicating virus with accumulation of immune complexes is responsible for the hemolysis and depletion of platelets from circulation as immune complexes are found bound to both platelets and red blood cell in the circulation. Inhibitory cytokines released during the immune response and acting on erythropoiesis and generation of platelet also play a role in the anemia or thrombocytopenia<sup>5</sup>. During asymptomatic phase, the virus continues to exist in macrophages and may escape immune surveillance with re-emerging symptomatology.

#### A2.2. Describe how the identity of the original virus was determined.

*The GMO to be administered is often based on a strain that was derived, sometimes by genetic modification, from the original virus. The properties of the original organism form the basis for the environmental risk assessment, which is why confirmation of the identity is important. Provide a description of the characteristics that determine the identity of the original virus. This may be based on bio-information analyses, such as sequence analysis, alignments or phylogenetic analysis. Information on the origins of the original virus, such as a micro-organism originating from the American Type Culture Collection (ATCC), may also be submitted.*

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<sup>3</sup> Leroux et al., Equine Infectious Anemia Virus (EIAV): what has HIV's country cousin got to tell us? Vet Res. 2004 Jul-Aug;35(4):485-512;

<sup>4</sup> US center for food safety and public heal, University of Iowa, 2009-  
[http://www.cfsph.iastate.edu/Factsheets/pdfs/equine\\_infectious\\_anemia.pdf](http://www.cfsph.iastate.edu/Factsheets/pdfs/equine_infectious_anemia.pdf)

<sup>5</sup> Mealey RH, Equine infectious anemia. In Equine Infectious Diseases (Second Edition); Sellon DC and Long M. 2014, pp232-238

The EIAV vector used in the study is based on a non-pathogenic infectious proviral EIAV clone pSPEIAV19 (accession number: U01866), a kind gift from Dr. SL Payne<sup>6</sup> to Oxford Biomedica Ltd as described<sup>7</sup>.

### **A2.3. What is the host range of the original virus?**

*Describe the hosts in which the original virus naturally occurs, also including hosts that serve as a reservoir. For each possible host, indicate the tissue and cell tropism.*

The natural habitat of EIAV is equidae (horses, ponies, donkeys, mules and zebras). EIAV is transmitted by bloodsucking insects, primarily biting flies. As EIAV has not been shown to multiply in insects, infected equidae appear to be the only reservoir of the virus<sup>5</sup>.

EIAV is endemic in the Americas, parts of Europe, the Middle and Far East, Russia, and South Africa. In the EU, it is a notifiable disease, though it has a low level of incidence and does not occur in all Member States. The organism occurs in Atlantic, Mediterranean and Continental ecosystems. According to the World Organisation for Animal Health no outbreaks of EIAV have been seen in The Netherlands in the last 10 years<sup>8</sup>.

The Animal Health Service (In Dutch: De Gezondheidsdienst voor Dieren) have been monitoring EIAV. First results of data from 2014 showed that no EIAV was found in 300 blood samples from horses<sup>9</sup>.

Furthermore, data of the Central Veterinary Institute (CVI) in The Netherlands showed that in 2000 to 3000 horses that were tested in 2015, no EIAV was found in these samples (information from unpublished data of the CVI).

EIAV is not a human pathogen and cannot be transferred to humans<sup>5</sup>.

### **A2.4. Provide relevant data on pathogenicity and possible attenuation and biological restrictions of the original virus.**

*What is the class of pathogenicity of the original virus? If this concerns an attenuated virus, the basis for attenuation must be described. If the organism is biologically restricted in another way, the grounds for the biological restriction must be substantiated.*

EIAV is the simplest of the known lentiviruses and a class 2 organism as it is an animal pathogen. Moreover, EIAV per se is biologically contained in nature as it has an extremely narrow tropism and only infects equidae<sup>5</sup>.

The parental organism is, therefore, not a human pathogen<sup>5</sup>.

### **A2.5. What are the pathogenic properties of the original virus and what are the available treatment methods?**

*Name the symptoms that are known or assumed to be caused by the original virus. Also indicate the treatment methods that are available to treat such an infection and provide an indication of the effectiveness of these treatments.*

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<sup>6</sup> Payne SL et al. Characterization of infectious molecular clones of equine infectious anaemia virus. J Gen Virol 1994; 75: 425–429

<sup>7</sup> Mitrophanous et al. Stable gene transfer to the nervous system using a non-primate lentiviral vector. Gene Therapy (1999) 6, 1808–1818

<sup>8</sup> [http://www.oie.int/wahis\\_2/public/wahid.php/Diseaseinformation/Diseaseoutbreakmaps](http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/Diseaseoutbreakmaps)

<sup>9</sup> <http://www.gddiergezondheid.nl/actueel/nieuws/2015/04/eerste-resultaten-monitoring-west-nile-virus-en-equine-infectieuze-anemie-bekend>

EIAV is not a human pathogen. In horses, the most salient disease features are fever, thrombocytopenia and anemia. There is no specific therapy for EIAV infection in horses as the disease is generally self-limiting. Treatment, when provided, is supportive with most animals surviving to become chronic carriers of the pathogen<sup>5</sup>.

#### **A2.6. What are the transmission routes of the original virus?**

*State all the observed and assumed transmission routes of the virus, and indicate the effectiveness of the transmission.*

The predominant route of EIAV transmission in nature is via bloodsucking insects, primarily biting flies. Less commonly, EIAV has also been shown to be transmitted between horses through aerosol, blood, saliva, milk, body secretions and materials contaminated with body secretions (e.g. bits, surgical equipment). Mares can transmit the disease to their foals via the placenta. The risk of transmitting the disease is greatest when an infected horse is showing symptoms, as the blood levels of the virus are then highest<sup>5</sup>.

#### **A2.7. How could the original virus survive outside the host?**

*State all survival options and the survival time of the original virus under optimal environmental conditions, and describe the factors that may be of influence.*

Outside its host, primarily horses, the EIAV mostly exists in the bloodsucking insects that normally transmit the disease to other horses. The virus does not replicate within insect cells, the insects serve only as mechanical vectors transferring blood on their mouthparts. The EIAV was demonstrated to survive and be transmitted by the horse fly only a few hours after the initial bite<sup>5</sup>. The virus is known to survive for up to 4 days on hypodermic needles held at room temperature<sup>5</sup>.

When present in blood outside a living animal, EIAV, like other enveloped viruses, is considered to survive poorly in the environment, especially in a dry condition. This is mostly supported by studies concerning HIV. A very well documented web site Public Health Agency of Canada Biosafety site<sup>10</sup> reports regarding HIV survival outside host: HIV can remain viable in blood in syringes at room temperature for 42 days, and in blood and cerebrospinal fluid from autopsies for up to 11 days<sup>11</sup>. Although drying in the environment is known to cause a rapid reduction in HIV concentration, under experimental conditions, Cell-free HIV dried onto a glass coverslip in 10% serum can survive for longer than 7 days, depending on the initial titre<sup>12</sup>.

The US Center for Diseases Control (CDC) document<sup>13</sup> reports: "The most extensive study on the survival of HIV after drying involved greatly concentrated HIV samples, i.e., 10 million tissue-culture infectious doses per milliliter. This concentration is at least 100,000 times greater than that typically found in the blood or serum of patients with HIV infection. HIV was detectable by tissue-culture techniques 1-3 days after drying, but the rate of inactivation was rapid. Studies performed at CDC have also shown that drying HIV causes a

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<sup>10</sup> <http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/hiv-vih-eng.php>

<sup>11</sup> Abdala, N., Reyes, R., Carney, J. M., & Heimer, R. (2000). Survival of HIV-1 in syringes: Effects of temperature during storage. *Substance use and Misuse*, 35(10), 1369-1383

<sup>12</sup> Van Bueren, J., Simpson, R. A., Jacobs, P., & Cookson, B. D. (1994). Survival of human immunodeficiency virus in suspension and dried onto surfaces. *Journal of Clinical Microbiology*, 32(2), 571-574

<sup>13</sup> Recommendations for prevention of HIV transmission in health care settings. *MMWR*, 36 (Suppl 2) (1987), pp. 1S-18S. <http://www.cdc.gov/mmwr/preview/mmwrhtml/00023587.htm>

rapid (within several hours) 1-2 log (90%-99%) reduction in HIV concentration. In tissue-culture fluid, cell-free HIV could be detected up to 15 days at room temperature, up to 11 days at 37 C (98.6 F), and up to 1 day if the HIV was cell-associated."

## The genetically modified viral vector

### A2.8. Describe the 'original vector or vectors'.

Describe the 'original' vector, and indicate – with or without the help of bio-information analysis – in what way the original vector deviates from the original virus. For the application of a viral system (e.g. lentiviral particles), in addition to the viral (transfer) vector, you must also describe for example packaging and pseudotyping plasmids. Provide a description of the characteristics that determine the identity of these original vectors. This can be done on the basis of bio-information analysis, such as sequence analysis, alignments or phylogenetic analysis.

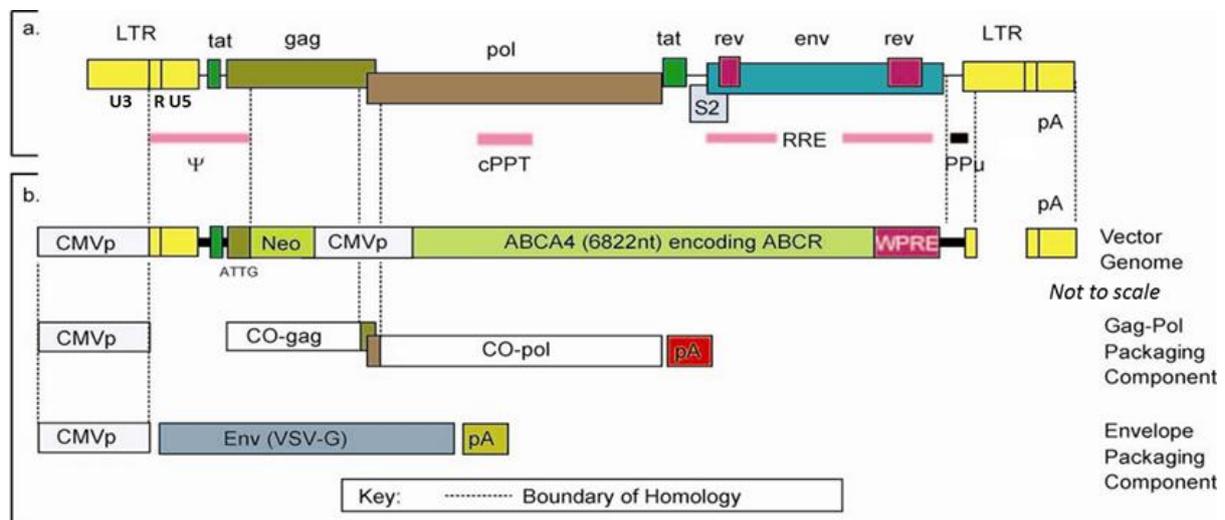


Figure 1: schematic representation of the wild type EIAV genome (1a), the vector system including SAR422459 vector genome, Gag-Pol packaging and Envelope packaging components (1b)

The SAR422459 vector only retains 861 nucleotides from the wild Type EIAV (figures 1a and 1b).

All genetic units <sup>314</sup>, encoding EIAV peptides have been removed, including 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; thought to be required for disease in horses<sup>15</sup>).

Other sequences on the viral nucleic acid that are involved in viral replication and gene expression and are embedded in the removed coding genes are also removed from the vector. This includes the central polypurine tract (cPPT), which is conserved among lentiviruses but its precise function is unknown and the Rev Response Element (RRE) which is

<sup>14</sup> Sellon DC, Fuller FJ, McGuire TC. The immunopathogenesis of equine infectious anemia virus as reference for various gene function in EIAV. *Virus Res* 1994; 32:111-138

<sup>15</sup> Fagerness AJ, Flaherty MT, Perry ST, Jia B, Payne SL, Fuller FJ. The S2 accessory gene of equine infectious anemia virus is essential for expression of disease in ponies. *Virology*. 2006 May 25;349(1):22-30 for role of S2

recognised by Rev protein and exports unspliced or partially spliced RNA's to the cytoplasm from the nucleus.

The retained sequences<sup>16</sup> are the ones necessary for the packaging of the vector genome in the production cell line, its reverse transcription and insertion in the target cell.

The EIAV vector production system is comprised of three plasmid constructs (figure 1b): the vector genome, the Gag/Pol packaging component and the envelope packaging component.

The vector genome component (EIAV Vector Genome (pONYKABCA4) comprises a Cytomegalovirus promoter (CMVp) which initiates transcription of the RNA vector genome at the beginning of the R region of 5' LTR, an EIAV LTR R-U5-packaging signal, a NeoR ORF, a 5'-UTR sequence, an ABCA4 cDNA, a WPRE element, an EIAV 3'PPT and self-inactivating (SIN) LTR.

ABCA4 cDNA contains 3 nucleotide synonymous variations with regards to reference sequences. The encoded sequence being preserved, no consequences are expected from these changes.

In addition, the plasmid backbone also contains a kanamycin resistance gene and a bacterial origin of replication (ori). These are not part of the vector genome and will not be integrated into the target cell genome.

Gag/Pol packaging component is expressed from pESGPK, a modified pCIneo-based plasmid<sup>17</sup> in which a codon-optimized gene encoding the EIAV Gag/Pol open reading frame is positioned downstream of a chimeric intron composed of the 5' -donor site from the first intron of the human  $\beta$ -globin gene and the branch and 3' -acceptor site from the intron of an immunoglobulin gene heavy chain variable region. This intron, as well as the one present in the pHGK vector described below, was inserted in the expression vector allow optimal expression of the transgene. Transcription is driven by the human cytomegalovirus (CMV) immediate-early enhancer/promoter and polyadenylation takes place at the SV40 late polyA signal. The plasmid backbone, containing the bacterial origin of replication (ori), has been modified to remove the ampicillin resistance cassette and replaced with the kanamycin resistance cassette.

In addition, the neomycin expression cassette has been removed. The use of the codon-optimized gene confers three benefits:

- High level expression of Gag/Pol is independent of Rev, thereby simplifying the vector system.
- The extent of homology within the "gag" region is minimized thereby eliminating the possibility of homologous recombination reactions between the Gag/Pol and vector genome components.
- The lack of EIAV sequence upstream of the Gag/Pol ORF and the alterations in the Gag sequence eliminate specific packaging of the Gag/Pol transcript and hence it's potential involvement in recombination or its transient translation in the target cell.

The envelope packaging component (expressed from pHGK) contains (in order from 5' to 3') a CMV promoter, an intron from rabbit beta-globin, an ORF for vesicular stomatitis

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<sup>16</sup> Rohll JB1, Mitrophanous KA, Martin-Rendon E, Ellard FM, Radcliffe PA, Mazarakis ND, Kingsman SM. Design, production, safety, evaluation, and clinical applications of nonprimate lentiviral vectors. *Methods Enzymol.* 2002;346:466-500.

<sup>17</sup> Promega: pCI neo mammalian expression vector protocol; Promega; revised 9/09; accessed August 2016; <https://www.promega.com/~media/files/resources/protocols/technical%20bulletins/0/pci%20neo%20mammalian%20expression%20vector%20protocol.pdf>

virus glycoprotein (VSV-G) which facilitates entry of vector particles into target cells, followed by a heterologous pA site<sup>18</sup>. VSV-G is the envelope glycoprotein from Vesicular Stomatitis Virus (VSV). VSV-G is commonly used to pseudotype lentiviral vectors as it conveys a broad tropism and because of the increased stability of the viral particles. In addition, the plasmid backbone also contains a kanamycin resistance gene and a bacterial origin of replication (ori).

The CMV promoter sequence in the transfer vector, originating from a Human Cytomegalovirus (CMV) (also known as Human herpesvirus 5 (HHV-5)) sequence) is similar to sequence present in GenBank accession number X17403.1. The CMV promoter sequences present in the pHGK and pESGPK packaging vectors also derive from a human CMV sequence. CMV promoter in pHGK vector is identical to the promoter present in the pHCMV-G vector<sup>18</sup>. CMV promoter in pESGPK is itself fully identical to the CMV promoter present in the pCI-NEO vector from Promega Corp. (GenBank Accession number U47120.2).

**A2.9. Regarding the pathogenicity of the original virus, have certain properties of the 'original vector' been altered that would determine the pathogenicity of the original vector?**

*Here, an elaboration could be given of the modifications made to the 'original vector', such as replication deficiency, which cause attenuation of the pathogenic properties.*

The GMO does not encode for any of the accessory genes known to be associated with pathogenicity in the wild type virus and no indications of vector pathogenicity have been observed during any nonclinical testing to date. In addition, the GMO is not replication competent which greatly limits the risk of dissemination of the GMO and eliminates its pathogenic potential (cf A2.8).

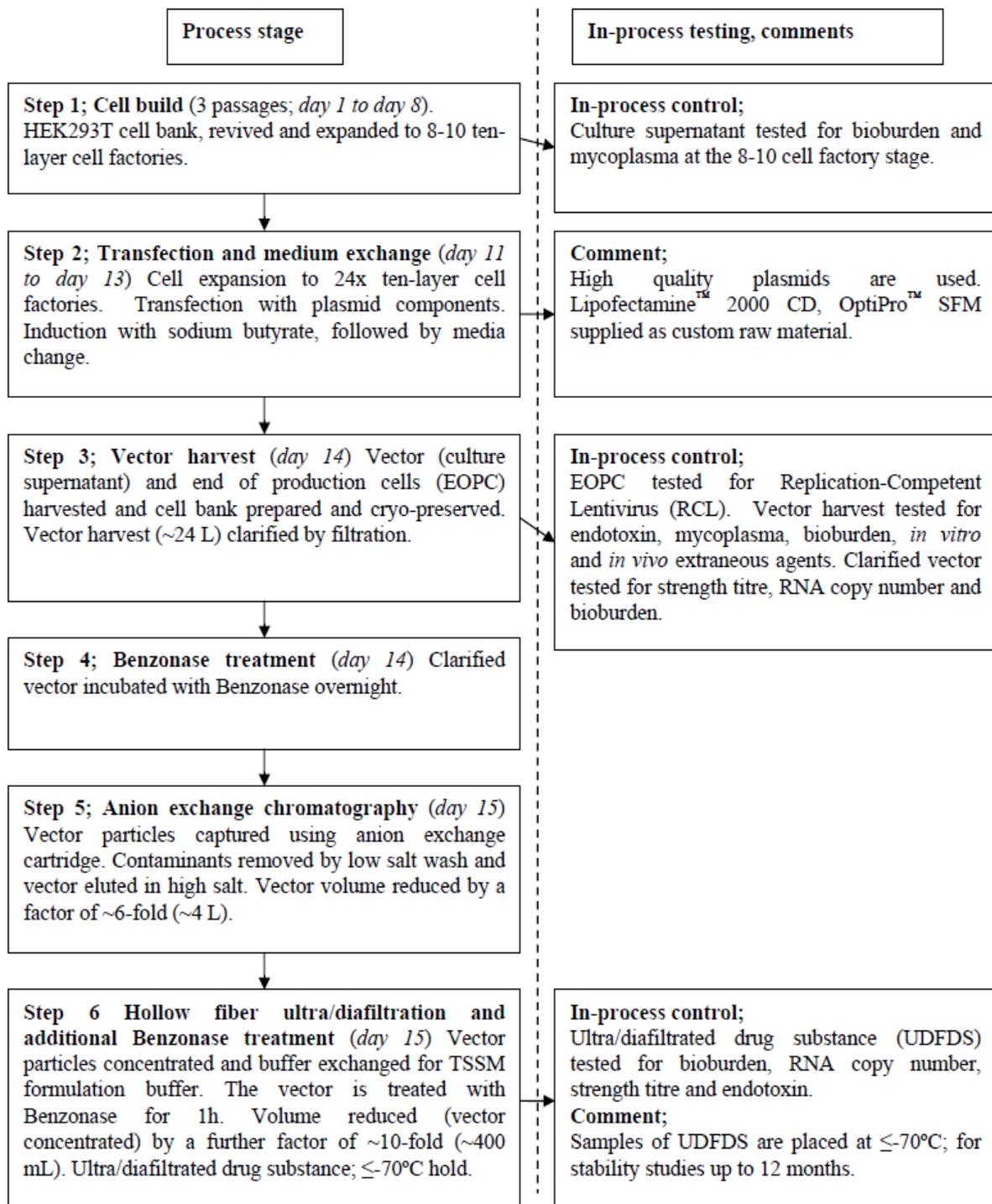
**A2.10. Describe the method of production of the clinical viral vector from the 'original vector or vectors'.**

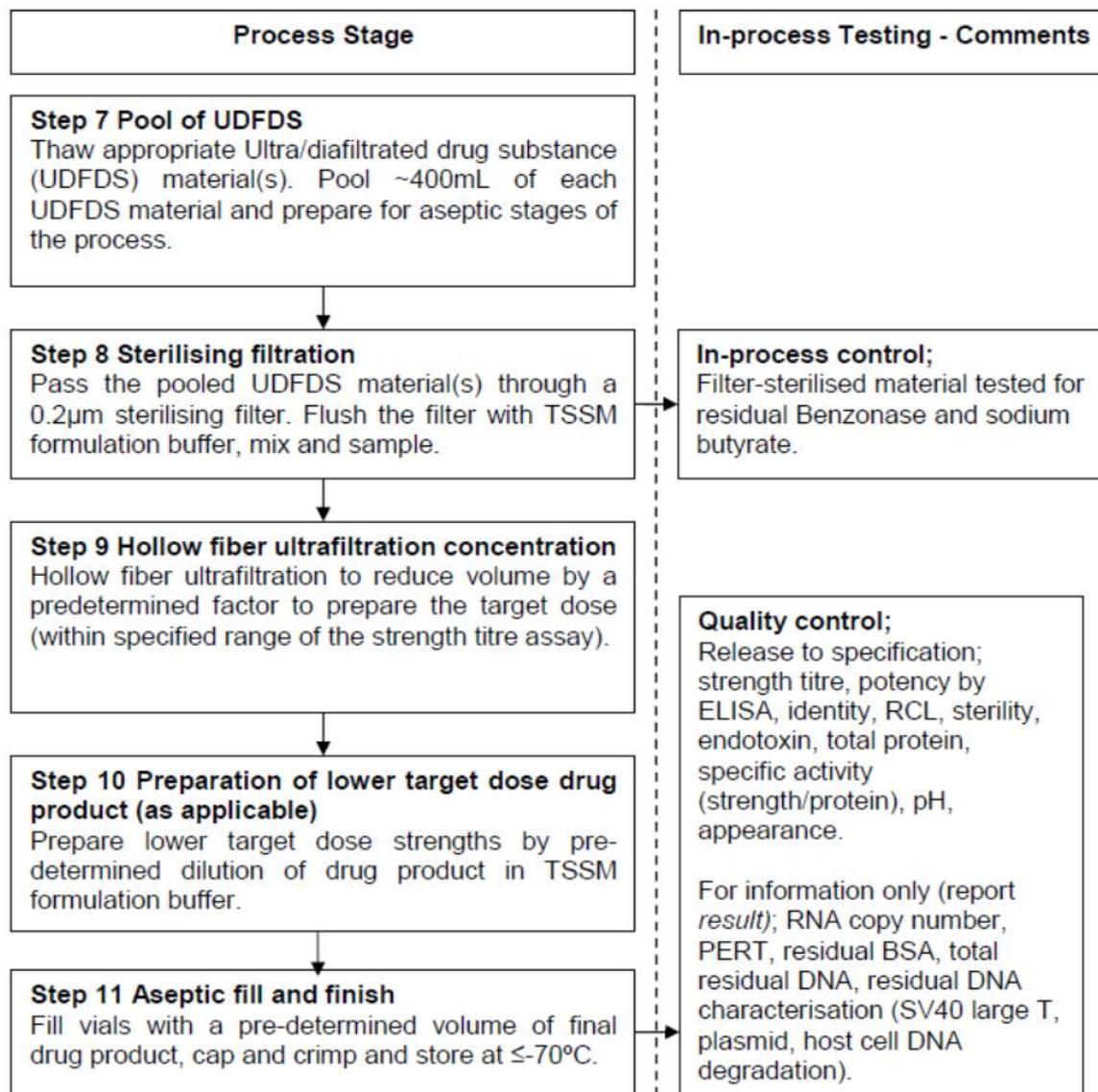
*Answer this question preferably by using a diagram that describes the various production steps.*

The diagram below describes the various production steps.

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<sup>18</sup> Yee et al., 1994. Generation of high-titer pseudotyped retroviral vectors with very broad host range. *Methods Cell Biol.* 43A:99-112





All the plasmids used in the manufacture of SAR422459 have been sequenced twice on each strand (four fold sequence coverage), revealing no differences from the expected sequence.

**A2.11. Describe the coding genes and the regulatory sequences present in the vector and in the DNA inserted into the vector.**

*A full description must be provided of the inserted or deleted genetic material, also discussing the functions of the sequences, for example:*

- *regulatory sequences, such as promoter, terminator, and enhancer sequences;*
- *structural genes;*
- *in case of insertion of a transgene: the function of the coded proteins in the donor organism (the donor organism is the organism from which the gene originally was isolated or in which it occurs naturally) and the expected function in the test subject;*
- *in case of deletion: the function of the deleted genetic material in the original organism;*
- *in case of point mutation(s): the effect of the point mutation or mutations on the function of the modified genetic material in the original organism and the expected function in the test subject;*

- whether the vector or the DNA inserted into the vector contains elements of which the origin or function is unknown.

The SAR422459 vector RNA genome (figure 2a) contains (in order from 5' to 3'):

- The EIAV LTR R-U5 region and  $\Psi$  region. The functions of these sequences have been described in section A2.8.
- An ORF coding for neomycin phosphotransferase II (NeoR) from Escherichia coli transposon Tn5. The presence of this ORF enables reliable manufacturing of high titre batches of lentiviral vectors in the absence of Rev gene, and this ORF has to be present downstream of the packaging signal ( $\Psi$ ) region, but upstream of the internal gene expression cassette for the target gene as discovered during development of this EIAV platform. Several sequences were tested for that purpose. The ORF coding for NeoR has been shown to be the most suitable candidate to ensure reliable production of high vector titers. It is not used as selection marker and it does not contain a promoter and downstream polyadenylation sequence.
- The Human Cytomegalovirus (CMV) immediate-early enhancer/promoter (CMVp). 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.
- The self-inactivating LTR U''-R regions (SIN LTR).

Upon reverse transcription and insertion in the target cell genome, the viral DNA genome (represented figure 2-b) unique modification is the duplication of the 3' LTR sequences at the 5' position).

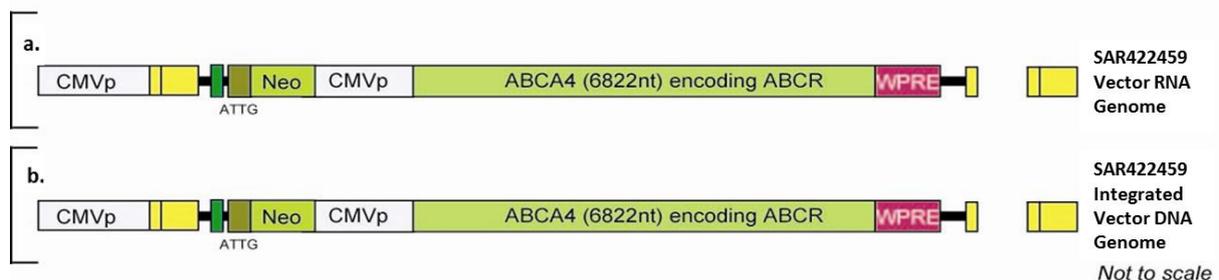


Figure 2: schematic representation of SAR422459 RNA genome (2a) and the Integrated DNA genome (2b)

As already described in previous section, sequences on the viral nucleic acid that are involved in viral replication and gene expression have been removed from the vector. This includes:

- The Gag gene which encodes a structural polyprotein
- The Pol gene which encodes the enzymes protease, reverse transcriptase, RNaseH, dUTPase, integrase. Gag and Pol are provided during production on a separate plasmid
- The Env gene which encodes envelope glycoprotein
- The tat Gene which encodes a transcriptional transactivator specific for viral enhancers contained within the LTR
- The Rev gene which encodes an RNA binding protein involved in RNA export to the cytoplasm
- The S2 gene which encodes a polypeptide of unknown function; thought to be required for disease in horses

Other sequences embedded in the previous genes are also removed

- the central polypurine tract (cPPT), which is conserved among lentiviruses but which precise function is still unknown
- the Rev Response Element (RRE) which is recognised by Rev protein and exports unspliced or partially spliced RNA's to the cytoplasm from the nucleus.

#### **A2.12. Provide a molecular characterization of the genetically modified viral vector.**

*A sequence analysis must be supplied for all inserted or deleted sequences, so that the precise location of the modification and, in case of insertion, the number of copies present and the orientation of the insertion cassette can be determined. The sequence to be submitted must contain the region of the intended modification as well as the recombination sequences used, also including the flanking sequences of the genome of the original organism. In the case of biological unrestricted GMOs, newly created fusion ORFs must be described and subjected to a bio-information analysis.*

*The complete genome of the viral vector must be characterized on a molecular level. This may be done according to a sequence analysis or a Southern blot analysis. During the production of a GMO, naturally occurring processes may lead to unintended modifications in the genome. These modifications may affect the fitness of the ultimate GMO and, thus, the results of the environmental risk assessment. Observed anomalies in the genome compared with the expected sequences, such as unexpected deletions, mutations or recombinations, must be described and interpreted. Phenotypic data may be used to support the data on the molecular characterization.*

*The sequence of the complete genome does not need to be submitted. A bio-information analysis must be carried out for the sequences and the annotated results must be presented. For more information, please refer to the COGEM advisory report CGM/130227-05.*

The description of the vector provided above derives from a full sequencing of the plasmid vector genome, performed on each batch of plasmid as part of the quality control. For each batch there is no deviation from the expected sequence.

The integrity of the vector genome at the level of the drug product is also quality controlled after reverse transcription of the RNA genome. The first set of primers amplifies a 817bp fragment between the CMV promoter and the ABCA4 cDNA. The second set of primers amplifies a 1422bp fragment from the ABCA4 cDNA to the WPRE region. The specific banding pattern for each digested PCR product after digestion with the restriction enzymes BssSI and XcmI is used to confirm the presence of the two amplified regions within vector genome.

With regards to the LTR which origin is already described in section A2.2, its sequence was corrected to include an extra nucleotide which was based on re-sequencing data and also confirmed by other publically available EIAV sequences of the same origin. This allows the use of a functional natural sequence.

Northern blotting was performed to characterize the packaged RNA using RNA extracted from vector particles. The internal (gene expression) transcript was also characterized by Northern blotting, using RNA extracted from transduced cells. Both characterizations were performed in parallel. Northern blotting analysis indicates that the correct length mRNA is generated and packaged into the SAR422459 viral particles and that following transduction the correct length mRNA transcript is made in the SAR422459 transduced cells. In addition, Southern blot analysis on SAR422459 transduced cells with various enzyme digests indicates that the integrated genome is of the correct size and has the expected integrated structure. The collective evidence from the Northern and Southern blotting indicated that the pONYKABCA4 vector leads to faithful delivery and integration of the expected gene

expression cassette within the genome of target cells, which in turn leads to the production of the expected gene expression transcript.

**A2.13. Describe the origins of the cells/cell lines in which the original viral vector is cultivated. Also indicate which of the genetic components of the cell could possibly cause complementation or recombination.**

*When answering this question, elaborate on the characteristics of all cell lines to be used. Describe which cell types this concerns as well as their origins (e.g. human kidney epithelial cells). Also discuss the possibility of the genetic material in the cells/cell lines causing a certain interaction with the original vector, such as by complementation or recombination.*

The Human Embryologic Kidney 293 T (HEK293T) cell line was developed from HEK293 cells<sup>19</sup> and was originally described in 1987<sup>20</sup>. The cell line was developed by transfecting a temperature sensitive SV40 large T-antigen mutant, tsA1609 into HEK293 cells. The HEK293 cells contain Adenoviral Type 5 (Ad5) DNA sequences (nucleotides 1-4344), that contain the E1a and E1b genes. HEK293T cells are more readily transfected than the original HEK293 line, and as such have been commonly used to produce high titre vectors by transient transfection.

The two sequences introduced in the 293T cell lines do not contain homology to the sequences contained in the parental virus, and a risk for homologous recombination is therefore excluded. The activities encoded by these sequences (Ad5 E1 region or SV40 largeT) also cannot substitute the sequences deleted from the EIAV to generate the parental vector.

The HEK293T cells are clear of HIV-1, HIV-2, HTLV-1 and -2, SIV and other non-human lentiviruses by *in vitro* (PCR) and *in vivo* assays.

**A2.14. Summarize the data in a diagram ('map') of the genetically modified organism. Also indicate any relevant helper sequences that may possibly be present.**

*Present clear maps of the genetically modified organism, such as plasmid maps, showing all the constituent parts of the vector. In this schematic depiction, the construction of the GMO must be clearly indicated.*

The SAR422459 vector RNA genome and DNA genome after reverse transcription and integration have been represented in figures 2a and 2b (see question A.2.11).

**A2.15. Indicate the degree to which the host range of the genetically modified viral vector has been or may be altered, relative to the original virus.**

*When answering this question, provide an argument that elaborates on the host range, host specificity and the tissue and cell tropism of the genetically modified viral vector, relative to the original virus. Also consider any modifications that were made in order to create the original vector.*

As already discussed in section A2.3, the parental EIAV virus has a very narrow host range, limited to equidae. The GMO vector particles are pseudotyped with the VSV glycoprotein to allow them to transduce the human photoreceptors (the intended target). Presence of the

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<sup>19</sup> Graham FL, Smiley J, Russell WC, Nairn R. Characteristics of a human cell line transformed by DNA from human adenovirus type 5. *J Gen Virol* 1977;36:59-72.

<sup>20</sup> DuBridg e RB, Tang P, Hsia HC, Leong PM, Miller JH, Calos MP. Analysis of mutation in human cells by using an Epstein-Barr virus shuttle system. *Mol Cell Biol*. 1987 Jan;7(1):379-87.

VSV-G envelop protein confers to the GMO a potentially broader host cell range<sup>21</sup>. The host range of the VSV includes humans, horses, cattle, pigs, mules, sand flies, grasshoppers, and rodents (list of VSV hosts as published for example on-line in the Pathogen Safety Data Sheets and Risk Assessment of Public health agency of Canada<sup>22</sup>). Although initial studies using pseudotyping with VSV-G demonstrated the non-mammalian retargeting of the vectors<sup>18</sup>, based on the numerous published studies using VSV-G pseudotyped lentiviral vectors, it is now also known that cells in most mammalian species including primates, pig, dog, cat, rabbit etc. can be also transduced<sup>23</sup>. However, in these transduced species the vectors would be replication incompetent as previously described.

#### **A2.16. What physiological (including pathogenic) effects may be caused by the genetically modified viral vector; and what are the available treatments?**

*Indicate which physiological processes may occur following the application of the GMO in the host.*

*A comparison must be made between its possible pathogenic properties and those of the original virus. In particular, the pathogenic properties that may be created specifically by the GMO should be considered.*

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 in transduced cells. This avoids viral spreading beyond the transduced tissues and induction of adverse pathogenic effects due to viral replication or presence of viral particles. The risk of the NeoR ORF present in the vector genome to trigger immune response or gene transfer in non-target cells has been evaluated in in-vitro and in-vivo studies. No evidence for immune response or gene transfer in non-target cells was found. In addition, the mechanism(s) for indirect transfer of NeoR resistance to bacteria from target cells (antibiotic resistance acquisition by pathogens after injection) is difficult to imagine, and would likely be highly inefficient.

The pharmacologic action of the GMO in host primary target cells, photoreceptors, is to allow expression of ABCA4 which is defective in the treated SMD patients<sup>2</sup>. As previously stated, the presence of this wild type ABCA4 is intended to correct for the defective transport of retinal compounds and the consequent formation of di-retinal compounds (such as pyridinium bis-retinoid [A2E], a major component of lipofuscin), which accumulate in SMD patients leading to the degeneration of PRs and retinal pigment epithelial (RPE) cells<sup>1</sup>  
<sup>24</sup>.

The lack of a physiologic or pathogenic effect resulting from the potential expression of ABCA4 in non-target tissues normally not expressing ABCA4 is postulated from:

- The absence of any toxicity in the eye demonstrated in the various toxicology species used during preclinical development of the product despite transduction and expression of ABCA4 in some RPE cells which do not normally express ABCA4.
- The absence of cytotoxicity demonstrated through a study which evaluated whether transduction of cells with the ABCA4 EIAV lentiviral vector had

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<sup>21</sup> Cronin et al, Altering the Tropism of Lentiviral Vectors through Pseudotyping; *Curr Gene Ther.* 2005 Aug; 5(4): 387–398.

<sup>22</sup> Pathogen Safety Data Sheets and Risk Assessment of Public health agency of Canada, <http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/stomatit-eng.php> consulted July 2015

<sup>23</sup> Farley et al, Factors that influence VSV-G pseudotyping and transduction efficiency of lentiviral vectors-in vitro and in vivo implications. *J Gene Med.* 2007 May;9(5):345-56

<sup>24</sup> Molday RS, Zhang K.. Defective lipid transport and biosynthesis in recessive and dominant Stargardt macular degeneration. *Prog Lipid Res.* 2010 Oct;49(4):476-92.

detrimental effects on the cell viability of human fibrosarcoma HT1080 and human retinal epithelial ARPE-19 cell lines, at multiplicity of infection (MOI) ratios of 0.4, 0.5, 4, 5, 10 and/or 20, in triplicate, based on the vector titers. Transduction of HT1080 cells with SAR422459 at MOIs of 0.5, 5 and 10 did not demonstrate any negative effect on proliferation. In ARPE-19 cells, a modest inhibition on cell proliferation was observed, but this was considered as nonspecific, and may have been a consequence of the high concentration of the VSV-G protein in the vector particles.

**A2.17. Indicate the possible transmission routes of the genetically modified viral vector.**

*Provide all the observed and assumed routes of transmission. Indicate the degree to which the transmission of the GMO could be made easier by helper functions, or by the presence of replication-competent virus. Furthermore, indicate the degree of impact on transmission due to the modification and the cells in which the GMO was cultivated or those infected with the GMO. Make a comparison with the original virus.*

The GMO may only be spread by 1) the entry of the GMO into non-target cells (i.e. non-retinal cells) in the intended subjects and 2) the entry of the GMO into cells in a non-target human or other species (accidental exposure). Once the GMO enters a cell it cannot be released or spread.

Entry of the GMO into non-target cells outside of the eye following the subretinal injection procedure is considered to have a low likelihood of occurrence as the GMO is inactivated by the human immune system (e.g. by potent cytotoxic T cell and antibody responses) and therefore its spread within the body will be limited to the injected eye as this site is immunoprivileged. The cytotoxic T cell response and antibody reaction will probably take place sometime after accidental exposure or subretinal injection to the patient, as the person would not have been exposed previously to the lentiviral vector. These reactions are assumed in view of the fact that anti-VSVG antibodies were generated following a second IV injection of SAR422459 in rabbits, and knowledge of the normal immunological mechanisms. Specific neutralizing assays for SAR422459 or more in depth investigations to the immune response noted in the rabbits have not been carried out, and so the potential involvement of complement in the immune response is unknown. However, VSV-G pseudotyped lentiviral vectors have been shown to be inactivated by the complement in human serum<sup>25</sup>.

Patients treated with SAR422459 have been sampled for blood and urine which has then been analysed by PCR for vector associated DNA (integrated vector) or RNA (non-integrated vector). So far, all samples (data up to 36 months following injection is available) have been negative for both vector associated DNA and RNA sequences. Further, immunogenicity testing has also been negative for SAR422459-associated antibodies (data up to 24 weeks following injection is available). Taken together, this data would suggest that the vector cannot get out of the eye following subretinal injection. This finding is supported by non-clinical studies with rabbits, where persistence, biodistribution and shedding studies demonstrated that SAR422459 remained within the ocular compartment after injection, and also did not cross into the contralateral eye. Further, this latter study and also a primate study showed that there is no significant seepage of vector from the injected compartment, and that there was no uptake in the gonads or other organs, such as the brain. For the rabbits only, immunological investigations demonstrated a positive response in around half the injected animals for some non-lentivirus associated proteins (i.e. VSV G2 envelope

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<sup>25</sup> DePolo NJ, Reed JD, Sheridan PL, Townsend K, Sauter SL, Jolly DJ, Dubensky TW Jr. VSV-G pseudotyped lentiviral vector particles produced in human cells are inactivated by human serum. *Mol Ther.* 2000 Sep;2(3):218-22.

protein and the HEK 293T-associated antigens). The presence of antibodies against the VSV-G2 protein and HEK293T-associated antigens in rabbits after injection may have been due to a short-term increase in choroidal capillary permeability in the subretinal bleb region following vector administration, which in turn may have led to escape of soluble, non-lentivirus associated proteins produced during manufacturing of the SAR422459.

Thereafter, in situations where vector could possibly escape from the eye, no spreading of the GMO can be expected because of the modifications incorporated in the viral vector as previously described in section A2.7 to A2.12. No replication competent particles have been observed during the SAR422459 vector production and also during the production of other vectors built from the same parental vector. Further SAR422459 is a self-inactivating vector with no transcription of encapsidation-competent RNA and that as it is derived from EIAV and as the wild type EIAV is not able to transduce human cells, no risk of recombination in target tissue exists.

Accidental exposure of non-target humans (e.g. healthcare personnel working on the study) is also considered to have a low likelihood of occurrence. Access to the GMO at the clinical site is restricted to qualified personnel and all site personnel will undergo comprehensive training as part of the site initiation. The GMO is inactivated if swallowed, will not infect cells through unbroken skin and is inactivated by human serum, so there are limited routes of exposure. Results from a nonclinical shedding study to assess the risk of shedding from the patients suggest that levels in measured samples are too low to pose a risk to healthcare and laboratory personnel.

No spreading of the GMO can be expected because of the modifications incorporated in the viral vector as previously described in section A2.7. to A2.12. No replication competent particles have been observed during the SAR422459 vector production and also during the production of other vectors built from the same parental vector.

Another more theoretical source of potential spreading for lentiviral vectors could be the co-infection of the transduced cell by a replication competent wild type virus. Such concern has been raised in the literature in the case of HIV based lentivirus but is of negligible concern here given that SAR422459 is a self-inactivating vector with no transcription of encapsidation-competent RNA and that as it is derived from EIAV and as the wild type EIAV is not able to transduce human cells, no risk of recombination in target tissue exists<sup>26, 27</sup>. Similar theoretical risks also exist with regard of human endogenous retroviruses (HERVs). HERVs sequences represent genomic "fossils" of ancient retrovirus insertion in vertebrates' genomes and account for 8% of the human genome<sup>28, 29</sup>. Although some full length provirus exists for representative of the HERV-K group, no replicating exogenous form of HERVs are known in humans<sup>30</sup> mostly because of accumulation of inactivating mutations or deletions in various components of the HERVs accumulated during the hundreds of thousands to millions years of genome evolution. In fact, a vast majority of HERVs sequences exists now in human genome as LTR only sequences<sup>31</sup>. With regards to potential recombination, human endogenous retroviruses are phylogenetically close to 3 retroviruses families, beta-, gamma-, and spuma- retroviruses, with low homology to EIAV<sup>30</sup>. The short remaining

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<sup>26</sup> Pauwels K, Gijssbers R, Toelen J, Schambach A, Willard-Gallo K, Verheust C, Debyser Z, Herman P. State-of-the-art lentiviral vectors for research use: risk assessment and biosafety recommendations. *Curr Gene Ther.* 2009 Dec;9(6):459-74.

<sup>27</sup> Rothe M, Modlich U, Schambach A. Biosafety challenges for use of lentiviral vectors in gene therapy. *Curr Gene Ther.* 2013 Dec;13(6):453-68.

<sup>28</sup> Löwer R, Löwer J, Kurth R.. The viruses in all of us: Characteristics and biological significance of human endogenous retrovirus sequences. *Proc Natl Acad Sci U S A.* 1996 May 28;93(11):5177-84.

<sup>29</sup> Hanke K, Hohn O, Bannert N. HERV-K(HML-2), a seemingly silent subtenant - but still waters run deep. *APMIS.* 2016 Jan-Feb;124(1-2):67-87.

<sup>30</sup> van der Kuyl AC. HIV infection and HERV expression: a review. *Retrovirology.* 2012 Jan 16;9:6

<sup>31</sup> Hughes JF, Coffin JM. 2004. Human endogenous retrovirus K solo-LTR formation and insertional polymorphisms: implications for human and viral evolution. *Proc Natl Acad Sci U S A* 101:1668–1672

portion of lentivirus genome in the SAR422459 vector makes recombination highly unlikely. Altogether, negligible risk is therefore also expected in term of potential mobilization by either trans-complementation, or recombination based on the defective nature of the both vector and HERVs.

### A3. Production of the GMO

#### A3.1. State under whose responsibility the production of the GMO is carried out.

**Answer:**

- Production will be under the responsibility of the applicant and forms part of this license application.
- Production will be by and under the responsibility of the applicant but does not form part of this license application:
  - A separate application for production will be submitted for contained use
  - Reference is made for the production to an existing license for contained use: (State here the number of the relevant GMO license)
  - Production will be under the responsibility of third parties. If production is in the Netherlands, please state the number of the relevant GMO license. Please state if production is outside the Netherlands.

Production of SAR422459 is performed outside of the Netherlands (by Novaseps rue des Prof. Jeener et Brachet, 12 Gosselies, 6041, Belgium). Production is under sanofi responsibility.

#### A3.2. During which steps of the production process does quality control take place, which test methods are used and how are the tests carried out?

*Give an overview of the production process of the GMO and describe the points in the production process at which quality control takes place. State which controls are carried out, the sensitivity of the tests and which methods are used for the controls.*

The quality control on the drug substance takes place at:

- The cell revival into tissue culture flasks and cell expansion into 8-10 ten-layer cell factories. The In process control are on Mycoplasma and bioburden.
- The transfection and medium exchange, cell expansion into 24 ten-layer cell factories and Plus a single control ten-layer cell factory.
- The vector harvest. The In process control are performed on endotoxin, mycoplasma, biodurben, in vitro extraneous agents, in vivo extraneous agents, bovine viral contaminant. The clarified vector is tested for strength titre, RNA copy number and biodurden.
- The Benzonaze<sup>®</sup> treatment.
- The anion exchange chromatography.
- The hollow fiber ultrafiltration/diafiltration and additional Benzonaze<sup>®</sup> treatment. The In process control on biodurden, RNA copy number, strength titre and endotoxin.

The quality control on the drug product takes place at:

- The pool of Ultradiafiltrated drug substance
- The Sterilizing filtration. The In process control on residual Benzonaze<sup>®</sup> and sodium butyrate
- The hollow fiber ultrafiltration concentration
- Aseptic fill and finish. The Quality control is the release to specification (strength titre, potency by ELISA, identity, Replication Competent Lentivirus, sterility, endotoxin, total protein, specific activity: strength/protein, pH, appearance)

The controls performed and the methods associated are listed in the table below:

Test	Method
Replication Competent Lentivirus	In house – details below
Identity	detection of the correct transgene by PCR

#### Replication Competent Lentivirus (RCL) testing on the viral vector

The absence of RCL in the vector is confirmed by a HEK293 cell-based test with PERT (Product Enhanced Reverse Transcriptase) detection (an indirect means to quantify vector particles). This test has several advantages over alternative assays formats, in that it is extremely sensitive and will detect reverse transcriptase from any retrovirus, enabling detection of RCL without prior knowledge of the virus.

It should be noted that the vector genome used for generation of SAR422459 had been modified so that all meaningful homology between the separated vector components has been removed (see A.2.8, A.2.12 and A.5.2). RCL is therefore considered only as a hypothetical possibility. To date no RCL has been detected in any batch manufactured.

SAR422459 is tested for RCL at two different stages of the manufacturing process: end of production cells (EOPC) and final drug product. A total of  $10^8$  representative EOPCs for each lot of final drug product and 5% of the purified final drug product is tested for RCL. RCL assay controls are a positive and a negative control as well as a spiked test article to test for inhibition. The PERT assay contains additional controls, include positive and negative controls. The cut-off for positive/negative PERT results is defined based on scrutiny of results from a series of PERT assay and cultured negative controls, since signals other than RT activity can contribute to a low level signal in the highly sensitive PERT assay.

#### Acceptance criteria:

- RT activity is detected in the positive control and spiked test article.
- RT is not detected in the negative control.
- RCL are absent if no RT activity is detected in the test article.
- If RT activity is detected in any of the test article flasks, additional testing is carried out to determine whether the RT signal was due to a replicating virus or a non-transferable entity. The additional testing involves inoculation of the filtered final passage supernatant fluid on fresh HEK293 amplification cells, followed by additional passages and PERT analysis.

#### Specificity:

The RCL assay control reactions all performed within expected limits.

#### Detection limit:

The most critical aspect of an RCL assay is that it is capable of detecting a minimal infectious dose of an RCL. This is accomplished by the use of a period in culture for amplification of replication competent virus, followed by the detection of RCL by the sensitive F-PERT assay. As no RCL was detected in any of the six (validation) assays performed, it is impossible to prove the detection limit based on detection of hypothetical RCLs. However, the positive control RCR RCL was used at a minimal infectious dose (80 PG-4 focus-forming units [FFU]); the minimum dose was calculated such that it was predicted to be detected in 100% of RCL assays). A pilot study, in which three different doses of ML V 4070A were used (8, 80, 800 FFU) that was performed prior to the full assay validation indicated that 8 FFU was detected.

With the highly stringent RCL testing method employed, there is an extremely low probability of a human dose of SAR422459 harbouring an RCL.

**Precision:**

The CT values obtained for positive controls in separate PERT analyses over a period of 12 months were similar across both assays.

**Robustness:**

The passage regime was specifically designed to ensure that the positive control and any putative RCL would be amplified, whilst ensuring that the RT activity from vector was removed.

Other quality control tests that are important for clinical have been performed by in house methods, including titre strength, RNA copy number, potency, residual benzonaze, residual sodium butyrate, specific activity and appearance. These quality controls are important for clinical use, but are not relevant for the environmental risk assessment.

**A3.3. Which criteria are imposed on a batch of the GMO before it is released for the application in question?**

*State which criteria are used to reject a batch.*

The drug product, before its release, is tested for strength, potency, identity, sterility, endotoxin, pH, appearance, replication competent virus, total protein content and specific activity such as:

- Absence of RCL has to be confirmed by a HEK293 cell-based test with PERT detection
- Strength is in the specification limits by quantitating the DNA that is integrated into the genome of target cells
- Potency and specific activity are above the specification limits
- Identity has to be confirmed (detection of the transgene by PCR)
- Sterility has to be confirmed
- Endotoxin level and total protein content are below specification limits
- Appearance has be confirmed by visual observation

All these criteria are defined as acceptance criteria for the release of batches of SAR422459. With the highly stringent RCL testing method employed, there is an extremely low probability of a human dose of SAR422459 harbouring an RCL.

## A4. Description of the research

### Administration

#### A4.1. How many test subjects will take part in the study?

*Here, the maximum number of test subjects to be treated should be indicated. This number can be higher than the intended number of test subjects. Please be aware of the fact that the number you enter limits the permit. For example, if you indicate that there will be 50 test subjects, then no more than 50 can be included in the study.*

Up to 500 patients will take part in the studies.

#### A4.2. Describe how the batch containing the GMO will be transported and prepared for administering to the test subjects.

*Describe how, after delivery, the GMO preparation will be transported to the hospital pharmacy for example. Also describe the way in which the GMO preparation will be handled, as well as the conditions under which they will be handled, in order to get the preparation ready to be administered. If this handling will be carried out under an existing permit for Contained Use (IG), the number of the GMO permit concerned must be stated. In addition, describe the way in which the GMO preparation will be transported to the test subject.*

The IMP will be delivered at a temperature of  $\leq -70^{\circ}\text{C}$ . The pharmacist will check the transport package (the IMP will not be unpacked at the pharmacy). The transport package with IMP will be transferred to the storage room. In the storage room, the IMP will be unpacked, checked and stored in a  $-80^{\circ}\text{C}$  freezer. This will be done within 30 seconds of removal from the transport packaging.

On the day of preparation, the correct number of vials is removed from the freezer. The vials will be transported to the MLII laboratory (less than a 5-minute walk) in a biosafe container (protected from light) according to Annex I of the GMO Regulations (G3 Regelung GGO). The IMP will be thawed at room temperature and prepared in the MLII laboratory. The prepared IMP (syringe) will be transferred at room temperature in a biosafe container (protected from light) to the operating room (less than a 5-minute walk). If the patient is ready to receive the treatment, the syringe will be taken from the container and the IMP will be administered. The syringe with the IMP will be used within three hours of removal from the freezer.

The used syringe will be discarded in accordance with the local hospital policy on genetically modified materials and biohazardous waste materials (I2 GMO waste procedures).

#### A4.3. How will the GMO preparation be administered to the test subject?

*State via which route and how the GMO is administered. Also state what aspects may affect the safety of human beings and the environment.*

SAR422459 will be administered to patients by subretinal injection. A subretinal injection of SAR422459 may result in a few transient ophthalmic changes, including vitreous and retinal opacities and/or retinal/choroid pigmentation. In non-clinical experiments, the severity of these changes was higher in the SAR422459 treated group when compared to the buffer-treated group; however, these changes did not result in any retinal complications. These types of changes are expected and occurred as a result of the physical separation between the neurosensory retina and the RPE at the time of injection. These observed ocular events were entirely attributable to the injection procedure and/or

administration of SAR422459 and consistent with what has been previously described and reported as common changes following subretinal injection of other lentiviral vector-based products. These events were not considered to be a source of additional risk to patients subretinally dosed with SAR422459. The administration of the IP will only be performed by qualified medical professionals in an operating room at an approved study site/facility using appropriate precautions, as described in A5.9.

**A4.4. Which doses will be administered and at what time points during the study will they be administered?**

*Indicate which doses of the GMO will be administered. Also provide an administration diagram, showing at what times they will be administered.*

Patients, including children 6 years of age or older, are planned to be administered with the highest dose of SAR422459 given to date in the worst eye, by a subretinal injection performed once on day 0 (surgery day).  
The administered dose range is  $10^4$  to  $10^7$  Transducing Units (TU).

**A4.5. What other medication will be administered to the test subject that may possibly affect the GMO to be administered?**

*Any medication that would affect the GMO and possibly also the environmental risk assessment should be named here. If, for example, a vaccination study is conducted, this may also include challenge with the wild-type virus.*

At this stage, there are no known effects of medications or diagnostic agents used during the procedure with the GMO. The diagnostic medications/agents that could be concurrently used with the GMO are fluorescein and dilating drops which are not known to affect the GMO. A standardized protocol for the use of perioperative antibiotics and anti-inflammatory medications such as steroids (topical, subconjunctival or systemic) will be followed. The perioperative medications are not known to affect the GMO.

## Sampling

**A4.6. Describe which of the samples taken from the test subject may contain GMOs.**

*Provide an overview of the samples and indicate whether GMO material is expected to be present in them.*

Samples taken from the patients are blood and urine.  
SAR422459 is not expected to be present in clinical samples. Staff should adhere to local Health and Safety guidelines applicable to biological sample collection, processing, and shipment. (I6\_Richtlijn veilig werken met biologische agentia.pdf)  
Patients treated with SAR422459, have been sampled for blood and urine which has then been analysed by PCR for vector associated DNA (integrated vector) or RNA (non-integrated vector). So far, all samples (data up to 36 months following injection is available) for all patients have been negative for both vector associated DNA and RNA sequences. Further, immunogenicity testing, has also been negative for SAR422459-associated antibodies (data up to 24 weeks following injection is available), with the exception of 2 patients found to have an antibody response against the VSV-G component of the vector. Taken together, this data would suggest that the vector is unlikely to get out of the eye following subretinal injection. This finding is supported by non-clinical studies with rabbits, where persistence, biodistribution and shedding studies demonstrated that SAR422459 remained within the ocular compartment after injection, and also did not cross into the contralateral eye.

Further, this latter study and also a primate study (following injection of SAR422459) showed that there is no significant seepage of vector from the injected compartment, and that there was no uptake in the gonads or other organs, such as the brain. Further, in the primates, there were no relevant immunological responses in sera.

The above mentioned non-clinical studies in rabbits and primates were pivotal combined GLP toxicology, biodistribution and shedding studies, performed following single subretinal injection of SAR422459 in rabbits and monkeys at  $1.4 \times 10^6$  or  $4.7 \times 10^5$  transducing units (TU)/eye, respectively. The studies demonstrated that the vector DNA persisted at the site of injection (eye) for at least 3 months in the rabbits, and that SAR422459 remained within the ocular compartment in both species and was not able to cross into the contralateral eye. Specifically, no vector was found in peripheral organs or gonads during the studies which lasted 6 months. However, following the subretinal injection in both species, there were some generally transient ophthalmic changes which did not evolve into any overt toxicity of the retina. All the observed ocular events were associated with the injection procedure and/or administration of SAR422459, and were consistent with what has been previously described following subretinal injection of other lentiviral vector-based products. In terms of immunological reactions, antibodies against the VSV-G envelope protein of the vector and HEK293T-associated antigens were present in rabbits only, but they did not translate into any toxicological findings.

The above findings are in agreement with those reported from the ongoing clinical trials with a similar vector, SAR421869, which is also being tested in patients following injection. Further, a non-clinical study performed following injection of SAR421869 to primates, showed that this vector remained in the eye and no immunological responses were detected in sera.

In terms of LOD for PCR, the following were used for the aforementioned non-clinical assays and for the clinical assays;

For the rabbit, the Lower Limit Of Quantification (LLOQ) for vector DNA was from 10 to 33 copies, with copies normalised to 1  $\mu\text{g}$  of DNA. For vector RNA in the rabbit the LLOQ was from 333 to 1000 copies.

For the primate, the LLOQ for DNA vector was from 10 to 1000 copies, depending on timepoint and tissue, with copies normalised to 1  $\mu\text{g}$  of DNA. For vector RNA, the LLOQ was 100 to 333 copies.

For patients, the RT-qPCR technique used to detect EIAV vector in fluid samples had a detection limit of 100 copies that is dependent on the volume of sample tested. The DNA qPCR assay was able to detect 10 copies/ $\mu\text{g}$  DNA (LLOQ).

For immunology in both non-clinical studies and patients, the ELISA method used was semi-quantitative and used a VSV-G peptide antibody to generate a standard curve to calculate the relative concentrations of EIAV vector associated antibodies in a test serum sample. Subsequently, the Western blot testing was a qualitative assay that was used to detect antibodies raised against the vector *in vivo*.

Regarding the ongoing clinical trials with SAR422459, a total of 23 patients have received a single subretinal injection of SAR422459 so far. Four SAE have been noted in total, of which only one (granulomatous panuveitis) was related to the test article. Prior to this case, minimal and transient inflammatory changes in the eye have been observed in the immediate follow-up of the injections in 16 patients, but they were related to the surgical procedure and were not IMP-specific. A specific antibody (Ab) response against the vesicular stomatitis virus G glycoprotein (VSV-G) component of the lentiviral vector was observed following an intermediate dose of SAR422459 in 2 of the 23 patients tested to date, but this was without any impact on the patient safety. Of note, no antibody response to the lentiviral vector was detected in the patient who developed the SAE described as granulomatous uveitis. No vector has been detected in any plasma, urine or blood samples collected so far.

Please refer to Section A4.7 for additional details.

#### **A4.7. Describe the method of sampling and how the samples will be subsequently processed.**

*In answering this question, also indicate how transmission of the GMO during sampling and testing will be prevented. For the subsequent processing, indicate what physical restrictions apply. In case the work concerned will be carried out in the Netherlands but is not part of the current permit application, then refer to the permit for work under Contained Use and provide the number of the GMO permit this concerns.*

Blood samples will be collected for immunology and for PCR to ensure effective monitoring of vector and product.

Urine samples will be collected for PCR to assess biodistribution.

SAR422459 is not expected to be detectable in the samples (LLOQ for PCR analysis of 10 copies of DNA for white buffy coat, and 100 copies of RNA for urine and plasma).

SAR422459 is not expected to be detectable in the samples (LLOQ for PCR analysis of 10 copies of DNA for white buffy coat, and 100 copies of RNA for urine and plasma).

For preclinical studies and clinical analysis, the following sample sizes were used for PCR;

- Preclinical and clinical samples; extraction of retroviral RNA from samples (e.g. csf, vitreous, swabs etc.); up to 4 mL of biological fluid is extracted, to give 60 µL eluent of which 55 µL is then DNaseI-treated in a final volume of 192 µL. Therefore, the assay is based on 8µL of extracted RNA per 0.15mL of biological fluid.
- DNA in buffy coat; the final result is adjusted to calculate the number of copies in 1 µg of sample. The extracted RNA will be analysed on basis of 8uL of viral vector RNA template per 0.14 mL of sample extracted.
- Quantifiable RNA results were finally recorded as copies of EIAV RNA per 1 mL of fluid.

Staff should adhere to local Health and Safety guidelines applicable to biological sample collection, processing, and shipment. In short, healthcare staff work aseptically and wear gloves and hospital clothing when taking the biological samples. In case of spills, normal disinfection procedures are followed (I2 Gene Therapy – basis for protocols). Samples are shipped in dedicated sample containers and transported in leak free plastic envelopes. Where necessary, samples are stored at -80°C in the kit boxes provided until shipment on dry ice to the analytical laboratory. Similar detailed procedures are described for preparation of the plasma and white buffy coat and collection of urine specimen.

Processing of the biological samples that is done locally will follow the normal Health and Safety guidelines for working with patient-derived material (I3 Basismaatregelen HIP).

#### **A4.8. How is the GMO preparation detected after being administered?**

*State, if applicable, when GMO components are detected during or after administration and why detection at that particular moment of the test is regarded as important. Describe the nature of the samples that are tested, the method used and the detection limit that can be achieved.*

The SAR422459 components are determined in blood and urine samples from patients using validated quantitative methods. The immunogenic potential of the SAR422459 components is determined from serum samples using a 2-tier approach. A semi-quantitative ELISA method is used for the screening of anti-SAR422459 components antibodies, followed by a qualitative Western Blot assay for the positive samples from the screening assay.

Oxford BioMedica (UK) Ltd. has developed quantitative reverse transcription polymerase chain reaction (RT-PCR) assays to detect the EIAV packaging signal in samples from patients as a highly sensitive measure for the detection of vector particles and transduced cells. The RT-qPCR technique can be used to detect EIAV vector in fluid samples and has a detection limit of 100 copies that is dependent on the volume of sample tested. The DNA qPCR assay is able to detect 10 copies/ $\mu\text{g}$  DNA (LLOQ).

The ELISA is a semi-quantitative method that uses a VSV-G peptide antibody to generate a standard curve that is used to calculate the relative concentrations of EIAV vector associated antibodies in a test serum sample. Western blot testing is a qualitative assay that is used to detect antibodies raised against the vector in vivo.

In patients, blood samples are collected for RT-PCR analysis at screening (28 days prior to Day 0/surgery) and at 60 minutes after surgery, at Day 1 and at Weeks 1, 2, 4, 12, 24, 36 and 48. Urine samples are collected for RT-PCR analysis at screening (28 days prior to Day 0/surgery) and at 60 minutes after surgery (where possible), at Day 1 and at Weeks 1 and 2. Samples for determination of anti-drug antibodies (ADA) are collected at baseline visit (Day -1) and at weeks 4, 12 and 24.

The sample collection schedule in patients allows detecting circulating SAR422459 sequences following subretinal injection, with potential peak on Day 1, followed by decay on the subsequent time points.

Immune response in animals is not predictive of the immune response in humans. The collection time points in patients were selected to allow detection of pre-existing ADA, transient and persistent ADA response as recommended in the recent White Paper on recommendation on assessment and reporting of clinical immunogenicity<sup>32</sup>.

## Waste management

### A4.9. Give an overview of the nature and quantity of the waste produced and describe how the waste will be disposed of.

*State which waste flows can be distinguished. State which waste flows may potentially contain the GMO and how the GMO is prevented from being released into the environment via the waste flows.*

The types of waste expected to be generated include general disposable surgical supplies (e.g. gloves, masks, gowns, dressings and swabs), injection device, vials and any 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 (a maximum of 500). Each patient is expected to generate a minimum amount of waste according to the list above.

GMO waste will be destructed by incineration or by local equivalent, in accordance with the local hospital policy on genetically modified materials and biohazardous waste materials and will be documented appropriately. Certificates of Destruction, or equivalent, must be completed for the used and unused vials, and copies maintained in the Trial Master File.

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<sup>32</sup> Shankar G, Arkin S, Cocea L, Devanarayan V, Kirshner S, Kromminga A, et al. Assessment and reporting of the clinical immunogenicity of therapeutic proteins and peptides-harmonized terminology and tactical recommendations. AAPS J 2014 Jul;16(4):658-73

## A5. Environmental risk analysis

### Environment-related information originating from earlier experiments

#### A5.1. Describe the results originating from earlier (pre-) clinical studies with the GMO and which are important for the environmental risk assessment.

*In answering this question, you should elaborate on results that were achieved using an identical or similar GMO, if these are relevant for the environmental risk assessment of the present application. Important data include data on shedding, duration of latent presence of the vector/GMO, transmission of the vector/GMO, and possible interaction with other micro-organisms (including viruses). For each of these results, describe the trial set up (e.g. doses used, method of administration, detection test and detection limit) and the relevance to the work in the present permit application.*

For the environmental risk assessment, two different validated PCR assays, developed by Oxford BioMedica, were employed to measure EIAV packaging signal ( $\Psi$ ) sequences to determine vector bio-distribution, shedding and persistence in biological samples from non-clinical species and human patients. Specifically, the biodistribution of SAR422459 vector was measured by analyzing DNA extracted from tissue (retina, gonads, heart, brain, liver etc. for non-clinical studies) and buffy coat samples (clinical and non-clinical studies) using a real-time PCR-based (PCR) assay for the presence of SAR422459-derived EIAV packaging signal ( $\Psi$ ) DNA sequences. Vector persistence was assessed by analyzing RNA extracted from plasma samples (clinical and non-clinical studies) using a reverse-transcriptase (RT) real-time PCR-based (qRT-PCR) assay for the presence of SAR422459-derived EIAV packaging signal ( $\Psi$ ) RNA sequences. In addition, vector shedding was assessed by analyzing RNA extracted from urine (clinical studies), and vitreous fluid, swabs of tears and saliva (the latter all for non-clinical studies only) using the qRT-PCR assay for the presence of SAR422459-derived EIAV packaging signal ( $\Psi$ ) RNA sequences. The same PCR method was used for the detection and quantification of EIAV  $\Psi$  DNA sequences in all tissues/fluids from rabbits, monkeys and human patients given SAR422459, and similarly the same qRT-PCR method was used for the quantification of EIAV  $\Psi$  RNA sequences in samples from all species given SAR422459.

The RNA and DNA from the various study samples were prepared according to validated SOP procedures. The same methods were used for preparing the DNA for analysis from the rabbit, monkey and human samples, and similarly the same method was used for preparing RNA in samples from the different species.

The primers and FAM labelled probes used in the analyses were the following;  
Forward primer sequence; ATT GGG AGA CCC TTT GAC ATT G  
Reverse primer sequence; ACC AGT AGT TAA TTT CTG AGA CCC TTG TA  
Probe sequence; (FAM)-CAC CTT CTC TAA CTT CTT GAG CGC CTT GCT-(TAMRA).  
TaqMan® Universal PCR and One-Step RT-PCR Master Mix Reagents were used.

The test DNA samples were assayed using 17 $\mu$ I template in 50 $\mu$ l PCR reaction, alongside controls to monitor levels of contamination and PCR inhibition. Dilutions of a plasmid standard (pONY8.OZ)<sup>16</sup> to specified copy numbers was used to construct a standard curve. The standard curve was used for quantitation of EIAV  $\Psi$  DNA copy numbers in the test DNA samples and as an indication of reaction efficiency. Seven standard samples of pONY8.OZ at known concentrations (1x10<sup>6</sup>, 1x10<sup>5</sup>, 1x10<sup>4</sup>, 1x10<sup>3</sup>, 1x10<sup>2</sup>, 33 and 10 copies of pONY8.OZ per reaction) were used for quantification and to check assay efficiency.

Extracted viral RNA samples were analysed after treatment with DNase I to remove residual DNA. Each 25µl PCR reaction contained the equivalent of 8µl of template RNA. There were 6 standards assayed in duplicate for the RT reactions; 1,000,000, 100,000, 10,000, 1,000, 333 and 100 copies of EIAV Ψ/CMV RNA per reaction.

Acceptance criteria;

Standards; the Ct (cycle threshold) of the (plus RT – for RNA) standard replicates must vary by less than or equal to 1.0 cycle, to be included in the construction of the standard curve. The trend line of the standard curve ( $y=mx+b$ ) must have an m value of between -3.0 to -3.8, and an  $R^2$  value of 0.98 or above. A minimum of three different standards must be used to construct the standard curve. The range of IVT RNA or plasmid copy numbers used to construct the standard curve set the upper and lower quantification limits of the assay. Test samples; results were recorded as copy numbers detected, based on the mean Ct values for each test sample referenced to the standard curve trend line. For quantification of (plus RT – for the RNA analysis) reactions; the difference between the Ct values for replicates, should be less than or equal to one Ct and the mean Ct value must lie within the linear range of the assay. If the Ct values for a given sample is outside the upper limit of quantification they must be analysed again following dilution by a factor suitable to ensure that the result will lie within the limits of quantification. Ct values returned from test samples must be m Ct values lower than any Ct values below 40 detected in NTC (no template control) and/or IPC (in process control) reactions.

Appropriate negative controls and DNA contamination controls (for the RNA analysis) were included as relevant. IPC were used to check for contamination during template preparation. NTC were used to check for contamination during template addition and also to monitor PCR inhibition.

With respect to the LOD for the PCR assay, this typically 10 copies of DNA per 50µl PCR reaction. The LOD for the qRT-PCR assay was typically 100 copies of RNA per 25µl PCR reaction. The LLOQ were determined in each assay depending on the lowest standard point that met the SOP acceptance criteria (the Ct values of duplicate standard points should be within 1Ct. If they were not within 1Ct they were removed from the standard curve). The starting sample sizes were typically up to 4 mls of biological fluid analysed in the qRT-PCR assay (RNA) (in plasma, urine, tears etc., in all species as relevant – though for tears and vitreous, the sample size was around 0.14 mL). For DNA (in buffy coat and organ/tissue samples such as liver, brain spleen optic nerve etc., in all species), the starting sample was up to 200µL; the sample analysed was normalised to 1µg DNA per reaction where sufficient sample was available.

The LLOQ values (copies of DNA or RNA per reaction) at the different timepoints for all the tissues analysed by q(RT)-PCR for the GLP rabbit and monkey single subretinal dose 6-month studies with SAR422459, and also for all samples from patients in the current TDU and LTS clinical trials with SAR422459, together with sample size and volume ranges, are shown in the tables below:

**Non-clinical  
Rabbit study**                      **Treated  
Group 2**

<b>Sample type</b>	<b>Timepoint analysed</b>	<b>Assay LLOQ</b>	<b>Sample volume range</b>
Plasma	D2	333	1.25 to 1.4 mls
Saliva swabs	D2	333	0.14 mls
Right eye tear swabs	D2	333	0.14 mls
Right eye tear swabs	D5	333	0.14 mls
Vitreous fluid	D3	333	0.14 mls
Plasma	D5	333	1.2 to 1.6 mls

Right eye tear swabs	D15	333	0.14 mls
Vitreous fluid	D8	333	0.14 mls
Vitreous fluid	D29	1000	0.14 mls
Buffy Coat	D2	33	103 to 1000 ng of DNA
Retina	D3	33	1000 ng of DNA
Sclera	D3	33	379 to 682 ng of DNA
Optic nerve	D3	33	447 to 548 ng of DNA
Optic Chiasm	D3	10	344 to 525 ng of DNA
Testis	D3	10	1000 ng of DNA
Ovary	D3	10	1001 ng of DNA
Liver	D3	10	1002 ng of DNA
Spleen	D3	33	1003 ng of DNA
Brain	D3	33	251 to 322 ng of DNA
Retina	D8	10	675 to 1000 ng of DNA
Sclera	D8	10	181 to 430 ng of DNA
Optic nerve	D8	10	322 to 624 ng of DNA
Optic Chiasm	D8	10	174 to 367 ng of DNA
Optic nerve	D29	33	243 to 422 ng of DNA
Retina	D29	33	1000 ng of DNA
Sclera	D29	33	137 to 432 ng of DNA
Retina	D92	10	753 to 1000 ng of DNA
Sclera	D92	10	194 to 307 ng of DNA
Optic nerve	D92	10	218 to 387 ng of DNA

**Non-clinical  
Monkey study**

**Treated  
Group 2**

<b>Sample type</b>	<b>Timepoint analysed</b>	<b>Assay LLOQ</b>	<b>Sample volume range</b>
Plasma	D2	333	1.2 to 2.3 mls
Saliva swab	D2	333	0.14 mls
Right eye tear swab	D2	333	0.14 mls
Plasma	D3	100	1.28 to 1.75 mls
Plasma	D5	333	1.2 to 2.0 mls
Buffy Coat	D2	33	52 to 429 ng of DNA
Buffy Coat	D3	10	30 to 714 ng of DNA
Buffy Coat	D5	33	407 to 983 ng of DNA
Optic Chiasm	D92	33	330 to 454 ng of DNA
Optic Nerve	D92	33	221 to 581 ng of DNA
Ovary	D92	33	1000 ng of DNA
Testis	D92	10	1000 ng of DNA
Liver	D92	10	837 to 1000 ng of DNA
Spleen	D92	10	1000 ng of DNA
Brain	D92	10	254 to 297 ng of DNA
Buffy Coat	D8	33	1000 ng of DNA
Buffy Coat	D15	33	284 to 526 ng of DNA
Buffy Coat	D29	1000	301 to 740 ng of DNA
Buffy Coat	D92	10	289 to 1000 ng of DNA

**Clinical studies TDU13583**

Sample type	Timepoint analysed	Assay LLOQ	Sample volume range
Plasma	ALL	100 to 333	0.9 to 3.2 mls
Buffy Coat	ALL	10 to 100	17 to 1000 ng of DNA
Urine	ALL	100 to 333	3.93 to 4.0 mls

**Clinical studies LTS13588**

Sample type	Timepoint analysed	Assay LLOQ	Sample volume range
Plasma	ALL	100 to 333	0.98 to 4.0 mls
Buffy Coat	ALL	10 to 100	18 to 1000 ng of DNA

With respect to non-clinical studies, the biodistribution and shedding of SAR422459 was investigated over a 3-month period in rabbits and macaques, as part of the respective 6-month GLP toxicology studies, following subretinal injection of SAR422459.

Tissues were analysed for vector RNA and DNA as mentioned above. SAR422459 DNA was primarily detected in the target retina (including the choroid layer) and some other ocular structures in the rabbit study (which had multiple tissue biodistribution sampling points), i.e. the sclera and optic nerve tissues. The average copy numbers detected were  $3.42 \times 10^4$  on Day 3 reducing to  $3.51 \times 10^2$  copies on Day 92 for the retina/choroid, and were in the ranges of  $4.73 \times 10^2$  to  $3.93 \times 10^3$  for the sclera and  $1.41 \times 10^2$  to  $4.52 \times 10^2$  for the optic nerve during the study. No vector was detected in the brain, gonads, liver, spleen, buffy coat, or saliva, even when the vector titers were at their highest, or in any of the uninjected contralateral rabbit eyes.

Vector RNA was detected in the plasma for 1/6 samples on Day 2 only at below the background level (83 copies detected in the sample) and the vitreous fluid (average,  $4.85 \times 10^8$  copies on Day 3 for 6/6 samples,  $1.38 \times 10^6$  copies on Day 8 for 5/6 samples, and below the Lower Limit Of Quantification (LLOQ) of 1000 copies on Day 29 for 1/6 samples; 52 copies detected in the sample), and tear swabs (mainly below LLOQ of 333 copies on Days 2 and 5, for 2/6 and 1/6 samples, respectively; 250,  $1.4 \times 10^5$  and 47 copies detected, respectively). The positive tear swab samples are believed to represent contamination of the ocular surface following the subretinal injection of SAR422459.

For the primate, SAR422459 DNA was detected in buffy coat samples from most SAR422459 treated animals at non-quantifiable levels throughout the study (around 33 to 1000 copies, depending on timepoint; copies detected were in the range 1 to 184), though levels were quantifiable on Days 2 ( $4.17 \times 10^2$  copies) and 5 ( $9.66 \times 10^1$  copies) in 1/6 animals. For all these animals, there was no evidence for a generalized increase in vector copy number in the buffy coat over time. SAR422459 was not detected in the brain, ovary, testis, liver, spleen, optic chiasm, and optic nerve on Day 92 (single sampling point in the study for tissue biodistribution). However, vector DNA below the LLOQ (10 copies) was detected in the liver from 2/6 animals (copies detected were 1) at this timepoint. Further, vector RNA at generally below the LLOQ (100 or 333 copies) was detected in plasma samples from 2/6 animals on Days 2 and 3, post-injection only (copies detected were in range 26 to 535). The positive responses in the primate in non-ocular tissues may be indicative of a damaged retina during dosing and subsequent vector leakage from the subretinal bleb into the blood supply at the back of the eye. No vector was detected in saliva. Overall, the data from both species show that the vector was retained in the injected retina/eye, and did not disseminate out to the systemic circulation and environment via

body fluids (tears, urine) following injection within a closed compartment (e.g. the eye). These findings are in accordance with those from other EIAV vectors, such as Retinostat (given by subretinal injection), SAR421869 (given by subretinal injection) and ProSavin (given by intrastriatal injection). Gene expression studies, using either reporter genes or ABCA4, have demonstrated that EIAV vectors are capable of transducing retinal cells, and that the transgene expression levels are maintained for at least 16 months.

Overall, the in life animal studies with SAR422459 have shown that the injected vector remains essentially in the injected eye. Further, in the same studies, except for the first two or three days post injection, no vector was present in plasma and at no time in the study was vector found in the gonads, suggesting that germline transmission is unlikely.

In the clinical setting in theory, if there is damage to the blood-retina barrier as a result of the subretinal injection or the vector is injected directly in to the choroid the vector could potentially access the blood stream. Therefore, to reduce this risk of vector dissemination the administration of SAR422459 will be performed by licensed vitreoretinal surgeons with prior experience performing the subretinal injection procedure. However, even in the event that the vector breaches the blood-retinal barrier or reaches the bloodstream as a result of an ocular surgical complication, the potential systemic consequences for the patient are expected to be minimal as the vector will be inactivated by the human serum and lacks the capacity to reconstitute competent viral particles or replicate. The major consequence of concern in the setting of an ocular complication associated with the subretinal injection in SMD patients is not the risk of systemic dissemination of the vector but rather the potential loss of visual function resulting from damage to the retina or other ocular tissues.

In addition to information given on SAR422459 (clinical and non-clinical), reference was also given to SAR421869 (UshStat), Retinostat and ProSavin (all non-clinical). In terms of vector, all quoted studies are relevant to the current study in that they use exactly the same vector as SAR422459 or they use vectors (i.e SAR421869, Retinostat and ProSavin) derived from the same lentiviral vector platform as SAR422459, in that only the coded protein (MYO7A for SAR421869, endostatin and angiostatin for Retinostat, and dopamine for ProSavin) is changed. Further, except for ProSavin which was given as a single intrastriatal injection, all other vectors mentioned including SAR422459 have been given as a subretinal injection to patients and animals (rabbits and/or monkeys), as for SAR422459. In terms of dosages for the current study, the dose levels used are based on the MTDs from the subretinal SAR422459 non-clinical studies in rabbits and monkeys. Specifically, all dose-levels used in the current clinical trial (after allometric scaling) are below the MTD in the rabbit, and either below (dose 1 and 2) or almost equivalent (dose 3) to the MTD in the monkey.

Further, the MTDs for SAR422459 in rabbits and monkeys are comparable to those of RetinoStat in the same species, and for SAR421869 in monkeys. Finally, in a pilot toxicology study with SAR422459 in NHPs, the no observed adverse effect level (NOAEL) for SAR422459 gives an allometric scaled NOAEL in humans which is similar to the NOAEL for RetinoStat in both rabbits and NHPs.

The one-day period, for protecting the eye with a bandage after injection, has been selected as it is standard of care (routine clinical practice) following invasive ophthalmic procedures in human patients, and in addition because it has been shown to be adequate to avoid contamination/shedding into the environment of the vector based on non-clinical studies. In both monkeys and human patients, following the subretinal injection of SAR422459 into the eye, no or negligible contamination of the ocular surface is anticipated after the subretinal injection procedure and no leakage via the tears is possible as there is no connection between the subretinal administration point and the tear extra-ocular tear producing organs. The absence of ocular contamination has been confirmed in non-clinical toxicity studies conducted with SAR422459, and similar vectors SAR421869 and Retinostat, following subretinal injection to rabbits and/or monkeys. Specifically, all these studies showed that there was no quantifiable signal for vector in tears from the injected eye at

one day after injection (or two days for SAR421869 in monkeys – as was the first sampling time point after injection in this study), except for 1/6 rabbits ( $1.4 \times 10^5$  copies on study Day 2) in the single subretinal dose 6-month study with SAR422459. However, as the monkey has the eye which is most similar to that of humans, the absence of vector in tears at one day after injection for primates given SAR422459 lends the strongest support to maintaining a bandage over the injected eyes in patients given this vector for only one day after surgery with respect to avoiding any environmental risks.

The presence of vector in tears in clinical studies has not been studied for SAR422459, SAR421869 or Retinostat.

Further with respect to contamination/shedding into the environment, SAR422459 is not expected to survive in a natural environment and is replication incompetent and therefore is not infectious, pathogenic, virulent or able to colonise other organisms either directly or via a vector. In addition, the GMO does not encode for any of the accessory genes known to be associated with pathogenicity in the wild type virus and no indications of vector pathogenicity have been observed during nonclinical testing.

The SAR422459 components are determined in blood (as white buffy coat and plasma) and urine samples from human patients using validated quantitative methods. The immunogenic potential of the SAR422459 components is determined from human serum samples using a 2-tier approach. A semi-quantitative ELISA method is used for the screening of anti-SAR422459 components antibodies, followed by a qualitative Western Blot assay for the positive samples from the screening assay.

Specifically, analysis of SAR422459 components in patient fluids (white buffy coat, plasma and urine) is performed by Oxford BioMedica (UK) Ltd., using the validated PCR methods for vector RNA and DNA as mentioned above..

No vector components have been detected so far in 22 patients treated in Cohorts 1-5 of the study TDU13583 by PCR (i.e., all levels were below the lower limit of detection in blood, at all timepoints from screening to Week 48, and in urine, from screening to Week 2), which is the hallmark for any biodistribution, persistence, and shedding of the lentiviral vectors. The LOQs for the PCR analysis were 10 to 100 copies of DNA for buffy coat and 100 to 333 copies of RNA for plasma and urine. In addition, no IMP related SAEs have been reported to date in the SAR422459 clinical program.

In the above section, and elsewhere in the document, reference is made to non-clinical and/or clinical studies performed with ProSavin, RetinoStat and UshStat. General public information on these lentiviral vectors can be found in the literature<sup>33,34,35,36</sup>.

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<sup>33</sup> Zallocchi M, Binley K, Lad Y, Ellis S, Widdowson P, Iqbal S, Scripps V, Kelleher M, Loader J, Miskin J, Peng YW, Wang WM, Cheung L, Delimont D, Mitrophanous KA, Cosgrove D. EIAV-based retinal gene therapy in the shaker1 mouse model for usher syndrome type 1B: development of SAR 421869. *PLoS One*. 2014 Apr 4;9(4):e94272.

<sup>34</sup> Binley K, Widdowson PS, Kelleher M, de Belin J, Loader J, Ferrige G, Carlucci M, Esapa M, Chipchase D, Angell-Manning D, Ellis S, Mitrophanous K, Miskin J, Bantsev V, Nork TM, Miller P, Naylor S. Safety and biodistribution of an equine infectious anemia virus-based gene therapy, RetinoStat(®), for age-related macular degeneration. *Hum Gene Ther*. 2012 Sep;23(9):980-91.

<sup>35</sup> Palfi S, Gurruchaga JM, Ralph GS, Lepetit H, Lavisse S, Buttery PC, Watts C, Miskin J, Kelleher M, Deeley S, Iwamuro H, Lefaucheur JP, Thiriez C, Fenelon G, Lucas C, Brugières P, Gabriel I, Abhay K, Drouot X, Tani N, Kas A, Ghaleh B, Le Corvoisier P, Dolphin P, Breen DP, Mason S, Guzman NV, Mazarakis ND, Radcliffe PA, Harrop R, Kingsman SM, Rascol O, Naylor S, Barker RA, Hantraye P, Remy P, Cesaro P, Mitrophanous KA. Long-term safety and tolerability of ProSavin, a lentiviral vector-based gene therapy for Parkinson's disease: a dose escalation, open-label, phase 1/2 trial. *Lancet*. 2014 Mar 29;383(9923):1138-46.

<sup>36</sup> Hacker CV, Vink CA, Wardell TW, Lee S, Treasure P, Kingsman SM, Mitrophanous KA, Miskin JE. The integration profile of EIAV-based vectors. *Mol Ther*. 2006 Oct;14(4):536-45.

## Risk analysis

### This is the most important aspect of the whole application!

*Give a detailed assessment of the expected effects of the GMO on human health and the environment on the basis of the answers to the above questions and in accordance with Appendix II of EU Directive No. 2001/18/EC and the corresponding guidance notes of the European Commission (2002/623/EC). Please take into account any direct, indirect, immediate and delayed effects of the GMO on human health and the environment.*

*A risk analysis should be carried out for each GMO included in this notification, as well as for combinations of the GMOs, if any. The risk analysis must cover the effects of the GMOs that are due to interactions between the GMOs and the environment(s) where they are introduced or where they may end up under the present activities. The effects in question are those which are relevant to safety to human health and the environment. Section A6 of this form describes those aspects which must at least be taken into consideration.*

*The risk analysis should include at least the aspects mentioned in Annex 1 of this form. The risk analysis includes the following sections, which should be given in the same order as shown below (see questions A5.2 – A5.5):*

- 1. List of the likely adverse effects;*
- 2. Estimate of the likelihood of these effects actually taking place;*
- 3. Evaluation of the risks and an estimate of the severity of the effects, based on Items 1 and 2 above. The severity can be estimated by comparing it with the severity assigned to similar risks, such as for example the effects that occur with non-GMOs in similar situations ('baseline principle');*
- 4. If you have concluded in Point 3 that the risk is high, you are requested to examine what measures can be used to mitigate the risk;*
- 5. Final conclusion of the risk analysis, stating the risk management measures that will be employed, and a conclusion as to the acceptability of the risks when these measures are put into operation.*

#### **A5.2. State which potentially harmful effects may be linked to exposure of human beings or the environment to the GMO.**

*Describe here the effects on human beings and the environment that could occur as a consequence of the use of the characteristics of the GMO described in earlier sections of this application. The aim here is identifying the hazards; the following questions deal with the chance that these hazards will actually occur.*

##### **A5.2.1 Potential harmful effects linked to the exposure of the environment to the GMO**

- No

##### **A5.2.2 Potential harmful effects linked to the exposure of human beings to the GMO**

- Immune response
- Mutagenesis and carcinogenicity and potential for delayed AEs

##### **A5.2.1 Potential harmful effects linked to the exposure of the environment to the GMO**

Even though there is a theoretical risk of spreading of the GMO into the environment via accidental exposure of healthcare professional/ laboratory personnel or other persons or via accidental needle stick injuries, or in case of broken vials, no harmful effect is expected.

EIAV has been extensively modified in order to produce SAR422459 so the GMO lacks the pathogenicity of the parent organism. Hence, the likelihood of creating a new reservoir of disease is extremely unlikely since the GMO is not associated with pathogenicity or disease and the GMO is also replication incompetent.

The risk of generation of Replication Competent Lentivirus is considered as very unlikely due the multiply deleted nature of the vector and the Self-Inactivating (SIN) configuration. A safety feature of the EIAV vector system is the codon-optimization of the gag/pol genes. This means that the codon usage across the entire coding sequence for these proteins, except for the frame shift site allowing for appropriately balanced synthesis of Gag and Gag/Pol polyproteins (this includes the region of overlap between the protease and the C-terminus of gag), has been optimized for maximal expression in human cells. The codon optimization of gag has minimised the region of sequence similarity between the vector genome and the gag region of the packaging signal ( $\Psi$ ) thereby reducing the chances of homologous recombination between these vector components. Furthermore, the removal of the packaging signal from the codon-optimized gag/pol mRNA means that it is not preferentially incorporated into virions. The EIAV plasmids used in this system have been designed to minimize the risk of replication competent lentivirus (RCL) formation. The EIAV vector genome contains a total of only 861 nucleotides of original wild-type nucleic acid. Therefore, there is no significant sequence similarity between the three vector components used to make the EIAV viral vector, making the possibility of homologous recombination to generate an RCL unlikely. Although the possibility of non-homologous recombination occurring to generate an RCL cannot be completely ruled out, to date no RCLs have been detected in post-production cell banks from 9 large-scale batches. All nine batches comprise SAR422459 EOPC and are tested with the same test as described in A.3.

Concerning the presence of CMV promoters, to our knowledge, CMV promoters have been used in a large proportion of gene therapy vectors used in human clinical trials worldwide and no events of recombination to a wild-type human CMV as ever been described. We therefore consider such risk as extremely improbable with no potential risk on environment.

With regards to WPRE, this sequence is present in Woodchuck Hepatitis Virus which normal host is *Marmota monax*. This animal is naturally present in Northern America, which make the suggested recombination extremely highly improbable, with no assessable risk on environment.

Moreover, the French Biotechnology Authority (Haut Conseil des Biotechnologies or HCB) has performed an evaluation of the program, before starting the French part of the clinical trial at the Hôpital des XV-XX in Paris. In its evaluation provided in 2011, the scientific council, presided by Prof. Jean-Christophe Pages stated on the topic of secondary mobilization of the vector that: "the transcomplementation of the vector is very highly improbable. One can even consider that it is not possible as the integrated vector is deleted in the U3 region of its ITRs, and therefore cannot produce a RNA containing an encapsidation sequence. Moreover, there is no sequence in human cells that can code for proteins able to encapsidate an EIAV genome."

Based on our own evaluation and this independent evaluation provided in France, we are therefore confident in considering such risk as extremely low.

## A5.2.2 Potential harmful effects linked to the exposure of human beings to the GMO

- **Potential risk of developing an immune response**
  - Following subretinal injection of SAR422459 in rabbits, antibody (Ab) responses were detected against the vector envelope protein, VSV-G2, and against a >250 kDa HEK293T-associated antigen in several animals. This Ab response did not translate into any toxicological findings in the rabbit, including in the ocular region. In NHPs, 1 anti-VSV-G2 response was reported. A specific antibody (Ab) response against the vesicular stomatitis virus G glycoprotein (VSV-G) component of the lentiviral vector has been observed. This response to VSV-G was identified following the injection of an intermediate dose of SAR422459 ( $6 \times 10^5$  TU/eye) in 2 of the 22 patients tested to date, without any impact on the patient safety.
  - Of the three other ongoing clinical trials using gene therapy compounds based on the same lentiviral vector as SAR422459 (i.e., RetinoStat for the treatment of the age-related macular degeneration, and ProSavin for the treatment of the Parkinson's disease, UshStat for the treatment of Usher syndrome, type 1B), a specific Ab response against the VSV-G component of the lentiviral vector has been observed only in one of the three studies. This response to VSV-G was identified in 4 patients with Parkinson's disease treated with ProSavin; 3 of these 4 patients also developed Ab responses to p26 (EIAV native capsid protein). No AEs have ever been identified as being associated with the presence of ABs to VSV-G or p26. To date no immunological response has been reported following the administration of RetinoStat to 21 patients and was negative for all the 7 patients in the UshStat program. In the SAR422459 clinical trials blood samples for immunology are taken at Weeks 4, 12, and 24 with additional testing performed only in patients with positive antibody response at Week 24 to document the return of the antibody levels to baseline values. Throughout the duration of the clinical program studies patients will be monitored for adverse immunological reactions. In the event of a reported adverse immunological reaction, additional immunological testing will be performed.
- **Potential risk of mutagenesis and carcinogenicity and potential for delayed AEs**
  - Carcinogenicity studies have not been conducted with SAR422459. SAR422459 is part of one of a family of non-replicating, recombinant lentiviral vectors which should not pose a risk of insertional mutagenesis or indeed carcinogenicity. However, two in vitro and one in vivo integration studies have been performed with the related lentiviral vector, ProSavin, or a marker gene vector expressing  $\beta$ -galactosidase coming from the same family. Given the common vector structural components used for the EIAV lentiviral vector platform, data on the integration of ProSavin and marker gene vector is considered to be relevant to inform upon the integration site profile of the vector system platform as a whole, including SAR422459. With respect to carcinogenicity, treatment with SAR422459 should offer very little opportunity to contribute to oncogenesis because the EIAV lentiviral vector system has been engineered to remove all of the viral genes and promoter sequences and is therefore unable to transactivate genes proximal to its insertional site. Further, during the production process, envelope components and other structural proteins required for vector packaging and transduction are supplied in trans and encoded on 3 separate plasmids to minimize the risk of generating a RCL, and current assays demonstrate that RCLs have not been found. Finally, two Benzonase® treatment steps are included in the SAR422459 manufacturing process to degrade DNA to prevent delivery of any intact gene impurities to target cells.

- There is a potential for gene therapy related delayed adverse events: The Food and Drug Administration (FDA) guidance has identified lentivirus vectors as potentially provoking in vivo modifications of cells and integration of vector sequences in the host cell genome and therefore as being at risk of delayed adverse events (AEs). The clinical conditions listed in the FDA guidance include new malignancy(ies), new incidence or exacerbation of a preexisting neurologic disorder, new incidence or exacerbation of a prior rheumatologic or other autoimmune disorder, new incidence of a hematologic disorder, and unexpected illness. These potential AEs are defined as AEs of special interest for SAR422459 clinical program and they are attentively monitored for a long term follow-up.

As mentioned above, to assess the potential risk of mutagenesis and carcinogenicity, three studies (two *in vitro* and one *in vivo*) have been conducted to characterize the integration site profile for EIAV vectors. These studies can be summarized as follows;

- Dividing cells *in vitro*; Analysis of 458 EIAV vector and 162 HIV-1 vector (control) integration sites in HEK293T cells was conducted using a multivariate regression model. Statistical comparison with a random data set was undertaken and it was demonstrated that EIAV-based and HIV-1-based lentiviral vectors behaved similarly; this work has been published; Hacker C et al. The Integration Profile of EIAV-Based Vectors. MOLECULAR THERAPY Vol. 14, No. 4, October 2006.
- Slowly-dividing primary astrocytes *in vitro*; As lentiviral vectors preferentially integrate in actively-transcribed genes, it is possible that the gene expression profile of the target cell may influence integration site selection. In order to test this hypothesis, the integration profile of EIAV within slowly dividing primary rat astrocyte cells was established . A total of 111 integration sites were mapped within rat astrocytes and the analysis indicated that the integration profile of EIAV within rat astrocytes was similar to the profile for EIAV within human HEK293T cells. No hot spots for integration, defined as more than one integration site located within a 100 kb region, were observed and there was no obvious preference for integration within specific chromosomes.
- Rat brain *in vivo*; In addition, a study to map the integration profile of EIAV in rodent brain tissue following intra-striatal administration was performed. This study used brain samples from a pilot study performed using an EIAV-LacZ vector pseudotyped with the VSV-G envelope. A total of 27 EIAV integration sites were mapped and although the number of sites mapped was not sufficient for meaningful statistical analysis, no obvious difference in in vivo site preference was observed when compared to the *in vitro* data in HEK293T cells or astrocytes.

As of January 2016, the SAR422459 development program has 2 clinical trials which are ongoing:

- The TDU13583 study is a Phase I/IIa Dose Escalation Safety Study of Subretinally Injected SAR422459, Administered to Patients with Stargardt's Macular Degeneration. The 48 week study currently conducted in France and the USA is designed to assess the safety and tolerability of ascending doses of SAR422459 in patients with Stargardt's Macular Degeneration and to evaluate for possible biological activity of SAR422459.
- The LTS13588 is an Open Label Study to Determine the Long Term Safety, Tolerability and Biological Activity of SAR422459 in Patients with Stargardt's Macular Degeneration. The purpose of the study is to examine the long term safety, over up to 15 years, of SAR422459 in patients that were treated in the TDU13583.

### **A5.3. State according to which scenario the GMO can disperse from the test subject into the environment.**

*State in your answer the scenarios in which the GMO may disperse in the environment. Explain the level of risk that spread will actually occur. Also describe in your answer whether the number of test subjects and/or the dosage to be administered affects the risks to be identified.*

There are three potential scenarios in which SAR422459 may disperse from patients into the environment: via needle stick injury during IMP administration (at preparation or surgery), via blood following needle stick injury (with active manipulation from a third party) or via shedding directly from the patient.

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 majority of SAR422459 remains within the eye after ocular administration; therefore shedding into the environment via body fluids (tears, urine) is extremely unlikely and 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 non-pathogenic and non-infectious, and replication incompetent, and also cleared rapidly from the body as shown in non-clinical studies.

Given the low number of patients expected to be exposed and the level of expertise and training of the medical persons allowed to manipulate the IP, to perform the surgical procedure at hospital and to obtain patient samples, it is very unlikely that the GMO will spread from the test subject into the environment as the levels of the GMO in the blood of the treated patient are barely detectable and the route of administration poses a negligible risk of shedding from patients. In any case, the vector is not expected to survive in the natural environment outside the body in blood spills, see section A5.5.1.

**A5.4. Give an estimate of the chance that the adverse effects described in A5.2 could actually occur.**

*Give a reasoned estimate of the chance (likelihood) of the aspects described in A5.2 and A5.3, also taking account of the number of test subjects and the dosage.*

<b>Potential harmful effects</b>	<b>Likelihood of occurrence</b>
<b>Potential harmful effects linked to the exposure of the environment to the GMO</b>	
<ul style="list-style-type: none"> <li>• No</li> </ul>	
<b>Potential harmful effects linked to the exposure of human beings to the GMO</b>	<ul style="list-style-type: none"> <li>• <b>Low</b></li> </ul>
<ul style="list-style-type: none"> <li>• Immune response</li> <li>• Mutagenesis</li> </ul>	<ul style="list-style-type: none"> <li>• Low</li> <li>• Low</li> </ul>

Preventive measures are taken to minimize the risk of spreading of the GMO in the environment (use of an approved area for preparation of GMOs, hospital staff equipment, discard of contaminated materials, decontamination of affected areas, etc. see section A.1.4). Spreading of the GMO the environment is considered as negligible. In case of spreading in the environment, as mentioned in section A.5.1 and A.5.2, EIAV has been extensively modified to produce SAR422459: all modifications performed will at the end make SAR422459 replication incompetent and thus it will not be infectious, pathogenic, virulent or be able to colonize other organisms. SAR422459 will be not able to survive in a natural environment. As mentioned in A.5.3, once SAR422459 is injected in the eye, studies show the GMO remains in the ocular compartment, therefore risk of shedding via body fluids into the environment is also considered unlikely. In addition, entry of SAR422459 into non-target cells will not have harmful effects as it will be inactivated by the human immune system.

The eye is considered as an immune privileged organ. Such immune privilege is thought to be an evolutionary adaptation to protect tissue that are indispensable, yet have limited

regeneration capacity, like the brain and the eye, from the potentially damaging effect of an uncontrolled inflammatory immune response<sup>37</sup>.

The knowledge on immune characteristics of the eye is derived from injection in the anterior chamber. Following injection, antigens in anterior chamber induce a mechanism called anterior chamber-associated immune deviation (ACAID) characterized by suppression of delayed-type hypersensitivity and inability to produce complement-fixing antibodies. An altered cytokine milieu is observed with down regulation of the pro-inflammatory cytokine IFN- $\gamma$ , and augmented levels of the immunosuppressive cytokines TGF- $\beta$ , IL-4, and IL-10<sup>38</sup>. The anatomical structure of the eye may have an influence in the mediating immune deviation due to absence of vessels and to numerous cellular and physical barriers, separating the ocular compartment from the blood supply.

In addition, studies with subretinal injection of lentivirus in animals show induction of Th2-type immune response and a significant induction of the Th2 cytokines IL-4, IL-10, and TGF- $\beta$ . No inflammatory cells were observed in injected retinal tissue after immunochemical examination and no neutralizing antibodies were formed in *in vitro* assays.

All these combined suggested that the likelihood to have a risk of immune response following use of SAR422459 in the eye is thus considered as low.

The risk of the insertional mutagenesis and carcinogenicity with lentiviral vectors such as SAR422459, is considered to be low. This is because SAR422459 is part of one of a family of non-replicating, recombinant lentiviral vectors which should not pose a risk of insertional mutagenesis or indeed carcinogenicity, as the native enhancer/promoters have been substituted with adequately designed enhancers/promoters so as to reduce the risks of insertional mutagenesis and proto-oncogene activation.

No actual integration studies with SAR422459 have been performed, but two *in vitro* and one *in vivo* integration studies have been performed with the related lentiviral vector, ProSavin, or a marker gene vector expressing  $\beta$ -galactosidase coming from the same family. Given the common vector structural components used for the EIAV lentiviral vector platform, data on the integration of ProSavin and marker gene vector is considered to be relevant to inform upon the integration site profile of the vector system platform as a whole, including SAR422459. Specifically, the integration studies with ProSavin were performed to determine the integration site profile of EIAV vectors *in vitro*, in HEK293T and cultured primary rat astrocytes cells, as well as *in vivo*, in rat brain (see, Assessment of the Risks of Insertional Mutagenesis, below).

With respect to carcinogenicity, it is believed that treatment with SAR422459 offers very little opportunity to contribute to oncogenesis because the EIAV vector system is fully "stripped out" and minimal, and has been engineered to remove all of the wild type viral genes and promoter sequences, and it is therefore unable to transactivate genes proximal to its insertional site. During the production process, envelope components and other structural proteins required for vector packaging and transduction are supplied *in trans* and encoded on separate plasmids. The different viral components are expressed by three different plasmids to minimize the risk of generating a replication competent lentivirus (RCL), and to date no RCLs have been found in any batch produced. Finally, two benzonase treatment steps to degrade DNA are included in the manufacturing process to prevent the delivery of any intact genes as impurities to the target cells (see, Carcinogenicity Risk, below).

#### Assessment of the Risks of Insertional Mutagenesis

Given that a single administration of SAR422459 should provide long-term (chronic) therapy, and that SAR422459 is an integrating vector with a finite risk of insertional

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<sup>37</sup> Benhar I, London A and Schwartz M (2012) The privileged immunity of immune privileged organs: the case of the eye. *Front. Immun.* 3:296

<sup>38</sup> Bennett, J, "Immune response following intraocular delivery of recombinant viral vectors," *Gene Therapy*, 10(11) 977-982 (2003).

mutagenesis leading to oncogenesis, it was appropriate to consider whether SAR422459 poses an undue carcinogenic risk when designing the nonclinical program. The EIAV integration site analysis studies mentioned above collectively demonstrated that EIAV vectors show no bias to integrate within any particular gene, class of genes, or genomic region. These studies were all conducted with VSV-G pseudotyped vectors using the same Gag/Pol expression cassette as used to produce SAR422459. SAR422459 shares a number of key features with the vectors that were used in these integration site studies (ProSavin, or marker gene vectors). Critically, the Gag/Pol expression cassette which encodes the vector particles and associated enzymatic activities (reverse transcriptase and integrase) is common to all the vectors studied. The SAR422459 vector genome sequence only differs from ProSavin and the marker gene vectors in the vector-specific central gene expression cassette. The self-inactivating long terminal repeats sequence, which is important for the integration process by virtue of the flanking attachment (att) sites that are recognized by the integrase enzyme, is identical in all of the EIAV-based lentiviral vectors, including SAR422459, RetinoStat, ProSavin and marker gene vectors produced with the optimized fully minimal system. Given the common profile observed for ProSavin and marker gene vectors and the common features across the EIAV lentiviral vector platform, the integration site studies described are therefore considered directly relevant to the platform, including SAR422459, rather than to a specific vector genome construct. However, it is acknowledged that the gene expression profile of the target cell will elicit a predictable influence on the integration site profile of the vector. The primary target cells for the SAR422459 clinical study are the terminally differentiated photoreceptor cells, which are not thought to divide in the fully formed eye, though RPE cells may be transduced as well. In the natural setting of the retina, RPE cells are considered to be either quiescent or very slowly dividing. Photoreceptor and RPE cells appear to be relatively resistant to oncogenic transformation under conditions of regular exposure to ultraviolet light, and retinal cancers derived from RPE or photoreceptor cells are relatively rare or non-existent. As a consequence of this, it is considered that SAR422459 will elicit a similar integration site preference to PR and RPE cells as for other cell types, with a tendency to integrate within genes that are active in the natural quiescent state. In addition, as the distribution of SAR422459 after subretinal administration is largely restricted to the retina, as shown by the biodistribution studies, any potential risks of insertional mutagenesis should be restricted.

#### Carcinogenicity Risk

Tumorigenic adverse events following retroviral gene therapy have been observed in three clinical settings: in a trial for chronic granulomatous disease (CGD), 3 out of 12 patients developed myelodysplasia with one death; in a trial for Wiskott-Aldrich syndrome, 1 out of 10 patients developed leukemia and is undergoing treatment; and in X-linked severe immunodeficiency (X SCID) trials, 5 out of 20 patients have developed leukemia with four patients successfully treated and one death. In the X-SCID trials the tumorigenic events have been attributed to insertional mutagenesis. Both the X-SCID and the CGD trials utilized gammaretroviral vectors which are different to the lentiviral vector used for SAR422459. The gene therapy community has concluded that it is likely that while other mutations contributed to these events, the proliferative advantage supplied by the vector was necessary to allow these secondary mutations to develop and be selected. The adverse events in these trials appear to be protocol, vector and transgene specific; the therapeutic gene favored proliferation, aspects of the protocol either required or allowed selective advantage of the transduced cells, and the patients were immuno-compromised. It is believed that treatment with SAR422459 offers very little opportunity to contribute to oncogenesis for the following reasons:

- The EIAV vector system has been engineered to remove all of the viral genes and promoter sequences and is thus fully 'stripped out' and minimal.
- During production, process envelope components and other structural proteins required for vector packaging and transduction are supplied in trans and encoded on separate plasmids.

- The complete vector system is split across three plasmids to minimize the risk of generating a replication competent lentivirus.
- The target cells are either non-dividing or slowly dividing and so genes involved in cell division will not be active in these cells.
- The therapeutic genes are not involved in cell survival or replication and so are unlikely to confer a proliferative advantage to the target cells.
- The risk of insertional mutagenesis is further reduced by using the self-inactivating vector configuration in SAR422459, meaning that lentiviral enhancer and promoter sequences are absent. In hematopoietic transplantation models in normal and tumor-prone mice using self-inactivating lentiviral vectors, no increase in the incidence of leukemia was reported<sup>39</sup>.
- The proposed patient population for SAR422459 is immunocompetent.
- Unlike gammaretroviral vectors, EIAV vectors do not integrate preferentially into the promoter region or the 5' end of the transcription unit, and are therefore less likely to up-regulate gene expression<sup>36</sup>.
- A benzonase treatment step to degrade DNA is included in the manufacturing process to prevent the delivery of any intact genes or impurities to the target cells.
- Finally, in an in vitro soft agar assay using WI38 cells (fetal human lung fibroblasts) to assess the potential of a viral vector to elicit anchorage independent growth, none was observed for EIAV vectors at high copy number

Taking the above points and features of the EIAV vector system together, these significantly mitigate the tumorigenic risks of SAR422459. In addition, SAR422459 nonclinical studies have demonstrated that it is safe and well tolerated. SAR422459 is very carefully targeted to the subretinal space, and biodistribution and shedding studies have indicated that SAR422459 remains confined within the ocular compartment, and >95% of SAR422459 is confined to the retina. There has not been any suggestion of carcinogenicity or chronic histopathology in the 6 month NHP and rabbit toxicology studies. A similar safety profile has been observed for ProSavin in a 9 month NHP and a 12 month rat toxicity study and for RetinoStat in 6 month rabbit and NHP studies.

**A5.5. Describe the risks that could occur as a consequence of the application of the GMO, taking into account the impact of any risk management measures taken.**

*Describe the risks in such a way that makes clear how the risks can be reduced through risk management. If risk management measures are necessary in order to limit the risks, these should be specified further in the questions below.*

**A5.5.1 Potential harmful effects linked to the exposure of the environment to the GMO**

No environmental plans are necessary as SAR422459's vector is not expected to survive in a natural environment. SAR422459 is not replication competent and therefore is not infectious, pathogenic, virulent or able to colonise other organisms either directly or via a vector. In addition, SAR422459 does not encode for any of the accessory genes known to be associated with pathogenicity in the wild type virus and no indications of vector pathogenicity have been observed during nonclinical testing.

**A5.5.2 Potential harmful effects linked to the exposure of human beings to the GMO**

- The risk of accidental exposure of non-target humans (e.g. healthcare personnel working on the study) is limited by restricted access to the GMO by qualified personnel; personnel will undergo comprehensive training as part of the investigator meeting and site initiation

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<sup>39</sup> Montini E et al. Hematopoietic stem cell gene transfer in a tumor-prone mouse model uncovers low genotoxicity of lentiviral vector integration. Nat Biotechnol. 24: 687-696, 2006

- The risk of detrimental immune response has been limited by not allowing any re-injection in the same patient of the TDU13583 study until more pre-clinical information is available to better evaluate this potential risk
- The potential risk of delayed adverse events has been taken into consideration as follows:
  - ✓ The following AEs of special interest (AESI) are defined to record the emergence of clinical conditions including new malignancy(ies), new incidence or exacerbation of a preexisting neurologic disorder, new incidence or exacerbation of a prior rheumatologic or other autoimmune disorder, new incidence of a hematologic disorder, and unexpected illness. The occurrence of these AESI should trigger immunogenicity and viral investigations as polymerase chain reaction (PCR) analysis [viral deoxyribonucleic acid (DNA and ribonucleic acid RNA) PCR]. Other AESI defined are infection (particularly opportunistic infection) and other AEs (e.g. inflammation, ischemia, cardiovascular event).
  - ✓ Patients will be followed for 15 additional years, every 6 months during the first 5 years and then yearly [visits include interview on AESIs, ophthalmologic examination and safety laboratory testing and PCR analysis (viral DNA and RNA PCR)].
  - ✓ A detailed record of all exposures to mutagenic agents and other medicinal products, and information about the AE will be collected for a total of 16 years in the program.

## Risk management

### A5.6. Which inclusion and exclusion criteria are adopted in the selection of test subjects and what is the effect of these criteria on environmental safety?

*Give an overview of the inclusion and exclusion criteria that are only necessary for the protection of the environment or which criteria might possibly have an effect on safety for human beings and the environment.*

Only patients with a diagnosis of SMD, with at least one pathogenic mutant ABCA4 allele on each chromosome confirmed by direct sequencing and family segregation analysis, are eligible for the study and treatment with the GMO via subretinal injection into the study eye. This inclusion criteria limits the risk to the environment by limiting the exposure of the GMO to only patients with the disease of interest that have a defective ABCA4 protein.

Also the exclusion of patients with a past medical history of HIV, or hepatitis A, B or C infection, removes the theoretical risk to the environment that the GMO could recombine with another competent virus.

Moreover, patients will be instructed not to donate blood, organs, tissues and cells after injection of SAR422459 in the eye for at least three months following SAR422459 administration.

### A5.7. Describe which measures are provided for in respect of the hospitalization of the test subject.

*When answering this question, please emphasize those aspects that are important in preventing spread in the environment of the test subject. Also indicate if, apart from medical reasons, hospitalization is prescribed as a way of protecting against possible effects for humans and the environment.*

Hospitalization for subretinal injection is not required but may be necessary for convenience of some patients. In the event of hospitalization, following the subretinal injection procedure patients could be admitted in a single room or in a room with other patients as the risk of shedding is considered negligible, standard hospital procedures will be instituted,

including the standard hospital procedures in the event of a spill of a patient sample (the vector is readily inactivated by hospital disinfectants such as 70% Ethanol). The likelihood of the GMO spreading outside of the hospital is considered to be negligible as the vector is replication defective, is not expected to survive in a natural environment and the route of administration poses a negligible risk of shedding from patients.

**A5.8. If the test subject is to be hospitalized, what are the criteria for his or her release from hospital?**

*Describe the criteria on the basis of which the test subject will be released from hospital.*

Hospitalization is not required but may be necessary for the subretinal injection procedure for convenience of some patients. In the event of hospitalization for the procedure, routine post-operative management will be followed and the patient released from the hospital when deemed appropriate for discharge by the operating surgeon.

**A5.9. Describe which measures will be taken to prevent the spread of the GMO to third parties (including medical personnel).**

*For example, give an overview of relevant (hospital hygiene) measures that will be taken. In case existing guidelines will be used, please indicate what they are (such guidelines must be attached to this application). Additional or deviating measures also must be described.*

All healthcare staff handling SAR422459 must wear an apron, gloves, mask and protective goggles (all specified in the hospital's hygiene and infection control, in Dutch: "Basismaatregelen infectiepreventie in een operatiekamercomplex").

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.

All materials contaminated with SAR422459 e.g., syringes, swabs, bandages, must be destroyed by incineration in accordance with hospital policy on genetically modified materials. Certificates of Destruction must be completed and copies maintained in the Study File.

Staff should adhere to local Health and Safety guidelines applicable to biological sample collection, processing, and shipment. ("Gentherapie Basis voor werkprotocollen")

The general hospital protocols for hospital hygiene and infection control (in Dutch: Basismaatregelen infectiepreventie in een operatiekamercomplex) and protocol for accidental exposure general GMO procedures for spills will be followed.

Venapunctures in exposed test subjects will be performed according to routine hospital procedures. Subretinal injections to administer the investigational product will be performed by an experienced ophthalmic surgeon.

No additional or deviating measures are needed, considering the low risk of occurrence and the absence of risk of spreading due to the non-replicative nature of the vector. The hospital's standard procedures for the containment of the GMO should be followed in the event of a spill. ("Gentherapie Basis voor werkprotocollen")

In case of any accidental event, the Environmental Safety Officer, the Responsible Clinician (RE-II), the Study Coordinator and the Sponsor (all specified in A7), will be notified immediately. If an accidental spill or needlestick/accidental exposure occur, the procedures described in the previous sections have to be followed to quickly and effectively address the spill or exposure. In summary: Any puncture or other wounds should be encouraged to bleed under warm running water, using soap. Wounds must not be sucked, squeezed or scrubbed as this may cause tissue damage and encourage the spread of any potential infection. Wounds should be covered with a dry dressing. Splashes to the eyes (after

removing contact lens, if appropriate), broken skin or mouth, should be washed immediately and liberally with water.

## **Procedure in case of unexpected situations and serious incidences**

### **A5.10. Describe the procedures to be followed if changes in the risk management are required for medical reasons.**

*This may concern situations in which a test subject needs to be removed from isolation; for example because he or she requires intensive care, or in situations when unexpected effects are being observed, including Suspected Unexpected Serious Adverse Reactions (SUSARS) or Serious Adverse Events (SAEs). Also consider the situation of a test subject having died and an autopsy being required.*

*All unexpected situations and serious incidences must be reported immediately. See the 'Procedure for unwanted incidences' ([www.loketgentherapie.nl](http://www.loketgentherapie.nl))*

As the GMO is replication defective, it encodes only a minimal EIAV sequence, as the risk of shedding from patients is negligible, no special measures are foreseen in order to treat patient for medical events related or not related to their ocular disease.

### **A5.11. Describe what aftercare will be given if a test subject ends his/her participation in the study prematurely.**

*Also state to what extent the aftercare deviates from the aftercare for test subjects who have completed the entire study.*

Following the administration of SAR422459 the patient's observation will continue until the end of the clinical program with follow-up visits including ophthalmological examinations and recording of adverse events will continue as needed. 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. In the event a subject elects to prematurely discontinue the study, the effect of the treatment cannot be discontinued. Subjects prematurely discontinuing the study may remain under the care of the investigator without completing all of the study assessments as required or may be referred to a local ophthalmologist for continued aftercare. In either event the patient will be asked to provide consent for the collection of information that would allow the assessment of delayed adverse events.

## **Monitoring**

### **A5.12. Describe how the monitoring will be set up to identify any spread of the GMO.**

*In answering this question, pay attention to the method followed, but also to the period during which any positive result can be expected in connection with the applicable scenario. Also state in this context during which period monitoring will take place.*

The SAR422459 components are determined in blood and urine samples from patients using validated quantitative methods.

Oxford BioMedica (UK) Ltd. has developed quantitative reverse transcription polymerase chain reaction (RT-PCR) assays to detect the EIAV packaging signal in samples from patients as a highly sensitive measure for the detection of vector particles and transduced cells. The RT-qPCR technique can be used to detect EIAV vector in fluid samples and has a detection limit of 100 copies that is dependent on the volume of sample tested. The DNA qPCR assay is able to detect 10 copies/ $\mu$ g DNA (LLOQ).

In patients, blood samples are collected for RT-PCR analysis at screening (28 days prior to Day 0/surgery) and at 60 minutes after surgery, at Day 1 and at Weeks 1, 2, 4, 12, 24, 36 and 48. Urine samples are collected for RT-PCR analysis at screening (28 days prior to Day 0/surgery) and at 60 minutes after surgery (where possible), at Day 1 and at Weeks 1 and 2.

In patients, the collection time points for identification of shedding in plasma and urine cover a much longer period than in the nonclinical shedding studies, in which SAR422459 was administered via the same route and with the same frequency. Blood samples are collected up to 48 weeks and urine samples are collected up to 15 days. It is expected that under these conditions multiple consecutive negative samples will be detected for SAR422459.

## A6. Conclusions of the possible environmental effects

*Directive 2001/18/EC Annex II under Point D.1 gives a number of aspects that should be used whenever applicable as the basis of the conclusions about the possible environmental effects of the introduction of the GMP into the environment. All these points should be taken into account when drafting the conclusions of the risk analysis.*

### **1. Likelihood of the GMO to become persistent and invasive in natural habitats under the conditions of the proposed release(s).**

The GMO is a non-replicative vector; therefore it is not possible that it becomes persistent and invasive in natural habitats.

### **2. Any selective advantage or disadvantage conferred to the GMO and the likelihood of this becoming realized under the conditions of the proposed release(s).**

The GMO is at a significant disadvantage to wild-type EIAV under the conditions of the release as it is a non-replicative vector and not possible that it persist for a significant length of time outside the patient.

### **3. Potential for gene transfer to other species under conditions of the proposed release of the GMO and any selective advantage or disadvantage conferred to those species.**

The GMO is to be administered in a controlled environment, where access to non-humans is limited. In addition, vector shedding from subjects is negligible and no selective advantages or disadvantages are conferred over untreated individuals.

### **4. Potential immediate and/or delayed environmental impact of the direct and indirect interactions between the GMO and non-target organisms (if applicable).**

No interactions between the GMO and non-target organisms are expected given the quantities involved, the nature of the release and the non-replicative nature of the GMO.

### **5. Possible immediate and/or delayed effects on human health resulting from potential direct and indirect interactions of the GMO and persons working with, coming into contact with or in the vicinity of the GMO release(s).**

It is hoped that some subjects taking part in the clinical trial will demonstrate clinical benefit from treatment with SAR422459 however the primary objective of the trial is the safety and tolerability of the GMO. No interactions between the GMO and persons working with or coming into contact with the GMO are expected given the quantities involved, the nature of the release and the non-replicative nature of the GMO.

### **6. Possible immediate and/or delayed effects on animal health and consequences for the feed/food chain resulting from consumption of the GMO and any product derived from it, if it is intended to be used as animal feed.**

No effects on animal health or consequences for the feed/food chain are expected.

### **7. Possible immediate and/or delayed effects on biogeochemical processes caused by possible direct or indirect interaction between the GMO and the target and non-target organisms in the vicinity of the GMO introduction(s).**

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 in transduced cells. This

avoids viral spreading beyond the transduced tissues and induction of adverse pathogenic effects due to viral replication or presence of viral particles. The pharmacologic action of the GMO in host primary target cells, photoreceptors, is to allow expression of ABCA4 which is defective in the treated SMD patients. The absence of any toxicity in the eye in various toxicology studies in several species as well as the absence of cytotoxicity in a study which evaluated whether transduction of cells with the ABCA4 EIAV lentiviral vector had detrimental effects on the cell viability makes any physiologic or pathogenic effect resulting from the potential expression of ABCA4 in non-target tissues normally not expressing ABCA4 very unlikely.

**8. Possible change in the current medical practice.**

To date, no change in the current medical practice is necessary with regard to possible environmental effects of this clinical research.