PART I

SUMMARY NOTIFICATION INFORMATION FORMAT FOR THE RELEASE OF GENETICALLY MODIFIED ORGANISMS OTHER THAN HIGHER PLANTS IN ACCORDANCE WITH ARTICLE 11 OF DIRECTIVE 2001/18/EC

A. General information

1. Details of notification

(a) Member State of notification	France
(b) Notification number	B/FR/13/GT04
(c) Date of acknowledgement of notification	17/06/2013
(d) Title of the project A Phase I/IIa Dose Escalation Safety Study of Subretinally Injected UshStat® Administered to Patients with Retinitis Pigmentosa Associated with Usher Syndrome Type 1B (US1/001/10)	
(e) Proposed period of release From approximately 01/09/2013 until 31/08/2015.	

2. Notifier

Name of institution or company	Oxford BioMedica (UK) Ltd.

3. GMO Characterisation

(a) Indicate whether the GMO is a:	viroid ☐ RNA virus ☒ DNA virus ☐ bacterium ☐ fungus ☐ animal - mammals ☐ - insect ☐ - fish ☐ - other animal ☐ specify phylum, class other, specify (kingdom, phylum and class)
(b) Identity of the GMO (genus and species)	Replication-defective vector based on a lentivirus, Equine Infectious Anaemia Virus (EIAV), pseudotyped with the vesicular stomatitis virus glycoprotein (VSV-G: rhabdovirus) envelope.

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

The UshStat® vector is produced by the transient transfection of HEK293T cells with three plasmids: (1) the recombinant EIAV UshStat® vector genome (pONYKMYO7A; pBSG477), (2) the synthetic EIAV Gag/Pol expression vector (pESGPK) and (3) the VSV-G envelope expression vector (pHGK). Replication competent lentivirus (RCL) could arise at this stage as a result of non-homologous recombination during production, before release of material.

Part of the optimisation of the EIAV vector system included reduction, where possible, of sequence similarity between the vector components in order to prevent generation of replication competent lentivirus (RCL) by site-specific recombination. To date no RCLs have been detected when testing post-production cell banks or final product from multiple manufacturing runs of all EIAV-based lentiviral gene therapy vectors using a validated assay.

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

Yes ☐	No ☒ (The same GMO has only previously been used under contained conditions.)
If yes insert the country code(s):	

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

Yes ☐	No ☒ (The same GMO has only previously been used under contained conditions in the UK and Belgium.)
If yes: - Member State of notification - Notification number	

6. Has the same GMO been modified for release or placing on the market outside the Community by the same or other notifier?

Yes ☐	No ☒ (The same GMO has only previously been used under contained conditions in the USA.)
If yes: - Member State of notification - Notification number	

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

UshStat® is replication-defective and the production system does not encode for EIAV viral genes, hence it is unlikely to be pathogenic and is only capable of transducing animal cells.

It is not expected that the vector will survive anywhere in the natural environment. The length of time that any particles remain active in the environment will be affected by the temperature (the vectors are thermolabile), the amount of UV, and cleaning procedures.

The vector is unlikely to interact with other organisms in the environment.

Real-time RT-PCR assays will be used to monitor potential shedding of the vector in urine from treated patients. Blood samples (plasma and buffy coat) will also be analysed using real-time PCR.

The total amount of the GMO present at the hospital site at any one time will be very limited. Overall the risk to the environment is considered to be negligible.

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

1. Recipient or parental organism characterisation:

(a)	Indicate whether the recipient or parental organism is a:	
viroid	☐	
RNA virus	☒	
DNA virus	☐	
bacterium	☐	
fungus	☐	
animal	☐	
	-mammals	
	-insect	
	-fish	
	-other animal (specify phylum, class)	
other,	Specify	

2. Name

(i)	order and/or higher taxon (for animals) Retroviridae
(ii)	Genus Lentiviridae
(iii)	Species Equine Infectious Anaemia Virus
(iv)	Subspecies
(v)	Strain
(vi)	Pathovar (biotype, ecotype race, etc.)
(vii)	common name

3. Geographical distribution of the organism

(a) Indigenous to, or otherwise established in, the community where the modification is made: Yes ☑ but at a low level of incidence No Not known and is an agricultural notifiable disease.
(b) Indigenous to, or otherwise established in, other EC countries: (i) Yes ☑ but not all If yes, indicate the type of ecosystem in which it is found: Atlantic ☑ Mediterranean ☑ Boreal Alpine Continental ☑ Macaronesian (ii) No (iii) Not known
(c) Is it frequently used in the country where the modification is made? Yes No☒
(d) Is in frequently kept in the country where the notification is made? Yes No☒

4. Natural habitat of the organism

(a)	If the organism is a micro-organism water soil, free-living soil in association with plant-root systems in association with plant leaf/stem systems in association with animals ☑ other, specify
(b)	If the organism is an animal: natural habitat or usual agroecosystem: The natural habitat of wild-type EIAV is horses and it is transmitted by bloodsucking insects, primarily biting flies. In the GMO, the majority of the EIAV sequences have been removed (~90%) to produce a replication defective minimal vector system. This GMO is an artificial laboratory produced replication defective viral vector and is therefore not considered to have a natural habitat.

5(a) Detection techniques

Oxford BioMedica (UK) Ltd. has developed real-time reverse transcriptase PCR assays to detect the EIAV packaging signal RNA sequence in samples as an indirect highly sensitive measure for the detection of UshStat® vector particles. These are complemented by real-time PCR assays to detect the DNA form of the vector following entry to target cells (as a measure of transfer of the vector, including transgene sequences). These techniques could also be used to detect the parental organism EIAV.

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

In the event of positive detection of the EIAV vector sequence in environmental or patient samples, additional PCR-based methods could be employed to further characterise and identify the sequences (e.g. the conventional RT-PCR based UshStat® identity assay that is performed as part of the QC testing strategy for UshStat® IMP).

5(b) Identification techniques

The techniques outlined in section 5(a) can also be used to identify EIAV and/or the GMO.

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

Yes No☑ (see below)

If yes, specify

The UshStat® vector is a replication defective vector and as such is not considered to pose a significant risk to human health or the environment.

The wild type EIAV on which UshStat® is based is not found in all EU countries. The features of EIAV that pose a risk to human health and/or the environment have where possible been removed from the vector system.

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

Yes☑	No	Unknown

If yes:

(a) to which of the following organisms: humans
animals ☑
plants
other

(b) give the relevant information specified under Annex III A, point II. (A)(11)(d) of Directive 2001/18/BC *pathogenicity: infectivity, toxigenicity, virulence, allergenicity, carrier (vector) of pathogen, possible vectors, host range including non-target organism. Possible activation of latent viruses (proviruses). Ability to colonise other organisms;*

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

8. Information concerning reproduction

(a) Generation time in natural ecosystems:

Not applicable, the GMO vector will not replicate in natural ecosystems or under artificial conditions (several features required for replication are absent from the vector and the vector production system).

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

Not applicable.

(c) Way of reproduction: Sexual Asexual ☑

(d) Factors affecting reproduction

Not applicable.

9. Survivability

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

(i) endospores
(ii) cysts
(iii) sclerotia
(iv) asexual spores (fungi)
(v) sexual spores (fungi)
(vi) eggs
(vii) pupae
(viii) larvae
(ix) other, specify

(b) relevant factors affecting survivability:

UshStat® is replication-defective and the production system does not encode for EIAV viral genes, hence is unlikely to be pathogenic and is only capable of transducing animal cells.

It is not expected that the vector will survive anywhere in the natural environment. The length of time that any particles remain active in the environment will be affected by the temperature (the vectors are thermolabile), the amount of UV, and cleaning procedures.

The vector is unlikely to interact with other organisms in the environment.

10(a) Ways of dissemination

The UshStat® vector can only transduce animal cells and is replication-defective, therefore dispersal will be limited to the first organism exposed. The route of transduction will usually be by injection however, other possible exposure routes are:

- contact with solution in opened vial
- contact with solution in syringe
- contact with material at injection site
- accidental exposure through injury (e.g. ‘needle stick’ injury)

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

10(b) Factors affecting dissemination

The UshStat® vector can only transduce animal cells and is replication-defective, therefore dispersal will be limited to the first organism exposed. Hence it is unlikely to survive in the environment. In addition, due to the replication defective nature of UshStat®, there is no potential for population increase within the environment.

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

None, since no deliberate releases have occurred. However, another EIAV-based minimal lentiviral vector (StarGen™) that is produced using the same manufacturing method is currently undergoing clinical evaluation in France.

C. Information relating to the genetic modification

1. Type of the genetic modification

(i) insertion of genetic material	☑
(ii) deletion of genetic material	☑
(iii) base substitution	☑
(iv) cell fusion	
(v) other, specify	

2. Intended outcome of the genetic modification

UshStat® is a gene therapy medicinal product developed by Oxford BioMedica for the treatment of retinitis pigmentosa associated with Usher Syndrome Type IB.

The disease is caused by a mutation in the gene encoding for the unconventional myosin (motor) protein VIIA, (MYO7A). MYO7A plays a critical function in the ear and eye. In the eye it plays a role in the development and stability of the retina by influencing the structure and function of both the rod photoreceptor cells, (PR), and supporting retinal pigmented epithelium, (RPE). Mutations that affect the normal function of the MYO7A genes can result in progressive retinitis pigmentosa and vision loss. The therapeutic rationale for treatment is to introduce a gene encoding normal MYO7A cDNA, which codes for the relatively large functional Myosin VIIa protein, directly into the retina of patients with retinitis pigmentosa associated with Usher Syndrome Type IB. The gene is delivered to the PRs within the eye where it integrates into PRs and supports RPE cells establishing local secretion of the functional Myosin VIIa protein thereby attenuating the deterioration in vision associated with Usher syndrome Type 1B.

To generate UshStat®, there has been extensive modification of the wild type EIAV genome including removal of 4 out of 6 of the EIAV genes in the EIAV vector production system to ensure that the GMO is safe (i.e. will not replicate and will not be pathogenic).

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

Yes ☑ The UshStat® GMO is manufactured using three different plasmids	No
If no, go straight to question 5.	

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

Yes ☑ But plasmid DNA is extensively degraded by Benzonase® during manufacture. This has the effect of degrading all DNA, including plasmids, into small fragments (>99% of DNA is degraded to fragments of less than 120bp) and will be present at extremely low levels.	No
If no, go straight to question 5.	

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

(a) Type of vector

- plasmid ☑
- bacteriophage
- virus
- cosmid
- transposable element

(b) Identity of the vector

The EIAV vector system, is comprised of three plasmid constructs: the vector genome, the Gag/Pol packaging component and the envelope packaging component. The Gag/Pol packaging component contains (in order from 5' to 3') a Cytomegalovirus promoter (CMVp) and a codon optimised ORF for Gag/Pol, linked by a non-codon optimised ribosome frame shift region required to maintain the correct balance of Gag and Gag/Pol proteins, followed by a heterologous pA site. The envelope packaging component contains (in order from 5' to 3') a CMVp, an ORF for vesicular stomatitis virus glycoprotein (VSV-G), and a heterologous pA site.

The minimal vector system design, in combination with the use of a codon-optimised Gag/Pol construct and a heterologous envelope glycoprotein, means that sequence homology between vector components has been minimized. This strategy was employed to reduce the chance of replication competent lentivirus generation.

UshStat® contains only 861 nt of EIAV sequence. No functional viral proteins can be expressed from this construct. The UshStat® vector genome, pONYKMYO7A (BSG477) contains (in order from 5' to 3') a CMVp-R-U5 region, the Ψ region (in which all the initiation codon [ATG] sequences have been mutated to ATTG), Neo (an open reading frame that enables high titre manufacture in the absence of Rev), an internal CMVp, an open reading frame (ORF)encoding myosin VIIA, (MYO7A), the modified woodchuck hepatitis virus post-transcriptional regulatory element (WPRE), and a self-inactivating LTR (SINLTR).

The UshStat® vector is produced by the transient transfection of HEK293T cells with three plasmids: (1) the recombinant EIAV UshStat® vector genome (pONYKMYO7A; pBSG477), (2) the synthetic EIAV Gag/Pol expression vector (pESGPK) and (3) the VSV-G envelope expression vector (pHGK).

The UshStat® GMO is manufactured by transfecting three different plasmids (encoding a genome, the VSV-G envelope and the EIAV Gag/Pol proteins) into HEK293T cells. The three plasmids used are based on pUC. Only the sequences from the insert in the genome plasmid (an RNA transcript that encodes a vector genome) are present in the final product. Any remaining plasmid DNA is degraded into small fragments (>99%, less than 120bp) by Benzonase® treatment at two stages of the production process and residual DNA is largely removed by vector purification processes.

(c) Host range of the vector

Plasmid vector.

E.coli and closely related species.

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

	Yes ☑	No
antibiotic resistance	☑	
Other, specify		

Indication of which antibiotic resistance gene is inserted:
Functional Kanamycin resistance genes are present on the UshStat® vector genome (pBSG477), Gag/Pol and envelope plasmids.

The coding sequence for the neomycin phosphotransferase II gene is present in the vector genome; however a promoter is not associated with this gene and hence expression is unlikely.

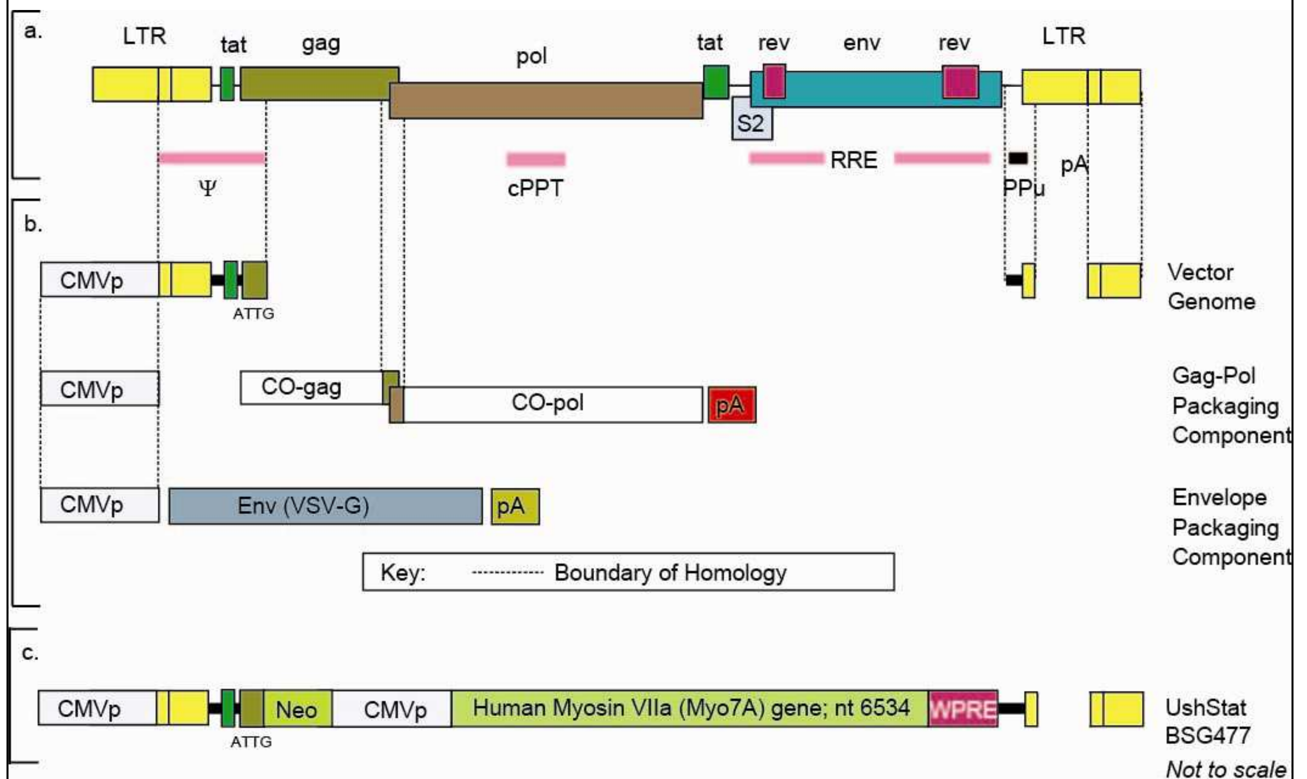
(e) Constituent fragments of the vector

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

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

The EIAV vector system, shown in Figure 1b, is comprised of three plasmid constructs: the vector genome, the Gag/Pol packaging component and the envelope packaging component. The Gag/Pol packaging component contains (in order from 5' to 3') a Cytomegalovirus promoter (CMVp) and a codon optimised ORF for Gag/Pol, linked by a non-codon optimised ribosome frame shift region required to maintain the correct balance of Gag and Gag/Pol proteins, followed by a heterologous pA site. The envelope packaging component contains (in order from 5' to 3') a CMVp, an ORF for vesicular stomatitis virus glycoprotein (VSV-G), and a heterologous pA site. Regions of the packaging components that share sequence homology with wild-type EIAV or each other are indicated with dotted lines. The minimal vector system design, in combination with the use of a codon-optimised Gag/Pol construct and a heterologous envelope glycoprotein, means that sequence homology between vector components has been minimized. This strategy was employed to reduce the chance of replication competent lentivirus generation. UshStat® contains only 861 nt of EIAV sequence. No functional viral proteins can be expressed from this construct. The UshStat® vector genome, pONYKMYO7A (BSG477) illustrated in Figure 1c contains (in order from 5' to 3') an CMVp-R-U5 region, the Ψ region (in which all the initiation codon [ATG] sequences have been mutated to ATTG), Neo (an open reading frame that enables high titre manufacture in the absence of Rev), an internal CMVp, an open reading frame (ORF) encoding myosin VIIA (MYO7A), the modified woodchuck hepatitis virus post-transcriptional regulatory element (WPRE), and a self-inactivating LTR (SIN LTR).

Figure 1: Schematic diagram showing the genetic structure of a. EIAV wild-type virus, b. the EIAV vector system and c. UshStat® (pONYKMYO7A, pBSG477)



Plasmid 1: The UshStat® vector genome, **pBSG477**.

The vector genome, pONYKMYO7A (BSG477) transcript contains only 861 nucleotides (nt) derived from EIAV. These include 640 nt from the 5'-end, including R-U5-leader-packaging signal (Ψ) sequence and 221 nt from the 3'-end, which includes 70 nt upstream of the U3 region of the 3'LTR and a self-inactivating (SIN) LTR which is deleted with respect to all transcription factor binding sites and the TATA-box. This is in order to confine transcription and expression to the internal transgene expression cassette in the target cell. This SIN LTR includes only 26 nt derived from the EIAV U3 region. In the interests of safety the EIAV-derived region of the vector genome has been altered to eliminate expression of full-length Tat, (exon 2 deleted), Rev, S2 and EIAV Env. The features ofpONYKMYO7A (BSG477) are described in table 1 below.

Table 1. Features of pONYKMYO7A (BSG477), the UshStat® vector genome plasmid (13260bp)

Feature	Comment
CMV(E): nucleotides (nt) 5-1149	Includes the enhancer region of the Cytomegalovirus CMV promoter. Is fused to EIAV R region and directs transcription of the vector genome. [nt 5 - 1149 are 'similar' to nt 174867 - 173732 of Gen bank accession number X17403.1]
EIAV R-U5-packaging signal region: nt 1150-1833	The R-U5 sequence is from nt 1150-1269. The sequence from 1270 to 1833 contains the packaging signal (ψ), including the primer binding site. This sequence corresponds to nt 268 to 897 from EIAV Gen bank Accession No. U01866 except there are additional C residues present after nucleotides 270 and 8178. These correspond to the 4th position of the R region and these additional residues are incorporated into the genome of pONYKMYO7A. The vector sequence also contains alterations in the analogue of the EIAV Gag encoding region which alter all ATG codons to ATTG thereby preventing expression of Gag or other peptides from this region.
Neomycin phosphotransferase gene: nt 1834-2628 (Accession No: V00618)	An open reading frame (ORF) is required for improved titres in the absence of Rev
CMVp: nt 2648-3432	Cytomegalovirus CMV promoter; drives transcription of the transgene expression cassette. In BSG477 plasmid feature table, nt 2648-2659 is the cPPT region, nt 2665 – 3432 is CMVp (nt.2706 – 3432 corresponds to nt. 174457 – 173731 of Gen bank accession number X17403.1)
5'untranslated region: nt 3433-3568	nt 3433 – 3509 corresponds to nt. 173730 - 173654 of Gen bank accession number X17403.1 (HCMV complete genome)
Human *MYO7A* gene: nt 3585-10118	Human *MYO7A* cDNA. The gene sequence is identical to nt273-6926 of Genbank Accession number NM_000260.
Woodchuck hepatitis virus post-transcriptional regulatory element (WPRE): nt 10190-10779	Enhances expression from the transgene cassette. Expression of the X-protein and peptides derived from it has been ablated by mutations in the promoter and at the translation start site.
EIAV 3'PPT and SIN LTR: nt 10908-11136	This includes the 3' U5 sequence which directs polyadenylation.
Plasmid backbone: nt 11137-13260 plus nt 1-4	Includes the bacterial Kanamycin selectable sequence and promoter in the complementary orientation (12296-13228) and the pUC origin of replication (ori) (11426-12093).

Plasmid 2: The Gag/Pol expression cassette, **pESGPK**.

Gag/Pol is expressed from a pCIneo-based (Promega) plasmid in which a codon-optimised gene encoding the entire EIAV Gag/Pol open reading frame is positioned in the multiple cloning site. Transcription is driven by the human cytomegalovirus immediate-early enhancer/promoter and polyadenylation takes place at the SV40 late polyA signal. The backbone has been modified to replace the ampicillin resistance cassette with that of Kanamycin from the plasmid pCR Blunt II (InVitrogen). In addition, the Neomycin expression cassette has been removed. The features of pESGPK are described in table 2 below.

Table 2. Features of pESGPK, the Gag/Pol expression plasmid (8249bp)

Feature	Comment
CMVp: nt 1-750	Drives transcription of the *gag/pol* encoding RNA.
Synthetic intron: nt 890-1022	Chimeric intron composed of the 5´-donor site from the first intron of the human β-globin gene and the branch and 3´-acceptor site from the intron of an immunoglobulin gene heavy chain variable region
EIAV syngp : nt 1103-5760	ORF for EIAV *gag/pol.* The gene sequence is codon-optimised except for the region which encodes the overlap between the C-terminus of Gag ORF and the N-terminus of the Pol ORF
SV40 late polyA signal: nt 5770-6026	Polyadenylation signal from SV40
Kanamycin resistance: nt 8074-7280	Complementary orientation
Ori: nt 6410-7077	pUC ori

Plasmid 3: The VSV-G expression cassette, **pHGK**.

The glycoprotein present in the particles is derived from Vesicular Stomatitis Virus rather than EIAV and allows the particles to enter human cells. The VSV-G transcript is expressed from the CMV promoter and includes intron B from the Rabbit beta-globin gene. Polyadenylation is directed by the SV40 early polyadenylation signal. The features of pESGPK are described in table 3 below.

Table 3: Features of pHGK, the VSV-G expression plasmid (6080bp)

Feature	Comment
CMVp: nt 1-752	Drives transcription of the VSV-G encoding RNA.
Rabbit beta-globin intron B: nt 760-1405	
VSV-G: nt 1437-2972	ORF encoding VSV-G
Rabbit beta-globin polyadenylation site: nt 3116-3638	NB. This is a different polyA signal to that used in pESGPK
Kanamycin resistance: nt 5685-4893	Complementary orientation
Ori: nt 4333-4686	pUC ori

(f) Method for introducing the vector into the recipient organism
(i) transformation
(ii) electroporation
(iii) macroinjection
(iv) microinjection
(v) infection
(vi) other, specify

Transfections are performed using lipofectamine™ 2000 CD.

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

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

(i) transformation
(ii) mikroinjection
(iii) microencapsulation
(iv) macroinjection
(v) other, specify

6. Composition of the insert

(a) Composition of the insert

UshStat® is derived from the Equine Infectious Anaemia Virus (EIAV). 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. A schematic of the UshStat® vector, which will be used to treat patients, is shown in Figure 1 below.

Figure 1: Schematic of UshStat® Vector Genome Plasmid (pONYKMYO7A, Plasmid BSG477)

The features of UshStat® are described in table 1 in section 4e

(b) Source of each constituent part of the insert

See above.

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

1) Human Cytomegalovirus (CMV): immediate-early enhancer/promoter (CMVp). The internal Human Cytomegalovirus immediate-early enhancer/promoter (CMVp) downstream of the Neo gene drives the production of an RNA transcript that encodes for the MYO7A protein in target cells. The 5’ CMV promoter produces the vector genome in the transfected producer cells but is not present in the packaged vector genome.
2) *Escherichia coli* transposon Tn5: neomycin phosphotransferase II (Neo) gene. Neo is not expressed in the target recipient cells but the open reading frame is required to obtain high vector titres in the absence of Rev during production.
3) Human: photoreceptor-specific ATP-binding cassette transporter (MYO7A) gene. This is the therapeutic transgene to correct for a non-functional form of MYO7A protein in patients with Usher Syndrome Type 1B and thereby attenuate deterioration in vision associated with these patients.
4) Woodchuck Hepatitis Virus (WHV): post-transcriptional regulatory element (WPRE). This is normally part of the coding sequence for the WHV X-protein. The WPRE enhances expression from the transgene cassette in UshStat®. The particular version of the element used has been engineered by mutations in the promoter and at the translation start site to prevent expression of the X-protein and peptides derived from it.

In addition a glycoprotein (but not coding sequence) is present from Vesicular Stomatits virus, this (VSV-G) allows the viral particles to enter human cells.

(d) Location of the insert in the host organism
— on a free plasmid
— integrated in the chromosome
— other, specify

The insert is present on the vector genome.

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

Yes | No☑

If yes, specify

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

1. Indicate whether it is a:

viroid
RNA virus ☑
DNA virus ☑
bacterium
fungus
animal ☑
— mammals ☑
— insect
— fish
— other animal (please specify phylum, class)
other, specify

2. Complete name

(i) order and/or higher taxon (for animals) • Herpesvirales • Not applicable • Primates • Hepadnaviridae (family) • Rhabdoviridae (family)
(ii) family name (for plants)
(iii) genus • Herpesviridae • Not applicable • Homo • Orthohepadnavirus • Vesiculovirus
(iv) species • Human Cytomegalovirus (CMVp) • Escherichia coli transposon Tn5 (Neo) • sapiens (MYO7A cDNA) • Woodchuck Hepatitis Virus (WPRE) • Vesicular Stomatitis virus (VSV-G)
(iv) subspecies
(v) strain
(vi) cultivar/breeding line
(vii) pathovar
(viii) common name

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

Yes	No☑	Not known
If yes, specify the following		
(a) to which of the following organisms?	Humans ☑ Animals ☑ plants Other ☑	Human Cytomegalovirus Vesicular Stomatitis virus Woodchuck Hepatitis Virus Vesicular Stomatitis virus Escherichia coli transposon Tn5
(b) are the donated sequences involved in any way to the pathogenic or harmful properties of the organism?		
Yes	No☑	Not known
If yes, give the relevant information under Annex III A, point II(A)(11)(d): *pathogenicity: infectivity, toxigenicity, virulence, allergenicity, carrier (vector) of pathogen, possible vectors, host range including non-target organism. Possible activation of latent viruses (proviruses).* *Ability to colonise other organisms;*		

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

Yes☑	No
If yes, specify • Human Cytomegalovirus classified as 2 • Vesicular Stomatitis virus classified as 2	

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

Yes	No☑	Not known

E. Information relating to the genetically modified organism

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

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

Yes☑	No	Not known

Specify

Whilst the wild type EIAV is pathogenic to equidae, the EIAV vector (UshStat®, the GMO) is not replication competent and therefore not infective / virulent / pathogenic or able to colonise other organisms either directly or via a vector. This means that survival would be limited to the initial number of particles released. As the vector is pseudotyped with the VSV glycoprotein rather than the natural EIAV glycoprotein, the vector is less thermolabile than the wild type. The vector is not expected to survive anywhere in the natural environment.

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

Yes☑	No	Not known

Specify

Whilst the wild type EIAV is pathogenic to equidae, the EIAV vector (UshStat®, the GMO) is not replication competent and therefore not infective / virulent / pathogenic or able to colonise other organisms either directly or via a vector. However since the vector is pseudotyped with the VSV glycoprotein it will enter a wider range of animal cells than the wild type EIAV.

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

Yes☑	No	Not known

Specify

Whilst the wild type EIAV is pathogenic to equidae and is spread via biting insects, the EIAV vector (UshStat®) is not replication competent and therefore not infective or able to colonise other organisms either directly or via a vector. However since the vector is pseudotyped with the VSV glycoprotein it will enter a wider range of animal cells than the wild type EIAV. However, once a cell that has come in contact with the vector has been transduced, there is no potential for dissemination.

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

Yes☑	No	Not known
Specify		

Wild type EIAV is pathogenic to equidae. However UshStat® is not replication competent and therefore not pathogenic or able to colonise other organisms either directly or via a vector. In addition, UshStat® does not encode for any of the accessory genes known to be associated with pathogenicity of the wild type parental virus and no indications of vector pathogenicity have been observed during nonclinical testing.

2. Genetic stability of the genetically modified organism

The vector is using a transient co-transfection system. Once the particles have been made they are replication defective and hence the sequence of the genome will not change until integration into the host genome occurs. The lentivirus integration process, which initiates with reverse transcription, may rarely introduce base changes which can occur during reverse transcription because reverse transcriptase does not possess 3' exonuclease activity capable of excising mispaired nucleotides and thus lacks the proof-reading function of cellular DNA polymerases. The fidelity of purified HIV-1 reverse transcriptase has been reported to be in the range of 1 misincorporation per 2,000 to 7,000 bases. The purified EIAV reverse transcriptase has been found to be similar to HIV-1 reverse transcriptase in vitro. Based on these data integrated copies of the UshStat® vector would be expected to contain very few or no base substitutions. Unpublished data from analysis of EIAV proviral vector sequences in transgenic chickens indicate the mutation rate is lower than predicted from in vitro studies and is less than 1 in 19,332 bp (Elizabeth Elliot and Helen Sang, personal communication). Therefore approximately 1 in every 2 integrated UshStat® vectors would be expected to contain a single base substitution. This number of base substitutions is unlikely to result in any phenotypic changes. As UshStat® vector particles are only capable of a single transduction event any mutations that occur are not cumulative.

Since this is an Investigational Medicinal Product stability testing programs are in place to monitor the stability (strength, potency etc) of the UshStat on storage.

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

Yes No☑ Unknown

(a) to which of the following organisms? humans
animals
plants
other

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

UshStat® is not replication competent and therefore is not infectious, pathogenic, virulent or able to colonise other organisms either directly or via a vector. Extensive testing of the vector in vivo in nonclinical studies have indicated no toxigenicity or allergenicity. Activation of latent viruses would not be predicted. The vector is only capable of transducing animal cells. Therefore in order for the GMO to replicate, it would require the cell to already contain a retrovirus. However, in this event, the UshStat® vector is unlikely to be packaged into chimeric vector particles as UshStat® itself is a self-inactivating vector and any RNA produced from the integrated genome is unlikely to contain a packaging signal and therefore would not be actively packaged into viral particles. If the RNA were to be packaged the efficiency of reverse transcription and integration would be severely compromised and therefore the likelihood of genetic exchange is negligible.

The potential risks associated with the use of UshStat® in this disease have been evaluated in formal toxicology studies. A combined toxicology / biodistribution study in non-human primates demonstrated that subretinal administration of UshStat® is safe and well tolerated and remains localised within the ocular compartment. The vector will not transduce plant cells.

(i) toxic or allergenic effects of the GMOs and/or their metabolic products;

The potential risks associated with the use of UshStat® as a treatment for retinitis Pigmentosa associated with Usher Syndrome Type 1B have been evaluated in a formal toxicology and biodistribution study in naïve non-human primates (NHPs). UshStat was safe and well tolerated. There were no UshStat®-related histopathological findings.

The potential for UshStat® to induce an immune response has been evaluated by monitoring serum from NHPs for antibodies to the vector and to the therapeutic gene products and by immunohistochemistry at the target site. Humoral responses against components of UshStat®, including the envelope protein VSV-G, the human transgene myosin VIIa protein, EIAV proteins or NeoPT, were not detected in serum samples collected at any time point from either UshStat®- or TSSM buffer control dosed animals, up to 3 months following treatment.

This data is in accordance with that generated for related product, RetinoStat®, administered by the same route where no antibodies were detected to any of the antigens tested (VSV-G, HEK293T production cells, EIAV proteins or the RetinoStat transgene products) in either rabbits or NHPs. Whereas no antibodies to EIAV proteins or the transgene products were detected in the sera of rabbits and NHP for other related product StarGen™, (also administered by the same route), antibodies against the envelope protein (VSV-G) were detected in 21 of 38 rabbits and against production cell (HEK293T) antigens in 17 of 38 by 3 months post-administration. The appearance of antibodies to VSV-G in the rabbits for StarGen™ but not RetinoStat® may due to the higher dose used in the StarGen™ study (ten-fold more transducing units of vector were administered in the StarGen™ rabbit study and three-fold more in the NHP study). For another lentivector product, ProSavin®, administered by stereotactic injection to the striatum, antibodies to VSV-G were observed in both some rodents (rats) and NHPs (one of four) and antibodies to the production cells (HEK293Ts) were detected only in the rat. Note that the production cells are human in origin. No antibodies against the EIAV proteins or the transgene products were detected in either species.

In clinical trials conducted to date with StarGen, RetinoStat, ProSavin and UshStat, (USA) no antibodies specific to any of the vector components have been detected in patients treated to date.

Single subretinal injection of UshStat® at a dose level of $9.08x10^5$ TU/eye into the right eye of rhesus monkeys followed by a 3-month (92-day) observation period resulted in ocular findings of anterior chamber inflammation/anterior uveitis with concomitant transient decreases in IOP, vitreal opacities, retinal opacities, retinal edema/haze and retinal vessel congestion. No signs of toxicity were noted at the level of the retina and most changes are as expected with the subretinal dosing procedure. All noteworthy ocular findings resolved during the 3-month observation period, with the exception of vitreous/retinal opacities and pigment changes in the retina/choroid. These findings are consistent with a transient and dose related inflammation response that has been seen with the family of EIAV-based lentiviral vector products when dosed subretinally in the eyes of Rhesus macaques in GLP regulatory safety studies.

(ii) comparison of the modified organism to the donor, recipient or (where appropriate) parental organism regarding pathogenicity;
Wild type EIAV is pathogenic in equidae. It cannot replicate in human cells. The GMO (UshStat®) is replication defective and therefore not pathogenic in either humans or equidae.

(iii) capacity for colonisation;
The GMO is replication defective. This is confirmed by the completion of assays to detect any replication competent lentiviruses in all GMP/clinical batches of UshStat®.

(iv) if the organism is pathogenic to humans who are immuno-competent:
No. Neither EIAV nor the GMO (UshStat®) is pathogenic in humans.

(v) other product hazards.
None known.

4. Description of identification and detection methods

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

Oxford BioMedica (UK) Ltd. has developed sensitive real-time reverse transcriptase PCR assays to detect the EIAV packaging signal RNA in test samples as a measure of vector particles. These assays are complemented by real-time PCR assays to detect the DNA form of the vector.

ELISA and Western blot techniques are available to detect antibodies raised against the vector in in vivo models.

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

F. Information relating to the release

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

No specific environmental benefits are expected from the conduct of a clinical trial "A Phase I/IIa Dose Escalation Safety Study of Subretinally Injected UshStat®, Administered to Patients with Retinitis Pigmentosa Associated with Usher Syndrome Type 1B, however it is hoped that the treatment will attenuate vision loss associated with the disease for these patients.

2. Is the site of the release different from the natural habitat or from the ecosystem in which the recipient or parental organism is regularly used, kept or found?

Yes☑	No	
If yes, specify The GMO will be administered to patients in an operating theatre at a hospital.		

3. Information concerning the release amid the surrounding area

(a) Geographical location (administrative region and where appropriate grid reference): The clinical trial will take place at: Centre Hospitalier Nationale d'Ophthalmologie des Quinze-Vingts 28 rue de Charenton 75 571 Paris FRANCE
(b) Size of the site (m^2): (i) actual release site (m^2): The GMO will be stored in a pharmacy room and administered in an operating theatre ($50m^2$). Patients are not required to stay in the hospital for a fixed period of time following treatment. Transport from the pharmacy room to the operating theatre will be in a sealed container. Any remaining GMO will be placed in a biotainer before being removed for analysis or destruction. (ii) wider release area (m^2):
(c) Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs), which could be affected: No proximity is expected as the GMO will be administered in a hospital.
(d) Flora amid fauna including crops, livestock amid migratory species which may potentially interact with the GMO Interaction with flora and fauna is considered to be highly unlikely as the GMO will be administered in a hospital and the product is replication defective.

4. Method amid amount of release

(a) Quantities of GMOs to be released:

Up to 18 patients will receive a single dose of one of three dose levels of Ushstat®. Three patients will receive 1.4 x 10^5 TU/eye, three will receive 4.7 x 10^5 TU/eye and three will receive 1.4 x 10^6 TU/eye, (Part A). Following completion of part A of the study three additional adult patients will receive the MTD determined from Part A, (Part B). Three months after completion of Part B and further to recommendations of the DSMB a further six patients aged ≥ 6 years will receive the MTD.

UshStat® will be provided in 100 µL aliquots in 0.3 mL vials. Drug supply to the hospital will be managed such that no more than 12 vials containing a maximum of 4.7x 10^5 TU per vial will be stored and available in the hospital at any one point in time. It is predicted that only 6vials will be removed from storage for each use as, allowing for overage, 5 vials contain sufficient vector to treat a patient with 300 µL of UshStat®.

(b) Duration of the operation:

The clinical study is already ongoing in the USA but is expected to start in France in September 2013 and continue for approximately two further years.

Following treatment, each patient will be followed for 48 weeks following administration of UshStat and after this period they will enter an open label safety study in which long-term follow-up visits and recording of adverse events will continue for 15 years.

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

The amount of UshStat® supplied to the site at any one time is limited and access to the product is restricted. The clinical protocol requires the use of appropriate protective equipment by personnel and there are written procedures in place to be used in the event of a spillage or when disposing of the product or contaminated materials. The route of administration has been chosen in order to minimise potential shedding from the patient and samples are taken to monitor this following treatment.

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

The GMO is to be administered to patients within an operating theatre in a hospital.

6. Relevant data regarding previous releases carried out with the same GMO, if any, specifically related to the potential environmental and human health impacts from the release.

The subject of this clinical trial application 'A phase I/IIa dose escalation safety study of sub-retinally injected UshStat® administered to patients with retinitis pigmentosa associated with Usher Syndrome Type 1B' is already ongoing in the USA. Four patients have been dosed to date, (3 in Cohort 1 and 1 in Cohort 2) and UshStat® has been found to be safe and well tolerated and no drug related adverse events have been reported. Further, UshStat® has been used under contained conditions during research and development studies, GMP manufacturing processes and nonclinical studies within the UK, Belgium, and the USA. These clinical, nonclinical and *in vitro* studies have not detected any adverse effects on the subjects/test animals / test systems which would indicate that the environment or humans would be at risk from this release.

In addition, two other related lentiviral vector products administered by the same route of administration have been approved for release and are currently being used in clinical trials in the USA, (RetinoStat® and StarGen™) and France (StarGen™). No adverse human health or environmental impacts have been noted to date in these clinical studies.

Another product (ProSavin®), based on the same lentiviral vector, was approved for release and was used in a recently completed clinical trial in Parkinson's Disease. This study was conducted in France and the UK and was administered to a total of 15 patients. The product was found to be safe and well tolerated and no adverse human health or environmental impacts were reported in that clinical trial.

G. Interactions of the GMO with the environment and potential impact on the environment, if significantly different from the recipient or parent organism

1. Name of target organisms (if applicable)

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

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

UshStat® introduces the normal gene for human MYO7A driven by an internal CMV/IE promoter permanently into the host chromosomes by a process referred to as integration. The transfer of the correct human MYO7A cDNA to the photoreceptor and retinal pigment epithelial cells is intended to restore normal cellular function and thereby attenuate and possibly reverse the pathophysiology which leads to retinitis pigmentosa associated with Usher Syndrome Type 1B.

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

The likelihood of the GMO spreading outside of the hospital is considered to be negligible as the vector is replication defective, is not expected to survive in a natural environment and the route of administration ensures minimal shedding from patients.

Data from nonclinical studies and clinical data collected from patients administered UshStat® and related lentiviral vector products administered by the same route of administration (analysis ongoing) indicate that shedding to the environment via body fluids (tears, urine) is negligible

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

Yes	No ☑	Not known
Give details The GMO is replication defective.		

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

None.

It is considered extremely unlikely that the GMO could be disseminated from the site of release. In addition, it is not expected that the vector will survive anywhere in the natural environment to become established.

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6. Complete name of non—target organisms which (taking into account the nature of the receiving environment) may be unintentionally significantly harmed by the release of the GMO

(i) order and/or higher taxon (for animals) None known.
(ii) family name (for plants) None known.
(iii) genus
(iv) species
(v) subspecies
(vi) strain
(vii) cultivar/breeding line
(viii) pathovar
(ix) common name

7. Likelihood of genetic exchange in vivo

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

A number of steps have been taken to minimise this possibility and it is considered to be extremely unlikely. UshStat® is replication-defective and the production system does not encode for EIAV viral genes, hence it is

unlikely to be pathogenic and is only capable of transducing animal cells. Therefore in order for the GMO to replicate, it would require the cell to already contain a retrovirus. However, in this event, the UshStat® vector is unlikely to be packaged into chimeric vector particles as UshStat® itself is a self-inactivating vector and any RNA produced from the integrated genome is unlikely to contain a packaging signal and therefore would not be actively packaged into viral particles. If the RNA were to be packaged the efficiency of reverse transcription and integration would be severely compromised and therefore the likelihood of genetic exchange is negligible.

(b) from other organisms to the GMO:

Since the GMO is replication defective and is produced under GMP conditions, where the cells used for production, excipients, the drug substance and the finished medicinal product are tested for the presence of adventitious agents, the likelihood of this is considered to be negligible.

(c) likely consequences of gene transfer:

The transfer of the normal functional human MYO7A cDNA to the photoreceptor and retinal pigment epithelial cells is intended to restore normal cellular function in patients with Usher Syndrome Type 1B thereby attenuating and possibly reversing the pathophysiology which leads to retinitis pigmentosa and vision loss in these patients. Expression of the relatively large MYO7A protein is not thought to constitute a risk, as borne out by the findings from nonclinical toxicology studies and the good safety profile reported in patients dosed with UshStat® to date.

8. Give references to relevant results (if available) from studies of the behaviour amid characteristics of the GMO and its ecological impact carried out in simulated natural environments (e.g. microcosms, etc.):

No studies of this type have been performed, as it is not considered that the GMO will survive anywhere in a natural environment.

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)

None known.

H. Information relating to monitoring

1. Methods for monitoring the GMOs

UshStat® Vector: Oxford BioMedica (UK) Ltd. has developed real-time reverse transcriptase PCR assays to detect the EIAV packaging signal RNA sequence in samples as an indirect highly sensitive measure for the detection of vector particles. These are complemented by real-time PCR assays to detect the DNA form of the vector following entry to target cells (as a measure of transfer of the vector, including transgene sequences). In the event of positive detection of the EIAV vector sequence in environmental or patient samples, additional PCR-based methods could be employed to further characterise and identify the sequences (e.g. the conventional RT-PCR based UshStat® identity assay that is performed as part of the QC testing strategy for UshStat® IMP).

Patients: Antibody responses to the UshStat® vector may be present in the treated patients; these will be monitored by ELISA and western blotting. Real-time RT-PCR assays will be used to monitor the potential shedding of the vector in urine from treated patients. Blood samples (plasma and buffy coat) will also be analysed using real-time PCR.

2. Methods for monitoring ecosystems effects

No additional methods have been developed to monitor the effects of the GMO in ecosystems.

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

A modified version of the real-time qualitative PCR assay mentioned in 1 (above) may be used to detect integrated vector genomes in a range of organisms. ELISA and western blot techniques may be used to detect immune responses to the vector.

4. Size of the monitoring area (m2)

There are no plans to directly monitor the site of administration or the operating theatre in which the patient is injected. However, samples are taken from treated patients in order to monitor potential shedding.

5. Duration of the monitoring

Blood samples are taken for PCR at screening 28 days prior to Day 0 and at 60 minutes after surgery, at Day 1 and at Weeks 1, 2, 4, 12, 24, 36 and 48.

Urine samples are taken for PCR at screening 28 days prior to Day 0 and at 60 minutes after surgery (where possible), at Day 1 and at Weeks 1 and 2, 4, 12, 24, 36 and 48.

6. Frequency of the monitoring

Blood samples are taken for PCR at screening 28 days prior to Day 0 and at 60 minutes after surgery, at Day 1 and at Weeks 1, 2, 4, 12, 24, 36 and 48.

Urine samples are taken for PCR at screening 28 days prior to Day 0 and at 60 minutes after surgery (where possible), at Day 1 and at Weeks 1 and 2, 4, 12, 24, 36 and 48.

I. Information on post-release and waste treatment

1. Post-release treatment of the site

At the end of the surgery all disposable surgical supplies used during the surgical procedure including needles, syringes and swabs along with the first dressing removed post-surgery, will be disposed of by incineration in accordance with hospital policy.
The operation room will be decontaminated in accordance with hospital policy on genetically modified materials.

2. Post-release treatment of the GMOs

None.

3(a) Type and amount of waste generated

The types of waste expected to be generated include syringes and general disposable surgical supplies (e.g. gloves, masks, gowns, dressings and swabs) 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. Each patient is expected to generate a maximum of 20 kg of waste.

3(b) Treatment of waste

All disposable surgical supplies; including syringes, gloves, masks, gowns, dressings, swabs and bandages used during the surgical procedure and the first dressing removed from the patients wounds following surgery will be destroyed by incineration or local equivalent according to hospital policy on genetically modified materials at the end of the operation. Following the injection, the needle should be removed from the eye and the entire device (cannula, tubing, and syringe) disposed of according to local hospital policy for biohazardous waste materials.

J. **Information on emergency response plans**

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

Standard hospital procedures will be used for the containment of the GMO in the event of a spill. The likelihood of the GMO spreading outside of the hospital is considered to be negligible as the vector is replication defective, is not expected to survive in a natural environment and the route of administration ensures minimal shedding from patients. The vector is readily inactivated by hospital disinfectants.

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

Spillages will be cleaned up with Virkon (method qualified with EIAV vectors) or locally approved disinfectant suitable for the inactivation of the HIV-1 lentivirus (e.g. Surfanios®). All contaminated materials will be disposed of locally by incineration or autoclaving.

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

The risk of this GMO spreading is minimal, in part due to the nature of the GMO itself. In addition, consideration has been given in order to reduce the risk of exposure as far as practicable. This includes limiting the amount of UshStat® IMP supplied to the site at any one time, the use of appropriate protective equipment and written procedures to be used in the event of a spillage or when disposing of the product or contaminated materials, which would include those examples given if necessary (although extremely unlikely).

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

No specific plans are in place for protecting human health in the event of an undesirable effect; there is no known method of vector removal should there be an error during UshStat® administration resulting in overdose or in the event of an undesirable effect in the patient or someone accidentally exposed to the product. However, the sub-

retinal route of administration has been chosen to allow local administration of the therapy to the photoreceptors and retinal pigment epithelial cells, whilst minimising systemic effects of the treatment and appropriate procedures are in place in order to minimise the risk of accidental exposure.

An environmental response plan is not necessary due to the nature of the GMO. Transmission from the first organism infected to other organisms is not possible as the GMO is replication-defective. It is not expected that the vector will survive anywhere in the natural environment and therefore the chance of any undesirable effect in the environment is considered negligible.