Environmental Protection Agency
An Ghníomhaireacht um Chaomhnú Comhshaoil

Headquarters, PO Box 3000
Johnstown Castle Estate
County Wexford, Ireland

Ceanncheathrú, Bosca Poist 3000
Eastát Chaisleán Bhaile Sheáin
Contae Loch Garman, Éire

T: +353 53 9160600
F: +353 53 9160699
E: info@epa.ie
W: www.epa.ie

LoCall: 1890 33 55 99

Dr Robin Henderson
Alan Boyd Consultants Ltd
Electra House
Crewe Business Park
Crewe
CW1 6GL
United Kingdom

17 April 2013

Request for further information under Article 19 (1) of S. I. No. 500 of 2003 with regard to notification for consent to release GMOs into the environment for purposes other than placing on the market (GMO Register G0498-01, Reference B/IE/13/01) – First Request

Dear Dr Henderson

I refer to the above mentioned notification, and request the following information which is required in order to assist the EPA (Agency) in their review of the application.

General

1. What is the relationship between Boyd Consultants, The Center for Cellular and Molecular Therapeutics at the Children's Hospital of Philadelphia and St James's Hospital?
   In the event that consent is granted who will the legal entity be and who/where will be the point of contact for the Agency, in Ireland?

2. Ireland is the only European site for this proposed trial. Why was Ireland selected as a site for this proposed trial and as the sole European site ?
   Please provide some information on the prevalence of the condition in Ireland.

3. Please provide some background information on Haemophilia B and how this proposed treatment will impact on the disease?

Study Objective

1. Please clarify the study objective in particular what precisely is meant by the measurement of biological and physiologic activity of the transgene product.
   Furthermore it is my understanding that this study does not purport to replace a defective gene rather to increase the level of circulating FIX. However, at the same time the patient's semen will be monitored for germline transmission. Please clarify.

2. How long is it envisaged that the GMO therapy will last once it is administered to the patient?

Dose

Please clarify the GMO dose to be administered to the patient?

Section 3.4 of the Environmental Risk Assessment (ERA) states that *"Doses to be administered for the proposed study in an inter-subject group dose escalation study design are 1×10^{12} vg/kg, 2×10^{12} vg/kg and 5×10^{12} vg/kg"*. At the same time the GMO will be administered by a single intravenous infusion. Please explain how "an inter-subject group dose escalation study" will operate, particularly given the very small number of participants (4-8).

Treatment of waste

1. Section 3.5 of the ERA states as follows:
   "Following administration of AAV8-hFIX19 at a medical facility, used (or partially used) vials of vector/empty capsids, syringes used in dose preparation, infusion bags and infusion sets are retained and stored in a labeled biohazard bag in the pharmacy at below -60°C for a period of no less than two months, per manufacturer's retention instructions (as specified in the Investigational Drug Data Sheet (IDDS) and Handling Instructions for AAV8-hFIX19). These items will then be returned to the sponsor or destroyed according to sponsor instructions".
   a. Why are contaminated materials retained in this way and for so long?
   b. Is it feasible that these items may be decontaminated within the St James's hospital site?
   c. If not, what is the procedure for the return of the items to the U.S.?
   d. What are the sponsor's instructions for the decontamination of the abovementioned items?

2. Section 3.5 of the ERA states as follows:
   "Any other disposable instruments or other materials used during the dose preparation procedure will be disposed of in a manner consistent with the standard practice of the institution for potentially biohazardous materials".
   a. Please clarify what the standard practice of the institution is or will be for the decontamination and disposal of GMM contaminated disposable and non-disposable materials.
   b. In the event that the procedure involves disinfection, please provide the name and concentration of disinfectant(s) to be used, and minimum contact times for both GMM contaminated solid and liquid wastes.
   c. Where the procedure involves autoclaving, please provide details of the location of the autoclave relative to the medical facility, and the decontamination cycle parameters (temperature, time and pressure).
   d. Please provide details of the off-site contractor where one is being used for purposes of waste disposal.

Antibiotic resistance

1. Section 3.3 of the ERA states as follows
   "The antibiotic kanamycin is used in the manufacturing process for production of the plasmid stocks. The kanamycin resistance genes encoded by the plasmids are not by design part of the final vector, but may be present as residual impurities. Residual plasmid DNA is analysed on a batch-by-batch basis by qPCR".
   a. What is residual plasmid DNA analysed for?
   b. What measures are taken in the event of a positive analysis?
   c. As residual impurities is there potential for the kanamycin resistance genes to be administered to the patient receiving AAV8-hFIX19?

2. According to section 4.1 of the ERA, residual plasmid DNA containing genes for kanamycin resistance and derived from the manufacturing process could contaminate the AAV8-hFIX19 preparation. The section continues that this contamination could lead to limited distribution of DNA sequences potentially containing genes for kanamycin resistance in the environment.
   a. How would this environmental contamination be brought about?
   b. Please clarify what the ensuing impact would be for the environment and human health.
   c.

Shedding

1. What is the potential for the recombinant vector to shed and over what period of time will shedding take place?

2. What risks to human health or the environment will arise from shedding?

3. What precautions (if any) can be taken to reduce the risk of shedding?

Chromosomal integration

Section G2 of the SNIF states that "*the vector* [once injected] *is expected to persist for months or years primarily as an episome but potentially by integration into the host cell genome* " Section 5.1.2 of the ERA states that "*integration will take place at some low level*". Since the vector lacks the AAV rep proteins, presumably this integration will take place at a chromosomal location other than chromosome 19.

a. Please clarify the recombinant vector's potential for integration, where integration will likely take place and the level at which it will take place (please expand on low level of integration)
b. What will be the impact of chromosomal integration?

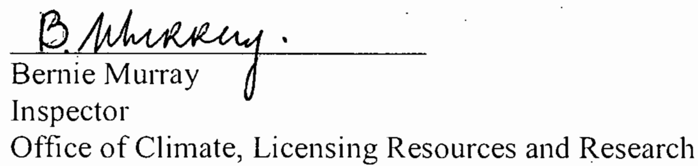
Containment

1. How will the GMO be stored prior to administration?
2. Where will it be stored?
3. What quantities of the GMO are anticipated to be stored and for what duration?
4. Where applicable, please provide details of temperatures at which the GMO will be stored.

Environmental Protection Agency
An Ghníomhaireacht um Chaomhnú Comhshaoil

Please note that in accordance with Article 8(1) of the GMO (Deliberate Release) Regulations, S.I. No 500 of 2003, "a period of time during which the Agency is awaiting any further information on a notification, which it may have requested from the notifier, shall not be taken into account", i.e. the clock is stopped until such time as all the requested information is received by the Agency.

Yours sincerely



Bernie Murray
Inspector
Office of Climate, Licensing Resources and Research

Center for Cellular and Molecular Therapeutics at
The Children's Hospital of Philadelphia (CCMT/CHOP)

AAV8-hFIX19

Responses to Request for further information under Article 19 (1) of S.I. No. 500 of 2003 with regard to notification for consent to release GMOs into the environment for purposes other than placing on the market
(GMO Register G0498-01, Reference B/IE/13/01)
First Request

Final – May 2013

General

1. What is the relationship between Boyd Consultants, The Center for Cellular and Molecular Therapeutics at the Children's Hospital of Philadelphia and St. James's Hospital?

In the event that consent is granted who will the legal entity be and who/where will be the point of contact for the Agency, in Ireland?

The Center for Cellular and Molecular Therapeutics at The Children's Hospital of Philadelphia (Sponsor) holds the Investigational New Drug Application (IND) with the United States Food and Drug Administration. The Children's Hospital of Philadelphia (a tax-exempt, non-profit academic healthcare institution) also provides the funding for the conduct of the study and is the legal entity responsible for all aspects of the clinical trial.

A Clinical Trial Agreement has been negotiated and will be entered into between The Children's Hospital of Philadelphia, 34th Street and Civic Center Boulevard, Philadelphia, PA 19104 USA (CHOP) and St. James's Hospital, P.O. Box 580, James's Street, Dublin 8, Ireland (St. James's) in which St. James's agrees to conduct the clinical research entitled: "A Phase 1 Safety Study in Subjects with Severe Hemophilia B (Factor IX Deficiency) Using a Single-Stranded, Adeno-Associated Pseudotype 8 Viral Vector to Deliver the Gene for Human Factor IX [AAV8-hFIX19-101]". St. James's will be reimbursed for agreed upon trial-related expenses, including effort of its investigators, in accordance with this Clinical Trial Agreement and budget attachment.

Alan Boyd Consultants Ltd, a Company based in the United Kingdom, provides the Sponsor with consultancy services according to the terms of a Consultancy Agreement. They will also serve as its legal representative in the European Economic Area to satisfy the requirement set out at Article 19 of the EU Clinical Trials Directive 2001/20/EC. It is the intention that, as the Sponsor's legal EU representative, Boyd Consultants will serve as the primary point of contact for the Agency.

2. Ireland is the only European site proposed for this trial. Why was Ireland selected as a site for this proposed trial and as the sole European site?

Please provide some information on the prevalence of the condition in Ireland.

The parent institution of the Center for Cellular and Molecular Therapeutics (CCMT), The Children's Hospital of Philadelphia (CHOP), prefers that non-US clinical trial sites for studies sponsored by CCMT be in English-speaking countries. This simplifies document preparation and regulatory submissions, as well as Clinical Trial Agreement negotiations, independent monitoring activities, and general study site management.

The study leaders were seeking a clinical site in Europe; however, a competing trial for the same indication (based at University College London (UCL) and sponsored by UCL in collaboration with St. Jude Children's Research Hospital) has sought collaborations with most haemophilia centres in the United Kingdom, eliminating them from consideration for CCMT. Therefore, the study leaders at CCMT contacted the investigators in Ireland; other countries in Europe are less desirable because of

the language issue. As described in the application, CCMT is also in the process of opening a clinical trial site in Australia.

In addition, there are other compelling reasons for wishing to perform the trial in Ireland, based on the prevalence of Haemophilia B in Ireland and consequently the potential benefits relating to recruitment of patients into the trial.

A paper published in the World Federation of Haemophilia journal *Haemophilia* in 2008 (Jenkins *et al.,* 2008), describes the prevalence of the condition as follows:

"Haemophilia B generally arises as a result of unique mutations within the F9 gene and occurs with a prevalence of approximately one case per 30,000 males worldwide. The population prevalence of haemophilia B in Ireland at one per 12,500 males is particularly high".

A study conducted between 1998 and 2006, and published in Haemophilia in 2011 (Stonebraker *et al.,* 2011), examined the prevalence of the condition (HB) for 105 countries from the World Federation of Haemophilia annual global surveys; they found that Ireland had the highest reported prevalence (8.07 per 100,000 males), with a strong trend of increasing prevalence over time. The mean prevalence among high income OECD countries ranged from 0.47 per 100,000 males in Luxembourg to 8.07 per 100,000 males in Ireland. This should be compared to the mean prevalence observed in the study: The HB prevalence (per 100,000 males) for the highest income countries was 2.69 ± 1.61 (mean ± SD), whereas the prevalence for the rest of the world was 1.2 ± 1.33 (mean ± SD).

3. Please provide some background information on Haemophilia B and how this proposed treatment will impact on the disease.

Haemophilia B is the bleeding diathesis that results from a deficiency of blood coagulation factor IX (FIX). The disease is X-linked and affects approximately 1 in 30,000 males worldwide. Current treatment of the disease is based on intravenous infusion of clotting factor concentrates, either recombinant or plasma-derived, in response to bleeding episodes. The major morbidity of the disease is arthropathy that results from recurrent spontaneous bleeds into the joints. A major cause of mortality is bleeding into critical closed spaces (e.g., intracranial, retropharyngeal, or retroperitoneal bleeds). The introduction of FIX concentrates and of home infusion protocols in the 1960s and 1970s represented a major advance in the treatment of haemophilia in the US and Western Europe. Factor IX concentrates remain largely out of reach for the haemophilia B population in developing countries because of cost. However, widespread use of contaminated plasma-derived clotting factor concentrates in the Western world has resulted in a high rate of hepatitis B, hepatitis C, and human immunodeficiency virus (HIV) infection among individuals with severe disease in these countries. HIV seroconversion studies document that most patients with haemophilia were infected between 1978 and 1984 (Ragni *et al.*, 1986). The development of viral inactivation procedures in the 1980s and of recombinant FIX protein in the 1990s provided an additional level of safety, and most haemophilia B patients born after 1987 are free of hepatitis C and HIV. However, these products have not circumvented all of the problems of protein-based therapies.

The majority of individuals with haemophilia B have severe or moderately severe disease, with FIX levels of ≤ 2%. A generation of clinical research in patients treated with clotting factor concentrates has documented that minimal elevations (a few percent) in the levels of circulating clotting factor are sufficient to prevent much of the morbidity of the disease. Some of the most comprehensive data are contained in the Swedish prophylaxis studies summarized in Lofqvist *et al.* (1997). Manco-Johnson and colleagues have confirmed these findings in a randomized controlled trial in a paediatric population; boys treated on a prophylactic regimen had markedly better protection from joint damage than those treated on an aggressive on-demand regimen (Manco-Johnson *et al.,*2007). Thus, the favourable effect on joint disease of keeping levels even marginally higher than baseline is clear.

Before the AIDS era, central nervous system haemorrhage was the most common cause of premature death in patients with haemophilia (Gilchrist *et al.*, 1989). In several retrospective analyses and in one prospective study, the prevalence of CNS bleeding ranged between 2.6 and 13.8%, with mortality rates between 20 and 50% (Eyster *et al.*, 1978; Andes *et al.*, 1984; Bray *et al.*, 1987) and morbidity rates (including seizure disorder, motor impairment, or mental retardation) of 40–50% in survivors (Eyster *et al.*, 1978). These bleeds can present with few or subtle premonitory symptoms and the bleed can impair the patient's ability to treat himself. The literature documents that these bleeds occur predominantly in patients with severe disease (Eyster *et al.*, 1978). Thus, it is reasonable to suppose that raising the levels of factor even slightly (> 3% of normal) would improve the chances for avoiding this life-threatening complication of the disease. The incidence of CNS haemorrhage can also be reduced by the use of prophylactic factor IX infusions (Gilchrist *et al.*, 1989), although patients remain at risk of breakthrough bleeding when factor levels are at the trough.

Prophylactic therapy for haemophilia is not widely practiced among adult patients in the United States due to the prohibitive expense and inconvenience. Gene therapy, a proposed alternative approach, would potentially allow patients to realize the benefits of prophylaxis, including reduced incidence of CNS bleeding and of haemophilic arthropathy, without the need for indwelling intravenous catheters (used predominantly in the paediatric population), which carry an additional risk of infection and/or local thrombosis (Manco-Johnson, 1996), or frequent factor IX infusion. Indeed, this prediction has been borne out in the recent St. Jude Children's Research Hospital-University College London gene therapy trial, in which 4 of 6 reported participants have been able to stop prophylaxis and yet remain free of spontaneous bleeding episodes after receiving gene therapy (Nathwani *et al.,* 2011b; personal communication, E.G.D. Tuddenham). Further, approximately 75% of individuals with haemophilia B around the world have no access to recombinant clotting factor, minimal to no access to plasma-derived clotting factor, and little hope of gaining access to these expensive therapies. Thus, availability of gene therapy may be a viable alternative to manage their disease.

It is not known if the doses used in this study will increase the level of circulating factor IX in participants, though based on prior clinical experience it is suspected that all doses tested will result in a detectable increase in factor IX levels. This would improve the disease from a severe phenotype to a moderate or mild phenotype, reducing the need for clotting factor infusions while preventing spontaneous bleeding episodes.

Study Objective

1. Please clarify the study objective in particular what precisely is meant by the measurement of biological and physiologic activity of the transgene product.

Furthermore it is my understanding that this study does not purport to replace a defective gene rather to increase the level of circulating FIX. However, at the same time the patient's semen will be monitored for germline transmission. Please clarify.

The primary objectives are to evaluate the safety and tolerability of a single peripheral intravenous administration, in an inter-subject group dose escalation, of AAV8-hFIX19 in adults with severe haemophilia B. Toxicity related to the administration of AAV8-hFIX19 will be evaluated locally and systemically, based on a comprehensive clinical monitoring plan.

The secondary objectives are as follows:

a) To assess the dose of AAV8-hFIX19 required to achieve stable expression of human factor IX above 3% of normal circulating plasma levels, a level which would significantly ameliorate the severe bleeding phenotype;

b) To describe the immune responses to the human factor IX transgene product and AAV capsid proteins following systemic administration of AAV8-hFIX19; and

c) To evaluate the safety and toxicity of an immunomodulatory regimen consisting of prednisolone, in those subjects who develop hepatic transaminase elevation after AAV8-hFIX19 administration.

The activity of the transgene product will be evaluated primarily by assessing circulating plasma factor IX levels, as measured by standard clinical laboratory coagulation tests (factor IX activity, aPTT, PT) and by factor IX antigen levels by ELISA. Additional evaluation will consist of reviewing participant diaries for the number of bleeding episodes, as well as the frequency of recombinant or plasma-derived factor IX concentrate administration during the active phase of the study.

The investigational product, AAV8-hFIX19, is designed to deliver a properly functioning version of the gene for normal human factor IX to individuals with a mutation in this gene. The delivered gene, which will only be expressed in hepatocytes (the site of biosynthesis for factor IX), will not replace the participant's defective gene within the individual's genome. Rather, the gene delivered by the recombinant adeno-associated viral vector is stabilized predominantly in a non-integrated, stable episome form within the nucleus of transduced cells, but outside of the genome (Miao *et al.,* 1998; Miao *et al.,* 2000). Of note, animal studies suggest that, even with this predominantly non-integrating vector, integration into the genome does occur at some low level (Li *et al.,* 2011; see question relating to chromosomal integration below for further details).

Vector sequences have been detected in semen of participants of prior AAV gene transfer studies for haemophilia B. A series of non-clinical studies was performed to demonstrate (1) the transient nature of this finding in both humans and animals, and (2) that vector sequences were not detected in motile sperm of humans (Arruda *et al.,* 2001; Couto and Pierce, 2003; Couto *et al.,* 2004; Manno *et al.,*

2006). A series of studies using rabbits, a valuable model for assessing germline transmission risk for humans, was performed and it was determined that following intravenous injection of AAV2, the duration of detection of vector genomes in the semen is dose-dependent and time-dependent, with genomes diminishing over time until they are completely undetectable; AAV infectious particles were only present up to day 4 post-injection and undetectable thereafter (Schuettrumpf *et al.,* 2006).

More recently (Favaro *et al*., 2009) data relating to the clearance of vector were obtained in rabbits injected by a peripheral intravascular route with AAV2 or with AAV8 vectors expressing human factor IX under the control of the liver-specific promoter utilized in the proposed study. Two doses were compared ($1x10^{12}$ vg/kg (low-dose) or $1x10^{13}$ vg/kg (high-dose)). The kinetics of vector clearance from semen samples were comparable between both serotypes. Overall the percentage of samples testing positive for AAV DNA was higher among the high-dose compared to low-dose cohorts. The last positive semen sample was between weeks 8 to 10 for the low-dose and weeks 10 to 13 for the high-dose groups. Together these findings show that the kinetics of AAV clearance is vector dose- and time-dependent but vector-serotype independent, at least for the two serotypes tested. Previously we reported that at similar doses of AAV2, the last positive semen was observed at day 104. Thus, for both AAV2 and AAV8, no vector DNA was found in semen after the third spermatogenesis cycle. The analysis of semen for an additional seven sequential spermatogenesis cycles in the AAV8 injected animals, i.e., a cumulative 63 cycles, revealed no late recurrence of vector DNA. These results were comparable to the earlier data for AAV2 in rabbits. Failure to detect recurrence of AAV in the semen argues against the possibility of transduction of an early spermatogenesis precursor exposed to AAV8 during the hematogenous dissemination to the gonads at the time of vector injection. Together these findings demonstrate that the presence of AAV8 vectors in the gonadal tissue at early and late times post injection is not associated with a measurable risk of germline transmission.

Lastly, using vasectomized rabbits, we have demonstrated that AAV vector sequences reached the semen in the absence of germ cells. The presence of AAV in the semen was vector-dose dependent but serotype-independent. Thus, fluid and cells from the genitourinary tract other than the germ cells also contribute to the vector shedding to the semen. Together these data provide evidence of low risk of germline transmission by AAV vectors.

This risk is further mitigated by the requirement (as specified in the Clinical Trial Protocol) that patients use an effective barrier contraceptive until at least two consecutive semen samples after vector administration are negative for vector sequences.

2. How long is it envisaged that the GMO therapy will last once it is administered to the patient?

It is expected that the delivered human factor IX gene, stabilized in a predominately episomal form, will persist and be expressed for the life cycle of the transduced hepatocytes. Several groups have established that AAV efficiently transduces hepatocytes in animal models of the disease following a single administration via the portal vein, hepatic artery, or by the IV route, resulting in long-term, (more than 10 years in haemophilia B dogs), dose-dependent transgene expression (Snyder *et al.*, 1997; Nakai *et al.*, 1998; Xiao *et al.*, 1998; Jiang *et al.*, 2006; Niemeyer *et al.,* 2009; Nathwani *et al.,* 2011a).

Results from prior AAV-mediated gene therapy study participants support this evidence for sustained transgene expression, in the absence or following successful treatment of an immune-mediated clearance of transduced hepatocytes by capsid-specific $CD8^+$ T cells; this immune-mediated clearance, observed following liver-mediated delivery of AAV vectors (Manno *et al.,*2006; Nathwani *et al.,* 2011a), can be successfully treated with the short course of immunomodulatory drugs described in the Clinical Protocol. Participants of the St. Jude Children's Hospital-University College London gene therapy study who received an AAV8 vector expressing human factor IX have now been followed for a periods ranging from four months to over three years, with observation ongoing, and all participants have exhibited sustained transgene expression.

It should be noted that expression levels may decline over time because hepatocytes are naturally turned over in the liver and daughter cells from hepatocytes that regenerate will not contain the non-integrated, stable transgene episome. A decline in factor IX levels has not yet been observed in the human subjects enrolled in the UCL/St. Jude trial.

Dose

Please clarify the GMO dose to be administered to the patient?

Section 3.4 of the Environmental Risk Assessment (ERA) states that "*Doses to be administered for the proposed study in an inter-subject group dose escalation study design are 1 x 10^{12} vg/kg, 2 x 10^{12} vg/kg and 5 x 10^{12} vg/kg*". At the same time the GMO will be administered by a single intravenous infusion. Please explain how "an inter-subject group dose escalation study" will operate, particularly given the very small number of participants (4-8).

This is a multi-centre, open label, non-randomized, inter-subject dose escalation safety study of three vector doses ($1x10^{12}$ vg/kg, $2x10^{12}$ vg/kg, and $5x10^{12}$ vg/kg) in adults with severe haemophilia B. There will be at least two weeks between vector administrations to successive intra-cohort subjects.

Study-wide enrolment is anticipated to be 9 to 15 eligible participants; 4 to 8 eligible participants are anticipated in Ireland. Participants who meet all eligibility criteria will receive a single peripheral intravenous infusion of AAV8-hFIX19 at one of the three doses described above. The vector dose will depend on the doses that previous study participants have received and whether those doses were safe and tolerated by the study participants.

Two to five participants will be enrolled per global dose cohort; if a given vector dose does not result in plasma FIX levels > 3% of normal in both of the first two individuals of the cohort, escalation to the next highest vector dose will occur in subsequently enrolled patients, provided there are no limiting toxicities. A level of > 3% in one but not both participants would trigger a dose escalation in subsequently enrolled patients. If a level of > 3% is attained in both participants, the dose cohort will be expanded to five participants. The investigators would still expect to enrol study participants in the next proposed dose cohort, since FIX levels may slowly decline over time and thus a higher level would be desirable. This means a total of 9 to 15 participants will be enrolled on the study at all sites, in the absence of safety concerns (*e.g.* 2+2+5=9 minimum to 5+5+5=15 maximum). This escalation

strategy is employed because viral vector administration may result in immune responses against AAV8 capsid that could preclude subsequent vector administration.

The determination of circulating FIX levels of > 3% will be based on two separate plateau level time points, at Weeks 4 and Week 8, provided these time points are each at least two weeks after the most recent infusion of clotting factor concentrate, and therefore reflect endogenous production. Factor IX levels from the next "clean" visit(s) will be used if exogenous FIX has been administered (*e.g.* Weeks 5 and 9).

Progression to the next cohort will be contingent upon independent DSMB review of safety and efficacy data from all participants in the previous cohort, through at least eight weeks following vector administration; therefore, there will be at least eight to ten weeks between the last participant infused in a given dose cohort and the first vector administration in the next higher cohort.

Treatment of waste

1. Section 3.5 of the ERA states as follows:

"Following administration of AAV8-hFIX19 at a medical facility, used (or partially used) vials of vector/empty capsids, syringes used in dose preparation, infusion bags and infusion sets are retained and stored in a labeled biohazard bag in the pharmacy at below -60°C for a period of no less than two months, per manufacturer's retention instructions (as specified in the Investigational Drug Data Sheet (IDDS) and Handling Instructions for AAV8-hFIX19). These items will then be returned to the sponsor or destroyed according to sponsor instructions".

a) Why are contaminated materials retained in this way and for so long?

b) Is it feasible that these items may be decontaminated within the St. James's hospital site?

c) If not, what is the procedure for the return of the items to the U.S.?

d) What are the sponsor's instructions for the decontamination of the abovementioned items?

a) Contaminated materials are retained for potential investigations subsequent to administration, should the need arise. For example, an unexpected lack of efficacy or an unexpected adverse event in a patient may trigger such an investigation. In these circumstances, samples obtained from the actual prepared dose would be important, to rule out dosing errors or unexpected effects such as contamination during dose preparation, or excessive absorption of virus particles onto the surfaces of contact materials (eg. infusion bags/ kits).

b) It is feasible to decontaminate these waste items at St James' hospital. Decontamination is most effectively achieved by incineration, in accordance with local practice for medical waste.

c) In the event that the aforementioned waste is returned to the US, the sealed bags will be securely packaged and shipped on dry ice according to CCMT/CHOP instructions. The packages will be labeled with a biohazard symbol and shipped by air in accordance with International Air Transport Association (IATA) regulations

d) Decontamination of these items will be achieved by incineration, in accordance with local practice for medical waste.

2. Section 3.5 of the ERA states as follows:

"Any other disposable instruments or other materials used during the dose preparation procedure will be disposed of in a manner consistent with the standard practice of the institution for potentially biohazardous materials."

a) Please clarify what is the standard practice of the institution is or will be for the decontamination and disposal of GMM contaminated disposable and non-disposable materials.

b) In the event that the procedure involves disinfection, please provide the name and concentration of disinfectant(s) to be used, and minimum contact times for both GMM contaminated solid and liquid wastes.

c) Where the procedure involves autoclaving, please provide details of the location of the autoclave relative to the medical facility, and the decontamination cycle parameters (temperature, time and pressure).

d) Please provide details of the off-site contractor where one is being used for purposes of waste disposal.

<u>Disposal of medical waste</u>

Disposable (non-sharp) materials potentially contaminated with the GMO will be placed in standard leak-proof bags/ rigid bins for 'risk' medical waste, which is collected twice weekly from the hospital and technologically treated and rendered inert by S.R.C.L. whose headquarters are in Beech Road, Western Industrial Estate, Dublin 12 and is EPA licensed. The inert waste is recovered and used as fuel.

'Special risk' waste is collected weekly by S.R.C.L. and exported for incineration to Belgium.

Disposable sharp materials will be placed in puncture-proof 'sharps' bins which are sealed when full and collected by S.R.C.L as described above.

All waste is traceable from source to destruction and S.R.C.L. provides certificates of destruction to the hospital.

Disinfection and autoclaving

Non-disposable equipment which may potentially be contaminated include surfaces of the biosafety cabinet and face protection (goggles/ splash-guards). These materials can be decontaminated by the application of the following as defined in the MSDS:

"*Effective disinfectants require a minimum of 20 minutes contact time. Use one of the following:*

- *Sodium hypochlorite (1-10% dilution of fresh bleach).*
- *Alkaline solutions at pH >9.*
- *5% phenol.*"

Alternatively, items may be autoclaved according to the standard procedures of the facility. There are two autoclaves in the laboratory in St James's Hospital for non-disposable biohazard material. The Hospital Sterile Services Unit in the hospital has autoclave machines for non-disposable materials.

The cycle parameters are as follows: 126°C for 30 min for waste and 134°C for 3 min for reusable material. A wash and disinfect pre-cycle is also used. St James's Hospital has a National Track and trace process on all their autoclaved items.

St James' Hospital has a number of policies on waste management and autoclaving which are available on request of EPA.

Antibiotic resistance

1. Section 3.3 of the ERA states as follows:

"The antibiotic kanamycin is used in the manufacturing process for production of the plasmid stocks. The kanamycin resistance genes encoded by the plasmids are not by design part of the final vector, but may be present as residual impurities. Residual plasmid DNA is analysed on a batch-by-batch basis by qPCR".

a) What is residual plasmid DNA analysed for?

b) What measures are taken in the event of a positive analysis?

c) As residual impurities is there potential for the kanamycin resistance genes to be administered to the patient receiving AAV8-hFIX19?

The active substance, AAV8-hFIX19 does not contain antibiotic resistance genes, nor are antibiotics used directly in the manufacturing process. AAV2-hRPE65v2 is a highly purified viral vector which contains an expression cassette (Human α1-antitrypsin (hAAT) liver-specific promoter coupled to the human apolipoprotein E (ApoE) enhancer/hepatocyte control region, Exon 1 from the human factor IX gene, a portion of the human factor IX intron 1, exons 2-8 of the human factor IX gene and Bovine polyadenylation signal sequence) flanked by AAV2 ITRs.

The three plasmids (starting materials) which are used in the transfection of the HEK293 cell line during manufacture of AAV8-hFIX19 contain the gene encoding aminoglycoside phosphotransferase (*APH*) conferring kanamycin resistance. The plasmid stocks of these starting materials are manufactured separately by bacterial fermentation, using a medium containing kanamycin in order to positively select for the retention of the plasmids during bacterial replication.

Residual levels of the kanamycin resistance gene in the final product are not intentional and are considered an impurity. Such sequences may include naked plasmid DNA, which is reduced during purification via digestion with an endonuclease (Benzonase™), anion exchange chromatography, CsCl gradient ultracentrifugation and ultra-filtration/ diafiltration (TFF). The Benzonase™ step was optimized to achieve efficient degradation of accessible DNA impurities. Lots of the Investigational Medicinal Product (AAV8-hFIX19) are analysed for the presence of residual plasmid-borne *APH*. The levels detected must be <100 pg *APH*/10^9 viral genomes or the batch is rejected. It is not possible to remove residual plasmid DNA completely, and this level is therefore chosen as the lowest level that can practically be achieved at this stage of process development. The calculation used for mass of residual *APH* assumes the molecular weight of the complete vector plasmid, providing a worst case but consistent calculation of this residual impurity; our best estimate of the actual mass is 5–10 fold lower than the value reported for each Lot. Residual naked DNA containing the gene for kanamycin resistance may therefore be administered to patients but would be expected to be quickly cleared from the human body through normal processes and does not have the capacity to persist.

A low level of packaging of plasmid backbone DNA into viral capsids, primarily due to unintended ‘reverse’ packaging of plasmid backbone from the ITRs that are present in the plasmid containing the expression cassette also occurs (Hauck, *et al*., 2009; Chadeuf, *et al*., 2005). This form of residual plasmid DNA impurity is not susceptible to digestion by endonuclease. Such packaged DNA impurities are present at a very low level in comparison with packaging of the correct (hFIX) expression cassette flanked by ITRs. Any residual packaged DNA encoding the *APH* gene is expected to be single stranded, and highly unstable following release from the AAV capsid particle (ie. following entry into the cell). The ITR containing vector plasmid was optimized with an oversized plasmid backbone exceeding the packaging capacity of AAV2, to minimize the amounts of such residual plasmid DNA impurities, and to render the low residual levels susceptible to rapid degradation.

Further, the method and route of administration are relevant to the assessment of risk posed by residual kanamycin resistance sequences in the preparation. The product is intended for single administration to the sub-retinal space of each eye where it remains largely localised. Virus particles which do not infect cells are quickly cleared from the body.

It is therefore considered that the risk of horizontal transmission of residual levels of the Kanamycin resistance gene to bacteria (which would need to actively infect the tissues where residual plasmid DNA was (transiently) present) is very low.

2. According to section 4.1 of the ERA, residual plasmid DNA containing genes for kanamycin resistance and derived from the manufacturing process could contaminate the AA V8-hFIX19 preparation. The section continues that this contamination could lead to

limited distribution of DNA sequences potentially containing genes for kanamycin resistance in the environment.

a) How would this environmental contamination be brought about?

b) Please clarify what the ensuing impact would be for the environment and human health.

CCMT/CHOP has specifically constructed the three plasmid starting materials to remove the gene conferring resistance to ampicillin, and replace with the *APH* gene for resistance to kanamycin. This removes the possibility of inadvertent administration of trace residual beta lactams, known to be associated with hypersensitivity reactions in a small percentage of the human population. Furthermore, kanamycin is deemed a more appropriate selection agent since it is less commonly used to treat human infections because of its numerous side effects.

Kanamycin is an aminoglycoside antibiotic. Aminoglycosides are useful primarily in infections involving aerobic, Gram-negative bacteria, such as Pseudomonas, Acinetobacter, and Enterobacter. In addition, some mycobacteria, including the bacteria that cause tuberculosis, are susceptible to aminoglycosides. Infections caused by Gram-positive bacteria can also be treated with aminoglycosides, but other types of antibiotics are more potent and less damaging to the host. In the past the aminoglycosides have been used in conjunction with penicillin-related antibiotics in streptococcal infections for their synergistic effects, particularly in endocarditis. Aminoglycosides are mostly ineffective against anaerobic bacteria, fungi and viruses.

As described above, the kanamycin resistance gene (*APH*) may be present in the Investigational Medicinal Product (AAV8-hFIX19) at low levels, either as naked DNA or packaged within viral capsids. There is therefore a theoretical risk that the environment could be exposed to these sequences, in the same way as the potential exposure to the Investigational Medicinal Product itself (ie. spills, contamination of equipment etc.). These risks are mitigated by the transport, handling and waste disposal procedures discussed elsewhere, and are therefore considered very low. Another route of potential exposure to the environment would be shedding of plasmid backbone DNA which has been packaged into viral capsids (see above). Such shedding from treated individuals is expected to be low level and transient (see below).

The release of DNA encoding the kanamycin resistance gene (*APH*) is considered to have no impact on the environment or on human health. Virus particles containing the *APH* DNA are unable to replicate under any circumstances and will rapidly be degraded in the environment. Antibiotic resistance genes confer no selective advantage to organisms in the non-clinical environment and as such would not be expected to perpetuate; thus no impact on human health is expected. Antibiotic resistance marker genes have been used in microbiological research globally for decades, with no detected impact on the environment.

Shedding

1. **What is the potential for the recombinant vector to shed and over what period of time will shedding take place?**

2. What risks to human health or the environment will arise from shedding?

3. What precautions (if any) can be taken to reduce the risk of shedding?

1. Potential for shedding

Data on the shedding of the experimental vector (AAV8-hFIX19) are not currently available. However, data are available on similar vectors, notably a self-complementary AAV8 pseudo-typed vector containing an hFIX gene (AAV8-LP1-hFIXco; Nathwani *et al.,* 2011a,b) and an AAV2 serotype vector containing hFIX (AAV2-hFIX16; Manno *et al.,* 2006). These data are summarised in Section II.C.2 (i).3. of the Technical Dossier (Annex III) submitted.

The shedding of AAV8-hFIX19 is not expected to differ from that observed with AAV8-LP1-hFIXco, since the route of administration is the same and the capsid (which determines its tissue tropism and clearance) is of the same serotype.

The main routes of viral shedding are via urine, stools, saliva and semen.

In the study of AAV8-LP1-hFIXco in human haemophiliacs, the vector genome was detectable in the saliva, semen and stools within 72 hours of vector infusion and up to but not after day 15 in all participants, with the exception of participant 1 whose semen remained clear of proviral DNA at all-time points assessed. The amount of vector DNA in the body fluids roughly correlated with the vector dose administered, as the highest level of viraemia (2.5-6.2x10^6vg/µl) was observed in the two participants that received 2x10^{12}vg/kg on the first day post gene transfer. Vector sequences were not detected in the urine of any of the participants at any time point after administration of vector.

It is therefore considered likely that shedding of AAV8-hFIX19 will occur following administration for a limited period of time (likely 2 weeks). This will be confirmed by sampling as part of the Clinical Trial Protocol.

2. Risks to human health

The consequences of the transmission of AAV8-hFIX19 to an unintended human recipient are expected to be no greater than those which may be anticipated in patients receiving the treatment.

Neither wild type AAV nor the experimental vector AAV8-hFIX19 is known to be pathogenic to humans. AAV8-hFIX19 is unable to replicate independently, even in the presence of a helper virus, since it lacks the *rep* and *cap* genes required for rescue/packaging.

The transgene encoded by AAV8-hFIX19 is a protein, and is identical to normal human coagulation Factor IX. The gene product is therefore expected to be metabolised naturally. It is clear, based on data from infusion of FIX concentrates (ie. the Factor IX protein) into patients with haemophilia B, that circulating FIX levels as high as 100% of normal are not associated with ill-effects since the protein circulates as a zymogen (inactive precursor). In patients receiving the full, high dose of AAV8-hFIX19, it is postulated that hFIX expression will lead to circulating levels of FIX in the region

of 2-10% of normal. Thus, the effect of expression from an inadvertently acquired 'dose' of AAV8-hFIX19 may be expected to have a negligible effect on circulating hFIX in a non-haemophiliac.

The possible effects of direct administration to patients are presented in Section 5.1.2 of the Environmental Risk Assessment submitted (Annex II). In the case of transfer of vector to an unintended human recipient, the risks are expected to be considerably reduced, since the vector is not able to replicate and the 'dose' which may conceivably be transferred (from e.g. aerosol, splashing or fomites) will be orders of magnitude lower than that received by patients.

Risks to the environment

Wild type AAV is not known to be involved in environmental processes. It does not respire and does not contribute to primary production or decomposition processes. In its virion form, it does not display any metabolic activity.

There are no known natural predators, preys, parasites, competitors or symbionts associated with wild type AAV (although it does require helper functions of co-infecting viruses for replication in nature as described above. Primate (human) AAV serotypes are not known to actively transfer genetic material to organisms other than primates under natural conditions, although an absence of zoonosis is not documented.

AAV8-hFIX19 is unable to replicate independently, even in the presence of a helper virus, since it lacks the *rep* and *cap* genes required for rescue/packaging. Therefore, the presence of the expression cassette is expected to confer a severe selective disadvantage to the GMO.

In conclusion, based on the nature of the GMO, the parental organism and the receiving environment, AAV8-hFIX19 is not anticipated to have any direct effects on the environment (other than humans).

3. Precautions to reduce the risk of shedding

The risk of shedding via semen is reduced by the requirement (as specified in the Clinical Trial Protocol) that patients use an effective barrier contraceptive until at least two consecutive semen samples after vector administration are negative for vector sequences (ie., the vector is confirmed as no longer present in seminal fluid).

The risk of shedding via saliva cannot be reduced. The risks of shedding via urine and stools could be mitigated further by instructing patients to use household bleach following each use of the toilet. However, given the absence of identified risks of exposure of the environment to AAV8-hFIX19, these additional precautions are not considered necessary.

Chromosomal Integration

Section G2 of the SNIF states that "*the vector* [once injected] *is exposed to persist for months or years primarily as an episome but potentially by integration into the host cell genome*". Section 5.1.2 of the ERA states that "*integration will take place at some low level*". Since the vector lacks the

AAV rep proteins, presumably this integration will take place at a chromosomal location other than chromosome 19.

a) Please clarify the recombinant vector's potential for integration, where integration will likely take place and the level at which it will take place (please expand on low level of integration).

b) What will be the impact of chromosomal integration?

When wild type AAV infects a human cell alone, its gene expression program is auto-repressed and latency ensues by preferential integration of the virus genome into a region of roughly 2-kb on the long arm (19q13.3-qter) of human chromosome 19 (Kotin *et al.*, 1990; Samulski *et al.*, 1991) designated *AAVS1* (Kotin *et al.*, 1991). However, it has been demonstrated that only approximately 1 out of 1000 infectious units can integrate (Tenenbaum *et al.*, 2003).

The mechanism of this site-specific integration involves AAV Rep proteins which are absent in AAV8-hFIX19. Accordingly, recombinant AAV (rAAV) do not integrate in a site specific manner.

It has been shown that, following liver transduction, rAAV is stabilized predominantly in a non-integrated form (Miao *et al.,* 1998; Miao *et al.,* 2000). However, integration into the genome of transduced cells does occur at some low level (Li *et al.*, 2011).

It is important to note that the integration mechanism of AAV differs from that of other viruses, such as retroviruses. Retroviral vectors contain the proteins needed to cause double-stranded DNA breaks for integration. AAV vectors do not contain such proteins and must rely on the cellular machinery. In fact, it is currently thought that AAV vectors may preferentially integrate into genomic DNA regions that are already broken (Miller *et al.,* 2004).

It is assumed that the greatest potential for integration would be into cells within the liver, but given the results of tissue distribution studies, the potential for integration into cells of other tissues also exists. The major potential risk resulting from integration into the host cell DNA is an enhanced risk of malignant transformation leading to cancer. A mouse model of AAV infusion into liver during the neonatal period led to an increased number of hemangiosarcomas and hepatocellular carcinomas (HCC) after prolonged periods, and for at least some of the HCC tumours, an integration event in mouse chromosome 12 was associated with the tumour tissue but not the normal adjacent tissue (Donsante *et al*., 2007). Other studies have not confirmed this association, although the studies may have been hampered by inadequate numbers of mice (Li *et al.,* 2011).

The site(s) of integration have been analysed using 454 pyrosequencing and bioinformatics analysis to characterize > 1000 integration sites from mouse liver injected at high doses with an AAV2-hFIX16 vector. These data (Li *et al*., 2011) confirmed earlier reports (Nakai *et al*., 2005) of preferential integration into actively transcribed genes, CpG islands, and GC-rich regions. Note that these data are relevant, since both AAV2 and AAV8 vectors utilize the AAV2 ITRs, the vector element involved in integration.

The risk of a malignant transformation leading to cancer is suspected to be low based on absence of such an event in large haemophilic animals. Long-term follow up of haemophilia dogs injected with

AAV-cFIX vectors as early as 1997 has revealed no evidence of tumour formation in liver as judged by ultrasound; follow up continues (Niemeyer *et al*., 2009; Nichols, Arruda, High, unpublished data). In addition, liver ultrasounds carried out in 4 of 7 clinical trial participants injected with AAV2-hFIX16 from 2001-2004 have shown no evidence of liver tumours. Of the remaining 3 participants: 1 has died of causes unrelated to gene transfer, and autopsy showed no evidence of tumour in the liver; 1 was lost to follow-up; and 1 has declined to undergo a liver ultrasound but remains in good health (Wellman *et al.,* 2012).

Containment

1. How will the GMO be stored prior to administration?

2. Where will it be stored?

3. What quantities of the GMO are anticipated to be stored and for what duration?

4. Where applicable, please provide details of temperatures at which the GMO will be stored?

A qualified pharmacist with specific training on the protocol will be responsible for gene transfer material receipt from the Sponsor, storage, documentation of traceability of product at the investigational site.

Adequate records of study drug receipt and disposition will be maintained by the study site Investigational Pharmacy, and records of receipts, investigational drug orders, dispensing records, and disposition forms will be examined during the course of the study. The purpose of these records is to ensure that the investigational new drug will not be distributed to any person outside the terms and conditions set forth in the Clinical Trial Protocol. The study medication is to be prescribed by the Investigator or designee and may not be used for any purpose other than that described in the Clinical Trial Protocol.

The investigational product will only be provided to sites on a subject-by-subject basis, following confirmation of subject eligibility and a review of registration documents/essential documents.

AAV8-hFIX19 vector is supplied as a frozen liquid at a volume of 1 mL in a 1.5 mL polypropylene sterile cryogenic screw cap vial. AAV8-hFIX19 is to be stored securely, frozen at less than -60°C (ie. in an ultra-low temperature freezer). Ultra-low freezers are located at St James' Hospital in the Cryobiology Laboratory and the Cancer Molecular Diagnostic Laboratory. Should these ultra-low freezers not be appropriate for storage of Investigational Medicinal Product (in addition to their normal function), an ultra-low freezer located either in the Hospital Pharmacy or the new Clinical Research Center will be dedicated to the storage of Investigational Medicinal Products such as AAV8-hFIX19.

The maximum total dose for an 80 kg individual patient is approximately 22 mL as a single treatment (approximately 40 x 10^{13} vg), therefore it is unlikely that more than 44 vials (2 patient's supply) will be stored at the site at any one time. Since the IMP is to be supplied 'on-demand', it is unlikely that it will be stored at the site in Ireland for more than 1 month prior to administration.

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Environmental Protection Agency
An Ghníomhaireacht um Chaomhnú Comhshaoil

Headquarters, PO Box 3000
Johnstown Castle Estate
County Wexford, Ireland

Ceanncheathrú, Bosca Poist 3000
Eastát Chaisleán Bhaile Sheáin
Contae Loch Garman, Éire

T: +353 53 9160600
F: +353 53 9160699
E: info@epa.ie
W: www.epa.ie

LoCall: 1890 33 55 99

Dr Robin Henderson
Alan Boyd Consultants Ltd
Electra House
Crewe Business Park
Crewe
CW1 6GL
United Kingdom

6 May 2013

Request for further information under Article 19 (1) of S. I. No. 500 of 2003 with regard to notification for consent to release GMOs into the environment for purposes other than placing on the market (GMO Register G0498-01, Reference B/IE/13/01) – Second Request

Dear Dr Henderson

I refer to the above mentioned notification, and request the following information which is required in order to assist the EPA (Agency) in their review of the application.

Treatment of waste

1. Your response to our first request for additional information (dated 17 April 2013) indicated that AAV8-hFIX19 contaminated waste is most effectively decontaminated by incineration. The Agency would not be in favour of shipping waste material to the US for purposes of decontamination. The alternative proposal was that the waste would be collected by SRCL Ltd and exported to Belgium for incineration.

   Wild type AAV is classified as a Risk Group 1 biological agent according to Directive 2000/54/EC on the protection of workers from risks related to exposure to biological agents at work under which it is defined as 'unlikely to cause human disease.' Recombinant AAV which is unable to replicate independently may be considered disabled, therefore, would it be feasible to decontaminate vector waste by autoclaving at 126°C for 30 minutes – the temperature / time parameters given for the decontamination of vector contaminated solid waste?

2. Please provide the corresponding pressure parameters for the decontamination of waste by autoclaving:

Waste type	**Temperature / time**	**Pressure (bar)**
Contaminated solid disposable waste	126°C / 30 minutes	
Contaminated non-disposable equipment	134°C / 3 minutes	

Please note that in accordance with Article 8(1) of the GMO (Deliberate Release) Regulations, S.I. No 500 of 2003, "a period of time during which the Agency is awaiting any further information on a notification, which it may have requested from the notifier, shall not be taken into account", i.e. the clock is stopped until such time as all the requested information is received by the Agency.

Yours sincerely

Bernie Murray
Inspector
Office of Climate, Licensing Resources and Research

Center for Cellular and Molecular Therapeutics at
The Children's Hospital of Philadelphia (CCMT/CHOP)

AAV8-hFIX19

Responses to Request for further information under Article 19 (1) of S.I. No. 500 of 2003 with regard to notification for consent to release GMOs into the environment for purposes other than placing on the market
(GMO Register G0498-01, Reference B/IE/13/01)
Second Request

Final – June 2013

<u>Treatment of waste</u>

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   Wild type AAV is classified as a Risk Group I biological agent according to Directive 2000/54/EC on the protection of workers from risks related to exposure to biological agents at work under which it is defined as 'unlikely to cause human disease'. Recombinant AAV which is unable to replicate independently may be considered disabled, therefore, would it be feasible to decontaminate vector waste by autoclaving at 126°C for 30 minutes – the temperature / time parameters given for the decontamination of vector contaminated solid waste?

2. **Please provide the corresponding pressure parameters for the decontamination of waste by autoclaving.**

The decontamination described in our first response is not a specific precaution for AAV8-hFIX19, which as stated is a disabled version of a wild-type AAV virus which itself is classified as Risk Group I. The decontamination procedure described is St James' Hospital's standard procedure for treatment of potentially infectious medical waste. The risk from such waste is considered to far exceed that posed by AAV8-hFIX19. Decontamination could be performed at St James by autoclaving, if stipulated by EPA. The standard pressures employed by St James in their autoclaves are provided below.

Waste type	Temperature/time	Pressure (bar)
Contaminated solid disposable waste	126°C / 30 minutes	2.4
Contaminated non-disposable equipment	134°C / 3 minutes	3.2

CCMT/CHOP do not intend to routinely ship materials back to US for decontamination. Material would only be shipped back to the US in the event that an investigation is triggered by (for example) an unexpected adverse reaction observed in a patient. Such incidents / investigations are rare, but if this did occur, CCMT/CHOP may wish to ship material back in order to rule out any product defect such as the presence of excessive impurities or possible contamination of the Investigational Medicinal Product. In this case, CCMT/CHOP would ship back the suspected material as biohazardous material (in accordance with IATA shipping recommendations). Note that decontamination of the material prior to shipping would render any such investigations obsolete. Provided that no investigation is triggered, decontamination will be performed by St James' Hospital or SRCL as per EPA stipulations.