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

AAV8-hFIX19

Technical and Scientific information on the GMO
(In accordance with Schedule III, SI 500/2003)

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TABLE OF CONTENTS

Appendices:

Clinical Trial Protocol synopsis
Investigational Drug Data Sheet (IDDS)/ Pharmacy Instructions
Patient Information
Handling Instructions
Material Safety Data Sheet (MSDS)

LIST OF ABBREVIATIONS

AAV	Adeno-associated virus
AAV-hFIX	Adeno-associated viral vector encoding human coagulation factor IX
AAV2-hFIX9	Single-stranded adeno-associated viral vector, serotype 2, expressing human factor IX under control of the cytomegalovirus promoter (muscle-directed)
AAV2-hFIX16	Single-stranded adeno-associated viral vector, serotype 2, encoding human factor IX under control of the human α1-antitrypsin promoter coupled to the human apolipoprotein E enhancer
AAV8-hFIX19	Single-stranded adeno-associated viral vector, serotype 8, encoding human factor IX under control of the human α1-antitrypsin promoter coupled to the human apolipoprotein E enhancer (construct changes from hFIX16 to hFIX19 include codon-optimization, removal of alternate open reading frames of the factor IX gene, and replacement of *amp* with *kan* resistance in the plasmid used for vector generation)
AAV8-LP1-hFIXco	Self complementary AAV vector, serotype 8, encoding human factor IX
Ad	Adenovirus
APH	Aminoglycoside 3'-phosphotransferase
ApoE	Apolipoprotein E
ARF	Alternate open reading frame
bp	Base pair
BSC	BioSafety Cabinet
BSL	Biosafety Level
CCMT	Center for Cellular and Molecular Therapeutics at The Children's Hospital of Philadelphia
cDNA	Complimentary deoxyribonucleic acid
CHOP	The Children's Hospital of Philadelphia
CRO	Contract research organization
DNA	Deoxyribonucleic acid
DSMB	Data and Safety Monitoring Board
ELISA	Enzyme-linked immunosorbent assay
EPA	Environmental Protection Agency
EU	European Union
FDA	Food and Drug Administration
FIX	Coagulation factor IX
GCP	Good clinical practice
GMO	Genetically Modified Organism
hAAT	Human alpha 1 antitrypsin
HCC	Hepatocellular carcinomas
HEK	Human embryonic kidney epithelial cell line
hFIX	Human coagulation factor IX
HPV	Human papilloma virus
HSV	Herpes Simplex Virus
IATA	International Air Transport Association
IBC	Institutional Biosafety Committee
IDDS	Investigational Drug Data Sheet

IM	Intramuscular
IMB	Irish Medicines Board
IND	Investigational new drug application
ITR	Inverted terminal repeats
IV	Intravenous
kan	Kanamycin
kb	Kilobases
kDa	kiloDaltons
LCA	Leber's Congenital Amaurosis
LOD	Limit of Detection
LOQ	Limit of Quantitation
LTFU	Long Term Follow-Up
MCB	Master Cell Bank
MSDS	Material Safety Data Sheet
Nab	Neutalising antibodies
NHP	Non-human primate
NPC	Nuclear Pore Complex
ORF	Open reading frame
PBMC	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
PPE	Personal Protective Equipment
QPCR	Quantitative polymerase chain reaction
rAAV	Recombinant adeno-associated viral vectors
RNA	Ribonucleic acid
SAE	Serious adverse event
ss	Single stranded
SUSAR	Suspected Unexpected Serious Adverse Reaction
vg	Vector genome
WBCT	Whole blood clotting time
WHO	World Health Organisation

I. GENERAL INFORMATION

A. NAME AND ADDRESS OF THE NOTIFIER

Center for Cellular and Molecular Therapeutics at The Children's Hospital of Philadelphia

5th Floor Colket Translational Research Building
3501 Civic Center Boulevard
Philadelphia, PA 19104-4319
USA

B. NAME, QUALIFICATIONS AND EXPERIENCE OF THE RESPONSIBLE SCIENTIST(S)

Dr Katherine High, MD
Director, Center for Cellular and Molecular Therapeutics, The Children's Hospital of Philadelphia
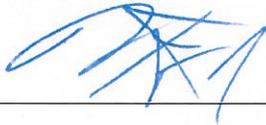
Dr Fraser Wright, PhD
Scientific Director, Clinical Vector Core, Center for Cellular and Molecular Therapeutics, The Children's Hospital of Philadelphia
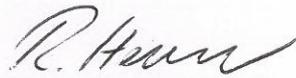
Dr Robin Henderson, PhD
Regulatory Consultant to CCMT/CHOP

C. TITLE OF THE PROJECT

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 [Protocol # AAV8-hFIX19-101].

II. INFORMATION RELATING TO THE GMO

A. CHARACTERISTICS OF PARENTAL ORGANISM

1.   SCIENTIFIC NAME

Adeno-associated virus (AAV).

2.   TAXONOMY

Group: Group II (ss DNA)

Family: *Parvoviridae*

Subfamily: Parvovirinae

Genus: *Dependovirus*

Species: Adeno-associated virus

3.   OTHER NAMES (USUAL NAME, STRAIN NAME, ETC.)

The parental virus concerned in this application is a primate (human) AAV. There are several naturally occurring serotypes of human or non-human primate adeno-associated virus (denoted AAV1 to AAV11) and further variants yet to be fully characterised. The serotype of AAV is determined by the capsid of the virion, which is integral to the species / tissue tropism and infection efficiency of AAV.

The capsid proteins of the experimental vector (AAV8-hFIX19) are derived from the AAV8 serotype.

AAVs have also been isolated from other animal adenovirus stocks; these include avian AAV (Dawson *et al.*, 1982; Yates *et al.*, 1973), bovine AAV (Coria and Lehmkuhl, 1978; Luchsinger *et al.*, 1970; Myrup *et al.*, 1976), ovine AAV (Clarke *et al.*, 1979), caprine AAV (Arbetman *et al.*, 2005) and snake AAV (Farkas *et al.*, 2004).

4.   PHENOTYPIC AND GENETIC MARKERS

Adeno-associated virus is a single-stranded DNA virus (Group II) which exists as a non-enveloped icosahedral virion with a diameter of approximately 25nm.

After entry into the host cell nucleus, AAV can follow either one of two distinct and interchangeable pathways of its life cycle: the lytic or the lysogenic.

Productive (lytic) infection develops in cells co-infected with a helper virus such as adenovirus (Atchison *et al*, 1965; Henry, 1973), Human papilloma virus (HPV) (Hermonat, 1994; Su and Wu, 1996; Hermonat *et al*, 1997), vaccinia virus (Schlehofer *et al*, 1986) or herpes simplex virus (HSV) (Salo and Mayor, 1979; Buller *et al*, 1981) to help its replication.

In contrast, a lysogenic state is established in host cells in the absence of a helper virus. Latency may manifest as a 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). Further work suggests that AAV DNA persists mainly as circular double stranded episomes in human tissues (Schnepp *et al.*, 2005).

The AAV genome is approximately 4.7 kilobase long and comprises inverted terminal repeats (ITRs) at both ends of the DNA strand, and two open reading frames (ORFs): *rep* and *cap* (Figure 1). The former is composed of four overlapping genes encoding Rep proteins required for DNA replication, and the latter contains overlapping nucleotide sequences coding for capsid proteins (VP1, VP2 and VP3) which interact together to form a capsid of an icosahedral symmetry.

Figure 1: Genome organisation of wild type adeno-associated viruses.
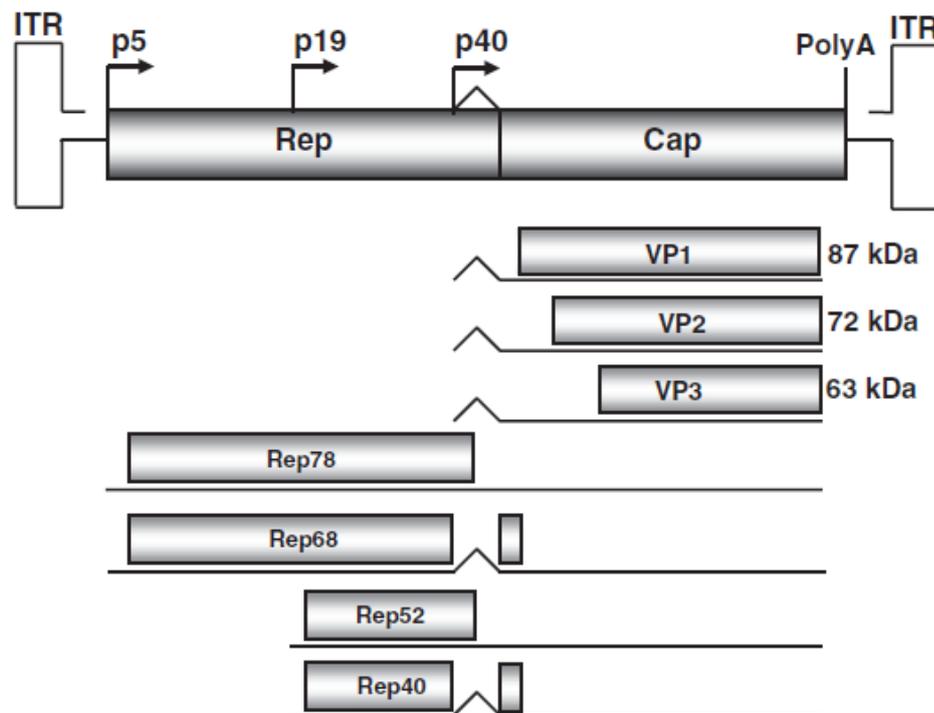
The single-stranded DNA genome of AAV. The inverted terminal repeats (ITRs) flank the two open reading frames *rep* and *cap*. The *rep* gene encodes four nonstructural proteins – Rep78, Rep68, Rep52, and Rep40. The *cap* gene encodes three structural proteins – VP1, VP2, and VP3. The location of the promoters, p5, p19, and p40 are depicted by arrows (Van Vliet *et al.*, 2008)

The Inverted Terminal Repeat (ITR) sequences comprise 145 bases each and contain all *cis*-acting functions required for DNA replication, packaging, integration into the host genome, and subsequent excision and rescue (Samulski *et al.*, 1989).

There are several serotypes of adeno-associated virus (Reviewed in Wu *et al.*, 2006). A serotype is, by definition, a newly isolated virus that does not efficiently cross-react with neutralizing sera specific for all other existing and characterized serotypes. Based on such, only AAV1–5 and AAV7–9 can be defined as true serotypes. Variants AAV6, 10, and 11 do not appear to fit into this definition, since the serology of AAV6 is almost identical to that of AAV1, and serological profiles of AAV10 and AAV11 are not well characterized.

The serotype of AAV is determined by the capsid of the virion, which is integral to the tissue tropism and infection efficiency of AAV. The AAV capsid is composed of 60 capsid protein subunits, VP1, VP2, and VP3, that are arranged in an icosahedral symmetry in a ratio of 1:1:10, with an estimated size of 3,900 kDa (Sonntag *et al.*, 2010). These structural elements are encoded by the *cap* gene of the AAV genome.

The *cap* proteins of the experimental vector (AAV8-hFIX19) are derived from the AAV8 serotype.

AAV serotypes 1 to 6 were isolated as contaminants in laboratory adenovirus stocks, with the exemption of AAV5, which was isolated from a human penile condylomatous wart. Among these, AAV2, 3, and 5 are thought to be of human origin based on the prevalence of neutralizing antibodies in the human population. In contrast, AAV4 appears to have originated potentially in monkeys since antibodies against AAV4 are common in nonhuman primates. Whether AAV1 originated from human or nonhuman primates remains inconclusive. While antibodies to AAV1 were found in monkey sera, AAV1 viral genomes have been isolated from human tissues. AAV6 is thought to be a hybrid recombinant between AAV1 and AAV2, since the left ITR and p5 promoter regions are virtually identical to those of AAV2, while the rest of the genome is nearly identical to that of AAV1.

In the past few years, several novel AAV serotypes, including AAV7, AAV8 and AAV9, and over 100 AAV variants have been found in human or non-human primate tissues. The genomes of AAV7, AAV8 and AAV9 were identified after amplification from monkey tissue (Gao *et al.*, 2002; Gao *et al.*, 2004); however, isolation of these viruses has not yet been reported.

Some of these isolates show enhanced transduction in comparison with previously identified AAV serotypes in several tissue types. For example, AAV8, isolated in non-human primates, displays a propensity for liver transduction (Gao *et al.*, 2002).

5.   **DESCRIPTION OF IDENTIFICATION AND DETECTION TECHNIQUES**

The presence of AAV may be detected in clinical samples in three ways:

1. Polymerase Chain Reaction (PCR). PCR can be used to detect vector genome sequences associated with AAV in a qualitative or quantitative manner, using primers specific for the *rep* or *cap* genes. Detection of a specific serotype, or any AAV-like sequence, as well as distinction between wild type AAV and recombinant AAV is possible, depending on the choice of primers. Note that the presence of vector genomes does not necessarily imply infectious virus particles.

2. Viral culture: Samples containing suspected infectious AAV particles may be cultured in vitro on a permissive cell line, in the presence of a helper virus.

3. AAV vector particles may be detected using Enzyme-Linked Immunosorbent Assay (ELISA) methods.   These methods rely on the generation of specific antibodies to the vector capsid proteins, and can therefore be specific to an individual serotype, or cross-react with several AAV serotypes. Detection of vector capsid particles does not necessarily imply infectious virus particles.

6. **SENSITIVITY, RELIABILITY (IN QUANTITATIVE TERMS) AND SPECIFICITY OF DETECTION AND IDENTIFICATION TECHNIQUES**

The sensitivity/ specificity of the three methods for detection of wild type AAV is not apparent in the scientific literature, likely due to a lack of clinical utility of diagnostic methods due to the apparent lack of pathogenicity of the virus. It is possible to estimate sensitivity of detection of wild type AAV based on what is known regarding recombinant AAV vectors; for example qPCR methods typically have a Limit of Detection (LOD) of 10 copies per sample, and a Limit of Quantitation (LOQ) of 100 copies per sample. Similarly, viral cultures (utilising a helper virus) may be able to detect a minimum 10^3 replication competent (wild type) AAV particles in 10^{11} viral genomes.

However it must be noted that such sensitivities are achieved in samples of purified vector, with essentially no interference from the sample matrix. Any clinical sample will be subject to tissue sample interference, which must be assessed and may reduce sensitivity.

7. **DESCRIPTION OF THE GEOGRAPHIC DISTRIBUTION AND OF THE NATURAL HABITAT OF THE ORGANISM INCLUDING INFORMATION ON NATURAL PREDATORS, PREYS, PARASITES AND COMPETITORS, SYMBIONTS AND HOSTS**

The human adeno-associated virus (AAV) was discovered in 1965 as a contaminant of adenovirus (Ad) preparations (Atchison *et al.*, 1965). It is a globally endemic infection of humans, as demonstrated by the cross-reactivity of antibodies in the population to one or more AAV serotypes. Seroconversion occurs during childhood and is usually concomitant with an adenovirus infection (Reviewed in Tenenbaum *et al.*, 2003).

Antibodies against AAV have been variously reported to be present in between 46-96% of individuals studied. Tenenbaum *et al.* (2003) cites studies of adults in Belgium and the USA (85-90% seropositive), France (63%), Brazil (55%), Germany (50%), and Japan (46%).

In a study by Chirmule *et al.* (1999), antibodies to AAV were seen in 96% of the subjects (patients with cystic fibrosis and healthy subjects). Boutin *et al.* (2010) report the prevalence of IgG cross-reactivity to specific serotypes: AAV1 (67%), AAV2 (72%), AAV5 (40%), AAV6 (46%), AAV8 (38%) and AAV9 (47%).

There are no known natural predators, preys, parasites, competitors or symbionts associated with AAV, although it does require helper functions of co-infecting viruses for replication in nature (see Section II.A.10 (b)).

8. **ORGANISMS WITH WHICH TRANSFER OF GENETIC MATERIAL IS KNOWN TO OCCUR UNDER NATURAL CONDITIONS**

AAV is thought to be spread in nature via inhalation of aerosolized droplets, mucous membrane contact or ingestion.

DNA replication occurs in the cell nucleus during lytic cycle. In its latent state, DNA is maintained either as a stable episome or by integration into the host cell DNA (see Section II.A.10 (b)).

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.   AAV can replicate in cells of a different species when infected with AAV *in vitro*, provided it is in the presence of a helper virus to which that species is permissive (e.g. human AAV may be replicated in canine cells if co-infected with a canine adenovirus) (Berns and Bohenzky, 1987).

Evolution of AAV viruses (like all viruses) is directed by spontaneous mutation or homologous recombination with other viruses of the same species, where such genetic modification confers a selective advantage.

Homologous genomic recombination may occur spontaneously in nature between the viral genomes of AAV strains only under circumstances where a cell of the host organism is infected simultaneously by two different strains of AAV and a helper virus which is permissive in that species (triple-infection).

9.   VERIFICATION OF THE GENETIC STABILITY OF THE ORGANISMS AND FACTORS AFFECTING IT

In general, DNA viruses have greater genetic stability than RNA viruses. Firstly, DNA is more thermodynamically stable than RNA; secondly, replication of DNA is a much less error-prone process than the replication of RNA; and thirdly, more mechanisms exist in the host cell for repairing errors in DNA than in RNA.

Homologous genomic recombination may occur spontaneously in nature between the viral genomes of AAV strains only under circumstances where a cell of the host organism is infected simultaneously by two different strains of AAV and a helper virus (triple-infection).

10.   PATHOLOGICAL, ECOLOGICAL AND PHYSIOLOGICAL TRAITS

(a) Classification of hazard according to existing Community rules concerning the protection of human health and/or the environment

Wild type AAV is not classified in Risk Groups 2, 3 or 4 in the European Union (EU) according to Directive 2000/54/EC on the protection of workers from risks related to exposure to biological agents at work (Appendix III). It is most appropriately designated a Risk Group 1 biological agent, defined in the EU as 'one that is unlikely to cause human disease'.

Similar classifications of hazard have been assigned to AAV according to the definitions of the World Health Organisation (WHO), and in the US, Canada and Australia as summarized in Table 1.

It should be noted that these classifications are based on the effects on healthy workers, and does not consider effects on individuals with altered susceptibility which may be as

a result of pre-existing disease, medication, compromised immunity, pregnancy or breast feeding.

Conversely, the classification does not consider genetically modified micro-organisms which are attenuated or have lost known virulence genes, or carry a transgene.

Table 1: Biosafety classifications for wild type AAV outside the EU

Territory	Category	Definition	Reference
WHO	**Risk Group 1** (no or low individual and community risk)	A microorganism that is unlikely to cause human or animal disease.	WHO Laboratory Biosafety Manual, 3rd Ed (2004).
US	**Risk Group 1**	Agents that are not associated with disease in healthy adult humans.	NIH Recombinant DNA Guidelines (USA, 2011). Appendix B-I. Not listed under 42CFR73.3 – Select Agents and Toxins.
Canada	**Risk Group 1** (low individual and community risk)	Any biological agent that is unlikely to cause disease in healthy workers or animals.	Canadian Laboratory Safety Guidelines (2004). Human Pathogens and Toxins Act. S.C. 2009, c. 24. Not listed in Schedules II, II, IV.
Australia/NZ	**Risk Group 1** (low individual and community risk)	A microorganism that is unlikely to cause human or animal disease.	Standard AS/NZS 2243.3:2010. Safety in laboratories Part 3: Microbiological safety and containment. Standards. Association of Australia/New Zealand. Not listed in Tables 3.4, 3.7 or 3.8.

(b) Generation time in natural ecosystems, sexual and asexual reproductive cycle

The lifecycle of wild type AAV has been summarised in two recent reviews (Daya and Berns, 2008; Gonçalves, 2005).

AAV enters cells by interaction of specific viral capsid epitopes with cell surface receptors, influencing the infection efficiency, host range and specific tissue tropism of a virus.

AAV2 gains entry into target cells by using the cellular receptor heparan sulfate proteoglycan. Internalization is enhanced by interactions with one or more of at least six known co-receptors including $\alpha_v\beta_5$ integrins, fibroblast growth factor receptor 1, hepatocyte growth factor receptor, $\alpha_v\beta_1$ integrin, and laminin receptor.

However, many clinically relevant tissues are not susceptible to infection by AAV2. Greater gene expression was seen in muscle, retina, liver, and heart using AAV serotypes 1, 5, 8, and 9, respectively. The cell surface receptors have been identified for only some of the many AAV serotypes: AAV2 (heparan sulfate proteoglycan), AAV4 (O-linked sialic acid), and AAV5 (platelet-derived growth factor receptor). In addition, a 37 kDa/67 kDa laminin receptor has been identified as being a receptor for AAV

serotypes 2, 3, 8, and 9. The attachment receptors for the other serotypes have not yet been identified (Daya and Berns, 2008).

The infectious entry pathway of AAV has been investigated in HeLa cells. Binding to a cell surface receptor initiates internalization (receptor mediated endocytosis) through clathrin-coated pits. Once internalized, the virus encounters a weakly acidic environment which is sufficient to allow penetration into the cytosol. AAV rapidly moves to the cell nucleus and accumulates perinuclearly beginning within 30 min after the onset of endocytosis. Escape of AAV from the endosome and trafficking of viral particles to the nucleus are unaffected by the presence of adenovirus. Within 2h, viral particles could be detected within the cell nucleus, suggesting that AAV enters the nucleus prior to uncoating. The majority of the intracellular virus particles remain in a stable perinuclear compartment and slowly penetrate into the nucleus, possibly through the Nuclear Pore Complex (NPC) (Bartlett *et al.*, 2000).

After entry into the host cell nucleus, wild type AAV can follow either one of two distinct and interchangeable pathways of its life cycle: the lytic or the lysogenic (Figure 2). The former develops in cells infected with a helper virus whereas the latter is established in host cells in the absence of a helper virus.

Figure 2: Schematic representation of wild type AAV lytic and lysogenic lifecycle
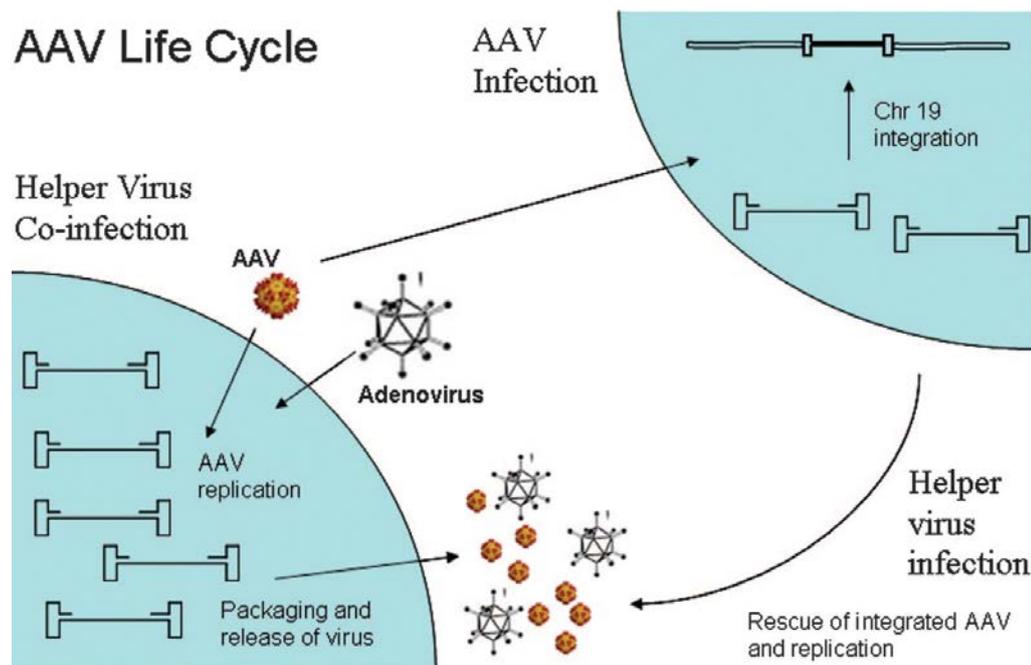
AAV life cycle. AAV undergoes productive infection in the presence of adenovirus coinfection. This is characterized by genome replication, viral gene expression, and virion production. In the absence of adenovirus, AAV can establish latency by integrating into chromosome 19 (AAVS1). The latent AAV genome can be rescued and replicated upon superinfection by adenovirus. Both stages of AAV's life cycle are regulated by complex interactions between the AAV genome and AAV, adenoviral, and host proteins (Daya and Berns, 2008).

Productive (lytic) infection develops in cells co-infected with a helper virus such as adenovirus (Atchison *et al*, 1965; Henry, 1973), HPV (Hermonat, 1994; Su and Wu, 1996; Hermonat *et al*, 1997), vaccinia virus (Schlehofer *et al*, 1986) or HSV (Salo and Mayor, 1979; Buller *et al*, 1981) to help its replication.

During this period, AAV undergoes productive infection characterized by genome replication, viral gene expression, and virion production. The adenoviral genes that provide helper functions regarding AAV gene expression have been identified and include *E1a, E1b, E2a, E4*, and *VA RNA*. Herpesvirus aids in AAV gene expression by providing viral DNA polymerase and helicase as well as the early functions necessary for HSV transcription. Although adenovirus and herpesvirus provide different sets of genes for helper function, they both regulate cellular gene expression, providing a permissive intracellular milieu for AAV productive infection (reviewed in Daya and Berns, 2008).

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). A similar locus has also been identified in nonhuman primates, and recently in rodents (Dutheil *et al.*, 2004).

Integration into host cell DNA enables the provirus DNA to be perpetuated through host cell division. The viral components needed for the site-specific integration reaction have been identified. They are composed in *cis* by the AAV ITRs and in *trans* by either one of the two largest Rep proteins (i.e., Rep78 or Rep68) (Gonçalves, 2005).

At high multiplicity of infection, wild-type AAV integrates into human chromosome 19 in ~60% of latently infected cell lines. However, it has been recently demonstrated that only approximately 1 out of 1000 infectious units can integrate (Tenenbaum *et al.*, 2003). Schnepp et al., 2005 have provided evidence that following naturally acquired infection, wild type AAV DNA may persist mainly as circular double stranded episomes in human tissues.

(c) Information on survival, including seasonability and the ability to form survival structures

Wild type AAV survives in the environment as a persistent infection in the host vertebrate species or as a latent infection in the nucleus of some infected cells, where it may remain inactive indefinitely, or be reactivated giving rise to secretion of virus.

Due to its reliance on a helper virus for replication (typically adenovirus) AAV may be considered to exhibit seasonality. Temperate regions experience seasonal vagaries in the occurrence of adenoviral infections, with the highest incidences occurring in the autumn, winter, and early spring.

Outside of the host, non-lipid enveloped viruses such as AAV are resistant to low level disinfectants, survive well outside of the laboratory environment and can be easily transmitted via fomites. AAV particles are resistant to a wide pH range (pH 3-9) and can resist heating at 56°C for 1 hour (Berns and Bohenzky, 1987). AAV does not form survival structures but can remain infectious for at least a month at room temperature following simple desiccation or lyophilization.

Effective disinfectants require a minimum of 20 minutes contact time. Adeno-Associated virus is susceptible to sodium hypochlorite (1-10% dilution of fresh bleach),

alkaline solutions at pH >9 and 5% phenol. Alcohol is not an effective disinfectant against AAV. AAV is inactivated by autoclaving for 30-45 minutes at 121°C (CCMT/CHOP MSDS; adeno-associated viral vectors).

(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

Although human infections are common, AAV is not known to be a pathogenic virus in humans (reviewed in Tenenbaum *et al*., 2003); AAV has never been implicated as an etiological agent for any disease (Blacklow *et al.,* 1968a,b, 1971).

The human immune response to AAV is reviewed in Daya and Berns (2008). Almost no innate immune response is seen in AAV infection (Zaiss *et al*., 2002). The host defense mechanism at the adaptive level is primarily made up of a humoral response (Xiao *et al.*, 1996). Pre-existing antibodies in patients, because of prior infection, account for the humoral response seen toward AAV. In a study by Chirmule *et al.* (1999), antibodies to AAV were seen in 96% of the subjects (patients with cystic fibrosis and healthy subjects), and 32% showed neutralizing ability in an in vitro assay.

The cell-mediated response functions at the cellular level, eliminating the transduced cells using cytotoxic T cells. Cell-mediated responses to AAV vectors have been documented, but this response may be dependent on the route of administration (Brockstedt *et al*., 1999).

Despite the lack of evidence for pathogenicity, correlations have been made between:

i). the occurrence of male infertility and the presence of AAV viral DNA sequences in human semen (Rohde *et al.,* 1999)

ii). the occurrence of miscarriage and the presence of infectious AAV in embryonic material as well as in the cervical epithelium (Tobiasch *et al.,* 1998; Walz *et al.,* 1997).

A clear association is hard to establish from these studies, given that co-incident evidence of human papillomavirus infection is present in most subjects (Malhomme *et al.,* 1997), and that AAV DNA can be detected in cervical samples in the majority of women (Burguete *et al.,* 1999) but is very dependent on differences in sample collection between studies (Erles *et al.,* 2001). The possible causal role of AAV in the occurrence of miscarriage is the finding that AAV2 interferes with mouse embryonic development (Botquin *et al.,* 1994). Furthermore, a significant correlation has been established between the presence of AAV DNA in amnion fluids and premature amniorrhexis and premature labour (Burguete *et al.,* 1999).

An additional, theoretical, risk of AAV infection is the risk of insertional mutagenesis caused by non-site specific integration of the AAV genome into the host-cell genome of infected cells. Such an event carries the risk of malignant transformation leading to cancer. There is no documented causal link between AAV infection and malignancies in humans, but it has been shown that wild type AAV may integrate at sites other than chromosome 19: Schnepp *et al*., 2005 identified an AAV-cellular DNA junction in a human tissue sample (tonsil) which was mapped to a highly repetitive satellite DNA element on chromosome 1. However, they also demonstrated that the majority of wild-

type AAV DNA persisted as circular double-stranded episomes in the tissues following naturally acquired infection.

AAV shows some species specificity, but can replicate in cells of a different species when infected with AAV *in vitro*, provided it is in the presence of a helper virus to which that species is permissive (e.g. human AAV may be replicated in canine cells if co-infected with a canine adenovirus) (Berns and Bohenzky, 1987). It is not known whether zoonosis occurs in nature.

(e) Antibiotic resistance, and potential use of these antibiotics in humans and domestic organisms for prophylaxis and therapy

Antibiotics are not effective in the treatment of viral infection, nor does wild type AAV present specific resistance to antibiotics. The wild type virus does not contain any gene that confers resistance to known antibiotics.

(f) Involvement in environmental processes: primary production, nutrient turnover, decomposition of organic matter, respiration, etc.

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.

11. NATURE OF INDIGENOUS VECTORS

The primary indigenous vector of primate (human) AAV serotypes is human beings.

AAVs have also been isolated from other animal adenovirus stocks; these include avian AAV (Dawson *et al*., 1982; Yates *et al*., 1973), bovine AAV (Coria and Lehmkuhl, 1978; Luchsinger *et al*., 1970; Myrup *et al*., 1976), ovine AAV (Clarke *et al.*, 1979), caprine AAV (Arbetman *et al*., 2005) and snake AAV (Farkas *et al*., 2004).

AAV shows some species specificity, but can replicate in cells of a different species when infected with AAV *in vitro*, provided it is in the presence of a helper virus to which that species is permissive (e.g. human AAV may be replicated in canine cells if co-infected with a canine adenovirus) (Berns and Bohenzky, 1987). It is not known whether zoonosis occurs in nature.

AAV may exist as a provirus in the infected cells of the host species (see Section II.A.10 (b)).

The presence of natural mobile genetic elements such as transposons or plasmids related to AAV has not been reported.

12.   HISTORY OF PREVIOUS GENETIC MODIFICATIONS

The experimental strain of AAV (AAV8-hFIX19) is ultimately derived from wild type DNA sequences from AAV2 (ITR and *rep* functions) and AAV8 (cap proteins). The experimental vector itself contains only the ITRs of wild type AAV2 and the transgene (hFIX) expression cassette, packaged in capsid proteins derived from wild type AAV8.

B. CHARACTERISTICS OF THE VECTOR (TRANSGENE)

1. NATURE AND SOURCE OF THE VECTOR

INFORMATION DELETED FOR CONFIDENTIALITY PURPOSES

2. SEQUENCE OF TRANSPOSONS, VECTORS AND OTHER NON-CODING GENETIC SEGMENTS USED TO CONSTRUCT THE GMO AND TO MAKE THE INTRODUCED VECTOR AND INSERT FUNCTION IN THE GMO

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3. FREQUENCY OF MOBILISATION OF INSERTED VECTOR AND/OR GENETIC TRANSFER CAPABILITIES AND METHODS OF DETERMINATION

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.

Homologous recombination between AAV8-hFIX19 and a wild type AAV could occur if both were present in the same cell. However, such recombination could only result in the exchange of the hFIX expression cassette with the *rep* and *cap* genes of the wild type virus. It is not possible for the AAV genome to contain both *rep/cap* genes and the transgene, as this is beyond the packaging limit of the virion.

Therefore the only mechanism by which the transgene could be mobilised is through a triple infection of the same cell by AAV8-hFIX19 (containing the transgene), wild type AAV (providing the rep and cap functions) and a helper virus. This scenario is expected to be a rare event, especially since the vector target cells (liver) are not the natural target cells of helper viruses. If it did occur, it would only result in the production of more wild type AAV and more AAV8-hFIX19 vector particles (which would still lack *rep* and *cap* genes and consequently could not be self-sustaining).

4. INFORMATION ON THE DEGREE TO WHICH THE VECTOR IS LIMITED TO THE DNA REQUIRED TO PERFORM THE INTENDED FUNCTION

The expression cassette is limited to the required elements designed to optimise expression of functional human coagulation Factor IX in the liver:

In order to potentially improve both the safety and potency of the vector, a codon-optimized hFIX gene construct was used; the hFIX cDNA was modified at the nucleotide sequence level, but NOT at the protein sequence level, to potentially achieve higher levels of expression at the same vector dose. The modifications of the hFIX cDNA sequence include amino acid codon usage optimization for expression in *Homo sapiens* and removal of alternate open reading frames (ARF) in the hFIX sequence.

The expression cassette therefore comprises:

- Human α1-antitrypsin (hAAT) liver-specific promoter coupled to the human apolipoprotein E (ApoE) enhancer/hepatocyte control region (Okuyama *et al.,* 1996; Nakai *et al*., 1999; Miao *et al.,* 2000);

- 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.

Small intervening DNA sequences are also present, derived from the process of assembling the genetic elements through recombinant DNA techniques. These sequences are non-functional and inactive.

The expression cassette is flanked by the 145 nucleotide inverted terminal repeats derived from AAV type 2.

C. CHARACTERISTICS OF THE MODIFIED ORGANISM

1. INFORMATION RELATING TO THE GENETIC MODIFICATION

(a) Methods used for the modification;

The plasmids used in the manufacture of AAV8-hFIX19 (see Section II.B.2) were constructed using standard molecular biological techniques for the precise excision and ligation of component elements using specific restriction enzymes followed by transduction and amplification in bacterial cells at each stage. The desired product plasmid was selected at each stage using antibiotic resistance marker genes.

The final plasmid stocks used in the manufacture of AAV8-hFIX19 carry the gene for resistance to Kanamycin for selection purposes, and have been amplified and tested to confirm the correct DNA sequence.

(b) Methods used to construct and introduce the insert(s) into the recipient or to delete a sequence;

The final vector, AAV8-hFIX19 is constructed from the plasmid stocks on a batch-by-batch basis by co-transfecting a Master Cell Bank (MCB) of Human Embryo Kidney (HEK) 293 cells with the three plasmid stocks:

1) Vector plasmid pAAV2-hFIX19 containing the hFIX expression cassette flanked by AAV2 ITRs;

2) AAV packaging plasmid pAAV8PKv3, containing the AAV2 *rep* and AAV8 *cap* genes coding for non-structural and structural proteins, respectively;

3) Adenovirus helper plasmid pCCVC-AD2HPv2, encoding the adenovirus type 2 genes E2A, E4 and VA RNAs required for AAV replication in HEK293 cells.

Note that the required helper functions are provided as a plasmid, NOT a viable adenovirus.

(c) Description of the insert and/or vector construction;

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(d) Purity of the insert from any unknown sequence and information on the degree to which the inserted sequence is limited to the DNA required to perform the intended function;

The sequence of the insert is limited to the DNA required to perform the intended function (see Section II.B.4). Each lot of AAV8-hFIX19 (containing the hFIX expression cassette) is tested for identity by PCR in combination with restriction digest to verify the integrity and identity of the insert. The entire 4275bp genome of AAV8-hFIX19 Lot A8FP1-A1109C has been sequenced (see Section II.C.1 (f)). For the region in which sequence data was obtained, the sequenced sample was 100% identical to the expected sequence.

(e) Methods and criteria used for selection;

Antibiotics are not used in the manufacturing process used to construct the AAV8-hFIX19 vector in HEK293 cells from the component plasmids.

The vector is purified according to the procedure outlined in Section II.C.1 (c) to remove contaminating process related raw materials including residual host cell proteins, host cell DNA, plasmid DNA, caesium, benzonase (used in purification) and bovine serum albumin (used in cell cultivation). Empty AAV8 viral capsids are also effectively removed, though a specified portion of these will be added back to the functional vector prior to administration (see Section II.C.1 (c)).

Each lot of AAV8-hFIX19 (and empty AAV capsids) is tested for identity, purity (including residual plasmid DNA) and freedom from adventitious agents (including replication competent AAV) and must meet specified acceptance criteria prior to use.

It is possible that replication competent (wild type) AAV particles are produced in the manufacturing process through recombination of AAV *rep*, *cap* and ITR components encoded by different production plasmids. The presence of such contaminants is analysed on a batch-by-batch basis. The recently established replication competent AAV assay (providing improved sensitivity, and replacing an established infectious titre assay used for AAV2 vectors), has an LOD estimated at 10^3 wild type AAV particles in 10^{11} viral genomes.

(f) Sequence, functional identity and location of the altered/inserted/deleted nucleic acid segment(s) in question with particular reference to any known harmful sequence.

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2.   <u>**INFORMATION ON THE FINAL GMO**</u>

(a) Description of genetic trait(s) or phenotypic characteristics and in particular any new traits and characteristics which may be expressed or no longer expressed;

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(b) Structure and amount of any vector and/or donor nucleic acid remaining in the final construction of the modified organism;

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(c) Stability of the organism in terms of genetic traits;

Based on the fact that long term therapeutic activity of the investigational drug is not dependent on replication of the recombinant AAV, and the known genetic stability of the parent wild type AAV, the genetic traits of the organism are expected to be stable.

(d) Rate and level of expression of the new genetic material. Method and sensitivity of measurement;

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(e) Activity of the expressed protein(s);

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(f) Description of identification and detection techniques including techniques for the identification and detection of the inserted sequence and vector;

In the proposed clinical trial, subjects will be monitored for the presence of the vector in blood, urine, saliva and semen according to the Clinical Trial Protocol (see Section V.1).

DNA will be extracted from the sample and the presence of AAV vector genomes in the sample quantitated using real-time polymerase chain reaction with reference to a standard curve of linearised plasmid DNA containing the target sequences. Spiked positive controls are included in the test procedure to detect sample matrix interference. A negative control (genomic DNA not exposed to AAV) is also included. All analyses are done in duplicate except semen which is performed in triplicate.

Transgene antigen/ activity tests will also be performed on blood samples as specified in the Clinical Trial Protocol (see Section V.1).

Factor IX protein will be detected and quantified by analysis of plasma samples using enzyme-linked immunosorbant assay (ELISA). A microtiter plate is coated with an antibody to human Factor IX, and plasma samples are loaded into the wells. If hFIX is present in the sample, it is recognized by a peroxidase-labeled anti-human FIX antibody. Detection is subsequently performed by an enzymatic reaction and quantified with reference to a standard curve. Factor IX activity is determined by a standard coagulation assay, in which formation of a fibrin clot is the endpoint. Activity is quantified by reference to a standard curve. Positive and negative plasma controls are included in all test procedures.

(g) Sensitivity, reliability (in quantitative terms) and specificity of detection and identification techniques;

The QPCR assay for the detection of vector genomes is being developed to elicit a demonstrated limit of quantitation of ≤50 copies of vector/ 1µg genomic DNA with 95% confidence, in accordance with FDA Guidance for Industry: Gene Therapy Clinical Trials - Observing Subjects for Delayed Adverse Events (2006).

The lower limit of detection of the ELISA assay for hFIX protein is 15ng/ml (0.3% normal level).

(h) History of previous releases or uses of the GMO;

The Clinical Trial Protocol which is the subject of this submission (Protocol # AAV8-hFIX19-101) is also being performed in the US and Australia. Approval was granted in the US in July 2012 (IND 15149) and is pending in Australia. To date, no patients have been treated.

In the US, the IND application is subject to categorical exclusion from the requirement to prepare an environmental assessment under 21 CFR 25.31(e).

(i) Considerations for human health and animal health, as well as plant health:

(1) Toxic or allergenic effects of the GMOs and/or their metabolic products;

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 possible effects of direct administration to patients are summarised below. 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.

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) in 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.

Potential risks of direct administration of AAV8-hFIX19 to human haemophiliacs

The long-term safety of recombinant AAV vectors in humans is unknown; however, AAV vectors have been delivered to several hundred human subjects to date, in trials for cystic fibrosis, rheumatoid arthritis, Leber's Congenital Amaurosis (LCA), α_1-antitrypsin deficiency, congestive heart failure, lipoprotein lipase deficiency, as well as haemophilia, and have been remarkably free of vector-related adverse events (Mingozzi and High, 2011).

In earlier trials of AAV mediated hFIX delivery in the treatment of haemophilia B, eight men were injected via the hepatic artery with AAV2-hFIX16 at doses ranging from 8 x 10^{10} vg/kg to 2 x 10^{12} vg/kg, during a period 2001-2004. Long-term follow-up has revealed no vector-related late complications (Manno *et al.*, 2006; Wellman *et al.*, 2012). In a subsequent trial conducted in 2009-2012, ten men were injected intravenously with doses of a self-complementary AAV8-hFIX (AAV8-LP1-hFIXco) ranging from 2 x 10^{11} vg/kg to 2 x 10^{12} vg/kg, again with no late complications noted to date (Nathwani *et al.,* 2011a; Davidoff *et al.*, 2012).

Potential immune response

Administration of AAV8-hFIX19 is likely to lead to the production of a humoral immune response to AAV and might lead to an antibody response to factor IX protein. In clinical trial results with similar constructs to date, anti-AAV neutralizing antibodies have been detected in all subjects after vector infusion; however, none have shown anti-FIX neutralizing antibodies (inhibitors). An antibody directed against the vector capsid may affect the response to a subsequent administration of vector and subjects in this trial may be precluded from subsequent treatment with AAV vectors. There is a possibility that subjects infused with AAV8-hFIX19 will develop neutralising antibodies to FIX, although this has not occurred in any of the eight subjects previously infused with an AAV2-hFIX16 vector via the hepatic artery (Manno et al., 2006), in any of the ten subjects receiving a self-complementary AAV8-hFIX vector (AAV8-LP1-hFIXco) by peripheral vein infusion (Nathwani et al., 2011a; Davidoff et al., 2012), or in the eight subjects injected with an AAV2-FIX9 vector in skeletal muscle (Manno et al., 2003).

A cell-mediated response to AAV8-hFIX19 may give rise to elevated transaminases. Two subjects infused with either $4x10^{11}$ vg/kg or $2x10^{12}$ vg/kg of AAV2-hFIX16 developed asymptomatic elevation of transaminases beginning four weeks after vector infusion and resolving to baseline by twelve weeks post vector infusion without specific treatment (Manno et al., 2006). Two of six reported subjects infused with AAV8-LP1-hFIXco, those at the high dose of $2x10^{12}$ vg/kg, developed elevation of hepatic transaminases (one above the upper limit of normal). Both of these subjects were treated with a short, tapering course of prednisolone and resolved the elevated transaminases; one suffered a modest reduction in FIX levels, but remained with a level greater than his baseline, while the other maintained his FIX level throughout the reported period of observation (Nathwani et al., 2011a). The level of transaminase elevations noted in previous trials are not high; this may be considered an issue that affects efficacy, rather than one that affects safety, since the transaminase elevation to date has been self-limited and asymptomatic.

Potential for insertional mutagenesis

AAV vectors have the potential to integrate into the genome of transduced cells. The major potential risk resulting from integration into the host cell DNA is an enhanced risk of malignant transformation leading to cancer.

It is assumed that the greatest potential for integration would be into cells within the liver, but given the results of tissue distribution studies of NHPs with similar (AAV8 pseudotyped) vectors (AAV8-LP1-hFIXco; Nathwani et al., 2011b; see Section II.C.2.(i) 3), the potential for integration into cells of other tissues also exists.

At high multiplicity of infection, wild type AAV integrates into human chromosome 19 in ~60% of latently infected cell lines. However, it has been recently 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 site-specifically. Random integration of vector sequences has been demonstrated in established cell lines but only in some cases and at low frequency in primary cultures and *in vivo*. In contrast, Duan et al., 1998 demonstrated prolonged persistence of head-to-tail circular intermediates in muscle tissue at 80 days postinfection, suggesting that a large percentage of rAAV genomes may remain

episomal. It has also been shown that, following liver transduction, (Miao et al., 1998; Miao et al., 2000) rAAV is stabilized predominantly in a non-integrated form; however, integration does occur at some low level.

The site(s) of integration have been analyzed 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. 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).

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 tumors, an integration event in mouse chromosome 12 was associated with the tumor 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). Still, the risk of such an event is suspected to be low based on absence of malignant transformation in large haemophilic animals (haemophilia B dogs) followed for over ten years after AAV vector infusion (Niemeyer et al., 2009; Tim Nichols, personal communications). In addition, liver ultrasounds carried out in 4/7 human subjects injected with AAV2-hFIX16 from 2001-2004 have shown no evidence of liver tumors. Of the remaining 3 subjects: 1 has died of causes unrelated to gene transfer, and autopsy showed no evidence of tumor 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).

Potential for germline transmission

In the AAV2-FIX16 liver gene transfer trial (Manno et al., 2006), vector genome sequences were detected in semen; semen samples from all eight subjects cleared within sixteen weeks. 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 spermatocytes (Arruda et al., 2001; Couto and Pierce, 2003; Couto et al., 2004; Manno et al., 2006). The sponsor has performed a series of studies using rabbits, a valuable model for assessing germline transmission risk for humans. 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). The sensitivity of the nested PCR used in these studies was greater than 1 copy per 6000 cells. Rabbit studies were also performed with AAV8 vectors (Favaro et al., 2009). In these studies, AAV2 and AAV8 vectors were compared, showing that the kinetics of vector clearance from semen was dose- and time-dependent but serotype-independent. Furthermore, AAV2 or AAV8 sequences were detected in the semen of

vasectomized animals that lack germ cells, leading to the conclusion that the genitourinary tract, as well as the testis, contributes significantly to vector shedding in the semen. These studies are supported by findings in humans (Nathwani *et al., 2011a;* see Section II.C.2.(i) 3), in which vector genomes were detected in semen only briefly after AAV8-LP1-hFIXco vector systemic administration; clearance was reported by 15 days post-administration. These results suggest that the risk of inadvertent germline transmission in males by AAV8 vectors is low, similar to that of AAV2.

(2) Comparison of the modified organism to the donor, recipient or (where appropriate) parental organism regarding pathogenicity;

Neither wild type AAV nor the experimental vector AAV8-hFIX19 is known to be pathogenic to humans.

(3) Capacity for colonisation;

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.

Homologous recombination between AAV8-hFIX19 and a wild type AAV could occur if both were present in the same cell in the presence of a helper virus (triple infection). However, such recombination could only result in the exchange of the hFIX expression cassette with the *rep* and *cap* genes of the wild type virus. It is not possible for the AAV genome to contain both *rep/cap* genes and the transgene, as this is beyond the packaging limit of the virion.

In the rare event that wild type AAV, supplying the requisite replication gene products, were to co-infect a hepatocyte, along with a helper DNA virus such as adenovirus or herpes simplex virus and AAV8-hFIX19 (a triple co-infection), it is possible that vector replication could occur. The likelihood of such an occurrence is extremely low, and the resulting virologic outcome would be increased synthesis of AAV8-hFIX19 and wild type AAV, both intrinsically non-pathogenic viruses.

The capacity for colonisation is also affected by the level and route of shedding of the GMO from patients following administration.

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 below.

Shedding of a self-complementary AAV8 pseudo-typed vector containing an hFIX gene (AAV8-LP1-hFIXco) following a single peripheral vein infusion of 2×10^{12} vg/kg to NHPs is described in Nathwani *et al., 2011b*. Clearance of vector from plasma, urine, saliva and stools was determined using a quantitative PCR assay on samples collected on Days 1, 3, 7 and 10 following administration. The results indicate that vector genome has cleared from the examined body fluids by Day 10 (Figure 9).

Figure 3: Clearance of self-complementary AAV vector (scAAV)2/8-LP1-hFIXco vector after peripheral vein administration of 2×10^{12} pcr-vector genomes (vg)/kg.
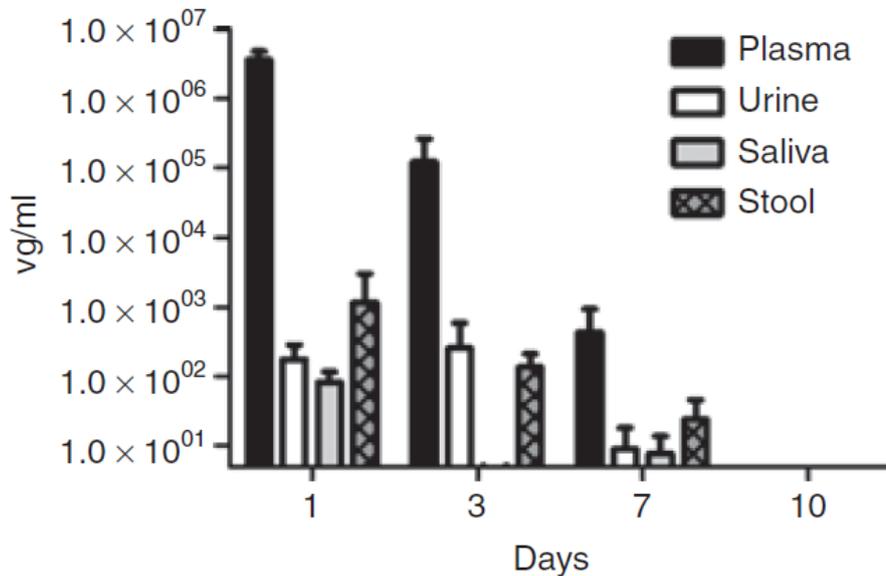
Results are expressed as mean transgene copy number (vg)/ml ± SE of sample obtained from three animals in the highest dose cohort (reproduced from Nathwani et al., 2011b)

The same vector was also administered by a single peripheral vein infusion to human haemophiliac patients, and data collected on shedding in plasma, stools, urine, saliva and semen using a qPCR assay to detect vector sequences (Nathwani et al., 2011a). The PCR primers were specifically chosen to amplify a region within the codon-optimized FIX transgene. Samples were collected from each participant on the stipulated days after peripheral vein administration of vector.

The vector genome was detectable in the plasma, 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 (Figure 10). The amount of vector DNA in the body fluids roughly correlated with the vector dose administered, as the highest level of viraemia (2.5-6.2×10^6 vg/µl) was observed in the two participants that received 2×10^{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.

Figure 4: Virus shedding post administration in Haemophilia B participants
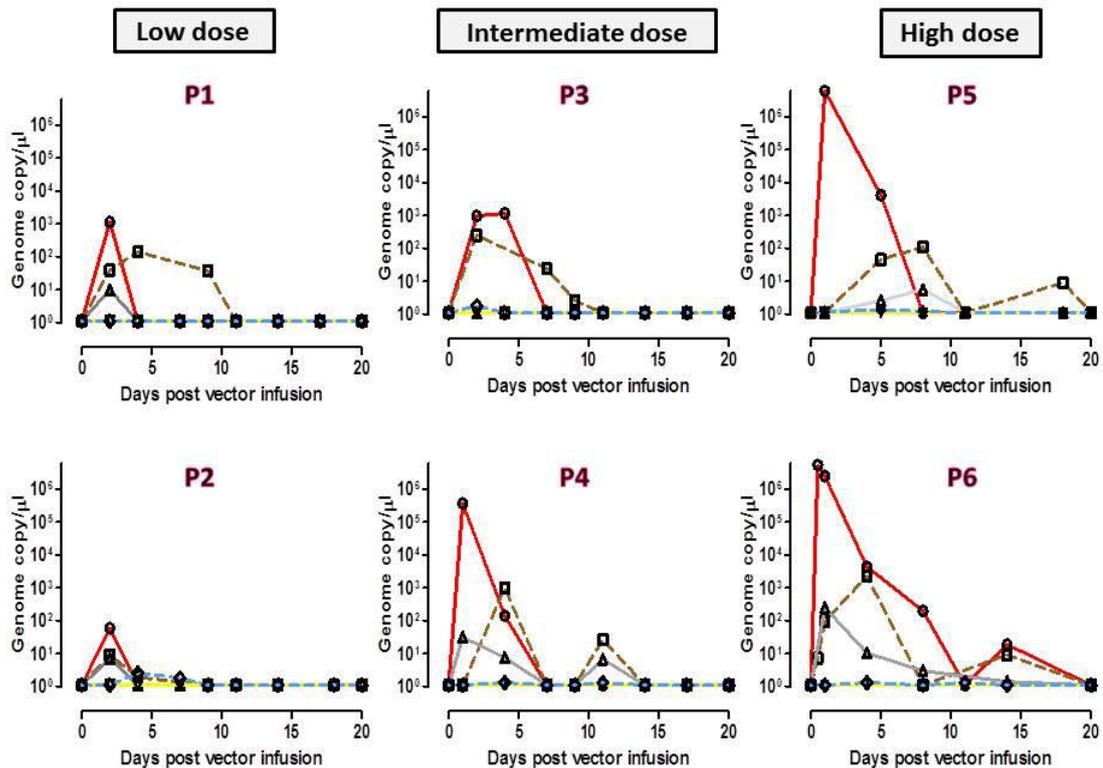
Detection of scAAV2/8-LP1-hFIXco DNA sequences in plasma, [redline], stool. [brown line], urine [yellow line] saliva [gray line] and semen [blue line]). Standards consisted of serial dilutions of scAAV2-8-LP1-hFIXco in naïve human plasma. Negative samples were spiked with vector plasmid and subjected to PCR to ensure that the sample did not inhibit the PCR reaction. Low dose = 2 x 10^{11} vg/kg, intermediate dose = 6 x 10^{11} vg/kg , high dose = 2 x 10^{12} vg/kg (Nathwani *et al.,* 2011a).

Shedding data have also been obtained from a study in human haemophiliacs following a single infusion of an AAV2 serotype vector containing hFIX (AAV2-hFIX16) into the hepatic artery (Manno *et al.,* 2006). Results from serum and urine suggested that time to clearing (PCR signal from the vector is no longer detectable) was dose dependent. Urine was the first body fluid to clear. There was no association between dose and time to clearing for semen. The last positive semen samples in the lowest dose cohort were at 10 weeks and 12 weeks, whereas the last positive samples in the five subjects in the two higher-dose cohorts occurred at a mean of 4.8 weeks. Age of the subject seemed to be the best predictor of time to clearing of semen, with young men on average clearing more quickly than older men. Semen fractionation, carried out for a single subject, showed no evidence of vector sequences in motile sperm. Peripheral blood mononuclear cells (PBMCs) showed the longest duration of positivity of any body fluid or tissue analyzed.

(4) If the organism is pathogenic to humans who are immunocompetent:
   Diseases caused and mechanism of pathogenicity including invasiveness and virulence, communicability, infective dose, host range, possibility of alteration, possibility of survival outside of human host, presence of vectors or means of dissemination, biological stability, antibiotic resistance patterns, allergenicity, availability of appropriate therapies.

Neither wild type AAV nor the experimental vector AAV8-hFIX19 is known to be pathogenic to humans.

(5) Other product hazards.

None known or anticipated.

III. INFORMATION RELATING TO THE CONDITIONS OF RELEASE AND THE RECEIVING ENVIRONMENT

A. INFORMATION ON THE RELEASE

1. DESCRIPTION OF THE PROPOSED DELIBERATE RELEASE, INCLUDING THE PURPOSE(S) AND FORESEEN PRODUCTS

Following the approval of the Clinical Trial Authorisation for AAV8-hFIX19, it is planned to make the product available to clinical trial site(s) in Ireland for use in accordance with a Clinical Trial Protocol 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' [Protocol # AAV8-hFIX19-101].

AAV8-hFIX19 will be administered by a single peripheral intravenous infusion into eligible, consenting adult males with X-linked severe Haemophilia B. Administration will be performed by a medical professional in a medical facility.

This is a Phase 1/2 safety and dose escalation study of intravenous administration of the AAV8-hFIX19 vector to subjects with severe Haemophilia B. The primary objectives are to evaluate the safety and tolerability of the treatment. A secondary objective is to measure biologic and physiologic activity of the transgene product. It is not known if the doses used in this study will increase the level of FIX in subjects, though based on prior clinical experience it is suspected that all doses tested will result in detectable factor IX levels. The information obtained in this study will guide the design of future studies in which a benefit to subjects may be anticipated.

2. FORESEEN DATES OF THE RELEASE AND TIME PLANNING OF THE EXPERIMENT INCLUDING FREQUENCY AND DURATION OF RELEASES

The use of AAV8-hFIX19 in Ireland will commence following approval of the Clinical Trial Authorisation by the Irish Medicines Board (IMB) and positive opinions from the Ethics Committee and Institutional Biosafety Committee. It is anticipated that the trial will start in Ireland in Q2 2013 and the last patient (worldwide) is expected to be treated by Q3 2015. This estimate is based on the global trial start date of Q3 2012 with an estimated 4 year duration for recruitment and completion of 1 year 'active phase' for the final patient (12 months after treatment).

3. PREPARATION OF THE SITE PREVIOUS TO THE RELEASE

No specific preparation of sites will be required prior to release, unless local procedures dictate otherwise. Information on handling will be available to medical professionals involved in product preparation via the Clinical Trial Protocol and Pharmacy and Handling Instructions. The Sponsor will provide pharmacy and handling training to those personnel involved in the clinical trial. Dose preparation is to be performed in a BioSafety Cabinet (BSC).

4. SIZE OF THE SITE

The clinical trial is expected to be performed at a single site in Ireland:

National Centre for Hereditary Coagulation Disorders
St. James's Hospital,
James's Street,
Dublin 8,
Ireland

Further sites (medical facilities) may be added in Ireland following appropriate ethical, Environmental Protection Agency (EPA) and Institutional Biosafety Committee (IBC) approval. The size of each medical facility will vary but it is important to note that contamination of the site at which the administration is performed is expected to be minimal, when suitable precautions as outlined in the Clinical Trial Protocol and Pharmacy Instructions are adhered to.

5.   METHOD(S) TO BE USED FOR THE RELEASE

AAV8-hFIX19 will be administered by a single peripheral intravenous infusion into eligible, consenting adult males with X-linked severe Haemophilia B. Administration will be performed by a medical professional in a medical facility.

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.

Eligible subjects will be admitted to the hospital on the day of AAV8-hFIX19 infusion. An intravenous catheter will be inserted into a peripheral vein, *e.g.* the median cubital vein, with normal saline fluid infusion. The vector will be thawed and prepared by the investigational pharmacy according to specific Pharmacy Instructions, and kept at room temperature prior to infusion. The vector dose, diluted in normal saline containing human serum albumin at a final concentration of 0.25% and in a volume of 200 mL, will be infused over one hour. The vector will be administered through the catheter using an appropriate infusion pump. On completion of the infusion the catheter will be flushed with saline.

After vector administration, the catheter will be removed approximately 20 ± 4 hours after the infusion. Haemostasis at the venipuncture site will be secured by applying pressure according to standard protocol for infusing FIX concentrates. Patients will remain in-hospital for approximately 24 hours of observation to monitor for adverse effects related to the procedure.

6.   QUANTITIES OF GMOS TO BE RELEASED

AAV8-hFIX19 vector is supplied as a frozen liquid containing $>0.5 \times 10^{13}$ viral genomes (vg) in a volume of 1 mL in a 1.5 mL polypropylene sterile cryogenic screw cap vial. The actual concentration of vector will vary between batches; the concentration of the currently available batch (Lot A8FP1-1109C) is 1.79×10^{13} vg/ml.

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. Pharmacy instructions will be updated to ensure the correct dose is given, depending on the concentration of vector in the batch supplied.

The maximum total dose for an 80 kg individual patient is therefore approximately 22 mL as a single treatment (approximately 40×10^{13} vg).

Purified empty capsid material will also be added to the purified full particle vector lot prior to administration at a defined ratio of empty:full capsid particles to allow liver transduction to occur even in the presence of a pre-treatment NAb titre (see Section II.C.1 (c)). Empty capsid particles do not contain vector genomes and are therefore genetically and metabolically inert.

The proposed clinical trial aims to recruit 15 evaluable patients across 4 sites in the US, Australia and Ireland. It is therefore reasonable to assume that approximately 4-8 patients will be treated in Ireland.

7.   DISTURBANCE ON THE SITE (TYPE AND METHOD OF CULTIVATION, MINING, IRRIGATION, OR OTHER ACTIVITIES)

There will be no disturbance at the sites of dose preparation/administration.

8.   WORKER PROTECTION MEASURES TAKEN DURING THE RELEASE

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.

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, preparation (dilution and combination of components) on the day of administration and disposal.

In addition, all study personnel will be trained on the Clinical Trial Protocol and Handling Instructions as part of the study site initiation.

AAV8-hFIX19 vector is derived from a virus and should be considered and handled as an infectious agent. Given the non-replicative nature of the modified organism, the nature of the transgene, and the fact that the parent organism is not known to be pathogenic, BSL-1 procedures are appropriate in the clinical setting. As a precaution, the Sponsor designates the AAV8-hFIX19 vector as BSL-2.

Key handling practices and containment requirements for BSL-1 and BSL-2 organisms obtained from globally available biosafety guidelines are presented in Table 2 and Table 3.

As a minimum, institutional risk assessments are required to determine appropriate precautions for the work tasks and processes.

Standard biosafety practices are similar amongst the guidelines and are typically followed by medical facilities when handling injectable medicinal products and medical waste, such as:

- Restricted access

- Secure storage
- Training of personnel
- Availability of Personal Protective Equipment (PPE; laboratory coats, gowns, gloves and safety glasses)
- Established routine practices for dealing with potentially biohazardous materials such as patient samples/fluids and medical waste (autoclaves, sharps bins, incinerators, disinfectants and appropriate cleanable surfaces).

The Investigational Drug Data Sheet (IDDS) provided with the Pharmacy Instructions instruct those involved with dose preparation to use universal precautions and appropriate Personal Protective Equipment. Dose preparation is to be performed in a BioSafety Cabinet (BSC) to reduce the risks posed by the possibility of generation and inhalation of aerosols. Once prepared, the dose (infusion bag) is labelled according to protocol (including a biohazard symbol), double bagged and taken to the administration area in a designated container.

Due to the minimal manipulations involved in dose administration it is not considered necessary to require further precautions at the point of administration, beyond the use of disposable gloves (as specified in the Handling Instructions).

Instructions for dealing with spills/breakages and accidental exposure are provided in the Handling Instructions.

The worker protection measures proposed for the preparation and administration of AAV8-hFIX19 are therefore in line with those recommended globally for the handling of BSL-1/2 organisms in a research setting. An additional precaution of dose preparation in a BioSafety Cabinet (BSC) is also specified.

Transportation

The International Air Transport Association (IATA) defines an Infectious Substance as 'A substance known to contain or can reasonably be expected to contain pathogens' and a Pathogen as 'Microorganisms (including bacteria, viruses, rickettsia, parasites, fungi) and other agents such as prions, that can cause disease in humans or animals'. Therefore AAV8-hFIX19 is not considered an infectious substance as defined by IATA (IATA Dangerous Goods Regulations 2012). The Investigational Drug Data Sheet (IDDS) and Handling Instructions for AAV8-hFIX19 accompany all shipments.

Table 2: Summary of Recommended Containment Precautions for BSL-1 Infectious Agents

Region	Practices	Personal Protective Equipment	Primary barriers	Secondary Barriers (Facilities)
WHO Laboratory Biosafety Manual (3rd Ed., 2004). Pages 2-3 and 9-15.	Good microbiological technique	Laboratory coats and gloves; eye, face protection, as needed	No primary barriers required	Laboratory bench and sink required
EU Directive 90/219/EEC as amended. Annex IV; Table IA	Good microbiological laboratory practices	Suitable protective clothing	No primary barriers required	Bench - Surfaces resistant to water, acids, alkalis, solvents, disinfectants, decontamination agents and easy to clean Autoclave on site
US Biosafety in Microbiological and Biomedical Laboratories (5th Ed., 2009). Page 59.	Standard microbiological practices	Laboratory coats and gloves; eye, face protection, as needed	No primary barriers required	Laboratory bench and sink required
Canada Canadian Biosafety Guidelines 3rd Ed., 2004. Page 19-22	Good microbiological laboratory practices	Laboratory coats and gloves; eye, face protection, as needed	No primary barriers required	Laboratory bench and sink required Autoclave available
Australia/ New Zealand AS/NZS 2243.3:2010 Safety in laboratories - Microbiological safety and containment Pages 38-40	Limited access Biohazard warning signs Staff shall be trained in the clean-up of microbiological spills	Laboratory coats and gloves; eye, face protection, as needed	No primary barriers required	Laboratory bench and sink required

Table 3: Summary of Recommended Containment Precautions for BSL-2 Infectious Agents

Region	Practices	Personal Protective Equipment	Primary barriers	Secondary Barriers (Facilities)
WHO Laboratory Biosafety Manual (3rd Ed., 2004). Pages 2-3 and 9-19.	BSL-1 plus: Limited access Biohazard warning signs	As BSL-1	BSCs for procedures with a high potential for producing aerosols	BSL-1 plus: Autoclave or other means of decontamination should be available in appropriate proximity
EU Directive 90/219/EEC as amended. Annex IV; Table IA	BSL-1 plus: Limited access Biohazard warning signs	As BSL-1 plus: Gloves (optional)	Minimise aerosol dissemination	BSL-1 plus: Autoclave in the building
US Biosafety in Microbiological and Biomedical Laboratories (5th Ed., December 2009). Page 59	BSL-1 plus: Limited access Biohazard warning signs "Sharps" precautions Biosafety manual defining any needed waste decontamination or medical surveillance policies	As BSL-1	BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials	BSL-1 plus: Autoclave available
Canada Canadian Biosafety Guidelines 3rd Ed., 2004. Page 22-23	BSL-1 plus: Limited access Biohazard warning signs Training and/or supervision of staff commensurate with their anticipated activities in the containment area Written procedures for emergencies (eg. spills/ clean up) must be available	As BSL-1	BSCs must be used for procedures that may produce infectious aerosols and that involve high concentrations or large volumes of biohazardous material.	As BSL-1
Australia/ New Zealand AS/NZS 2243.3:2010 Safety in laboratories - Microbiological safety and containment. Pages 40-44	BSL-1 plus: Instruction and training in handling infectious microorganisms shall be provided to laboratory personnel with regular updates	As BSL-1	If the work produces a significant risk from the production of infectious aerosols, a biological safety cabinet shall be used.	BSL-1 plus: Autoclave available

9.   POST-RELEASE TREATMENT OF THE SITE

Post-release treatment of the site will not be necessary, provided the precautions outlined in the Investigational Drug Data Sheet (IDDS)/ Pharmacy Instructions and Handling Instructions are adhered to when preparing or administering the product or when dealing with accidental spillages and breakages.

10.   TECHNIQUES FORESEEN FOR ELIMINATION OR INACTIVATION OF THE GMOS AT THE END OF THE EXPERIMENT

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.

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.

In the medical facility, this will involve temporary containment in sharps bins or clearly marked bags (e.g. biohazard, medical waste) prior to autoclaving and/or incineration either on or off site as per local institutional guidelines for handling potentially infectious materials.

All non-disposable equipment and other materials used during the procedure will be cleaned using a chemical disinfectant capable of virucidal activity for the required duration of contact, or sterilized by autoclaving consistent with local institutional guidelines for handling potentially infectious materials.

Typically, standard operating procedures for disposal within medical facilities (where the potential for contamination from other agents is potentially much more hazardous than that presented by AAV8-hFIX19) will be consistent with the guidance given in the WHO Laboratory Biosafety Manual, 3rd Ed (2004) for BSL1/2 as outlined below:

Contaminated (infectious) "sharps":
Hypodermic needles, scalpels, knives and broken glass; should always be collected in puncture-proof containers fitted with covers and treated as infectious.

After use, hypodermic needles should not be recapped, clipped or removed from disposable syringes. The complete assembly should be placed in a sharps disposal container. Disposable syringes, used alone or with needles, should be placed in sharps disposal containers and incinerated, with prior autoclaving if required. Sharps disposal containers must be puncture-proof/-resistant and must not be filled to capacity. When they are three-quarters full they should be placed in "infectious waste" containers and incinerated, with prior autoclaving if laboratory practice requires it. Sharps disposal containers must not be discarded in landfills.

Contaminated (potentially infectious) materials for autoclaving and reuse:
No pre-cleaning should be attempted of any contaminated (potentially infectious) materials to be autoclaved and reused. Any necessary cleaning or repair must be done only after autoclaving or disinfection.

Contaminated (potentially infectious) materials for disposal:
Apart from sharps, which are dealt with above, all contaminated (potentially infectious) materials should be autoclaved in leak-proof containers (e.g. autoclavable, color-coded plastic bags), before disposal. After autoclaving, the material may be placed in transfer containers for transport to the incinerator. If possible, materials deriving from healthcare activities should not be discarded in landfills even after decontamination. If an incinerator is available on the laboratory site, autoclaving may be omitted; the contaminated waste should be placed in designated containers (e.g. color-coded bags) and transported directly to the incinerator. Reusable transfer containers should be leakproof and have tight-fitting covers. They should be disinfected and cleaned before they are returned to the laboratory for further use.

11. **INFORMATION ON, AND RESULTS OF, PREVIOUS RELEASES OF THE GMOS, ESPECIALLY AT DIFFERENT SCALES AND IN DIFFERENT ECOSYSTEMS**

Information on previous releases is provided in Section II.C.2 (h).

Information relating to the expected shedding of AAV8-hFIX19, obtained from similar viral vectors, is provided in Section II.C.2 (i).3.

B. INFORMATION ON THE ENVIRONMENT (BOTH ON THE SITE AND IN THE WIDER ENVIRONMENT):

1. **GEOGRAPHICAL LOCATION AND GRID REFERENCE OF THE SITE(S) (IN CASE OF NOTIFICATIONS UNDER PART C THE SITE(S) OF RELEASE WILL BE THE FORESEEN AREAS OF USE OF THE PRODUCT)**

Following the approval of the Clinical Trial Authorisation for AAV8-hFIX19, it is planned to make the product available to the following clinical trial site in Ireland for use in accordance with the Clinical Trial Protocol [Protocol # AAV8-hFIX19-101]:

National Centre for Hereditary Coagulation Disorders
St. James's Hospital,
James's Street,
Dublin 8,
Ireland

Further sites (medical facilities) may be added in Ireland following appropriate ethical, Environmental Protection Agency (EPA) and Institutional Biosafety Committee (IBC) approval.

2. **PHYSICAL OR BIOLOGICAL PROXIMITY TO HUMANS AND OTHER SIGNIFICANT BIOTA**

AAV8-hFIX19 will be administered to the patient by a medical professional in a medical facility. The product itself will be stored prior to administration in a secure environment (pharmacy).

Subjects are corrected to 100% normal circulating FIX with concentrate products prior to vector infusion. Haemostasis at the venipuncture site will be secured by applying pressure according to standard protocol for infusing FIX concentrates. This patient population therefore poses no increased risk of shedding through blood loss after the infusion.

Given the nature of the product administration (intravenous), and the transient/ low levels of shedding expected (see Section II.C.2 (i).3), the risk of unintended exposure to AAV8-hFIX19 to humans and other biota is minimal.

Treatment of waste will be affected as described in Sections III.A.10 and V.C.

3. **PROXIMITY TO SIGNIFICANT BIOTOPES, PROTECTED AREAS, OR DRINKING WATER SUPPLIES**

Given the nature of the product administration, scale of release and procedures for waste treatment, the exposure to significant biotopes, protected areas and drinking water supplies is expected to be negligible.

4. CLIMATIC CHARACTERISTICS OF THE REGION(S) LIKELY TO BE AFFECTED

The clinical trial of AAV8-hFIX19 will occur in Ireland which has a temperate climate. The risk of release of AAV8-hFIX19 in to the environment is unrelated to climatic characteristics.

The stability of AAV8-hFIX19 in the environment is unchanged from that of wild-type AAV (see Section II.A.10 (c)).

5. GEOGRAPHICAL, GEOLOGICAL AND PEDOLOGICAL CHARACTERISTICS

The risk of release of AAV8-hFIX19 in to the environment is unrelated to these characteristics. The stability of AAV8-hFIX19 in the environment is unchanged from that of wild-type AAV (see Section II.A.10 (c)).

6. FLORA AND FAUNA, INCLUDING CROPS, LIVESTOCK AND MIGRATORY SPECIES

Given the nature of the product administration (intravenous) and the transient/ low levels of shedding expected (see Section II.C.2 (i).3), the risk of unintended exposure of flora and fauna to AAV8-hFIX19 is minimal.

Treatment of waste will be affected as described in Sections III.A.8, III.A.10 and V.C.

7. DESCRIPTION OF TARGET AND NON-TARGET ECOSYSTEMS LIKELY TO BE AFFECTED

No ecosystems are targeted in the use of AAV8-hFIX19. Ecosystems are not expected to be affected.

8. A COMPARISON OF THE NATURAL HABITAT OF THE RECIPIENT ORGANISM WITH THE PROPOSED SITE(S) OF RELEASE

The natural habitat of wild type AAV2/8 is primates. AAV8-hFIX19 will also be administered to human beings, though the genetic construct is deleted for the *rep* and *cap* genes, rendering it unable to replicate, even in the presence of a helper virus (see Section II.C.2 (i) 1).

9. ANY KNOWN PLANNED DEVELOPMENTS OR CHANGES IN LAND USE IN THE REGION WHICH COULD INFLUENCE THE ENVIRONMENTAL IMPACT OF THE RELEASE

None known.

IV. INFORMATION RELATING TO THE INTERACTIONS BETWEEN THE GMOS AND THE ENVIRONMENT

A. CHARACTERISTICS AFFECTING SURVIVAL, MULTIPLICATION AND DISSEMINATION

1. BIOLOGICAL FEATURES WHICH AFFECT SURVIVAL, MULTIPLICATION AND DISPERSAL

AAV8-hFIX19 is a disabled version of a non-pathogenic wild-type AAV, modified by deletion of the *rep* and *cap* genes rendering it unable to replicate, even in the presence of a helper virus.

As a derivative of primate (human) AAV2/8, the primary indigenous vector of AAV8-hFIX19 is human beings.

AAV shows some species specificity, but can replicate in cells of a different species when infected with AAV *in vitro*, provided it is in the presence of a helper virus to which that species is permissive (e.g. human AAV may be replicated in canine cells if co-infected with a canine adenovirus) (Berns and Bohenzky, 1987). It is not known whether zoonosis occurs in nature, nor whether other species can act as carriers or vectors under natural conditions

The survival of AAV8-hFIX19 outside of the host is expected to be the same as wild-type AAV (see Section II.A.10 (c)).

2. KNOWN OR PREDICTED ENVIRONMENTAL CONDITIONS WHICH MAY AFFECT SURVIVAL, MULTIPLICATION AND DISSEMINATION (WIND, WATER, SOIL, TEMPERATURE, PH, ETC.)

Environmental conditions which may affect survival of AAV8-hFIX19 outside the host are temperature, pH and environmental humidity (see Section II.A.10 (c)). AAV8-hFIX19 is a disabled version of a non-pathogenic wild-type AAV, modified by deletion of the *rep* and *cap* genes rendering it unable to replicate, even in the presence of a helper virus.

3. SENSITIVITY TO SPECIFIC AGENTS

The genetic modifications made during the construction of AAV8-hFIX19 from wild type AAV2/8 are not expected to affect its sensitivity to physical and chemical inactivation.

Physical inactivation:

Wild-type AAV virus is inactivated outside the host by exposure to pH > 9 and by autoclaving for 30-45 minutes at 121°C (CCMT/CHOP MSDS; adeno-associated viral vectors).

Chemical inactivation:

Adeno-Associated virus is susceptible to sodium hypochlorite (1-10% dilution of fresh bleach) and 5% phenol. Alcohol is not an effective disinfectant against AAV (CCMT/CHOP MSDS; adeno-associated viral vectors).

Susceptibility to anti-viral agents:

There is no specific anti-viral agent against AAV.

B. INTERACTIONS WITH THE ENVIRONMENT

1. PREDICTED HABITAT OF THE GMOS

The predicted habitat of AAV8-hFIX19 is humans where it is expected to persist in a lysogenic state. AAV8-hFIX19 is a disabled version of a non-pathogenic wild-type AAV, modified by deletion of the *rep* and *cap* genes rendering it unable to replicate, even in the presence of a helper virus.

2. STUDIES OF THE BEHAVIOUR AND CHARACTERISTICS OF THE GMOS AND THEIR ECOLOGICAL IMPACT CARRIED OUT IN SIMULATED NATURAL ENVIRONMENTS, SUCH AS MICROCOSMS, GROWTH ROOMS, GREENHOUSES

AAV8-hFIX19 is a replication-incompetent virus derived from AAV2/8. The genetic modifications do not affect its natural host and tissue tropism.

No specific studies have been conducted regarding transmission of AAV8-hFIX19 between humans or animals.

Shedding has however been monitored in both humans and animals following administration of similar vectors to AAV8-hFIX19 (see Section II.C.2 (i) 3).

3. GENETIC TRANSFER CAPABILITY

AAV8-hFIX19 is a replication-incompetent virus derived from AAV2/8. The genetic modifications do not affect its natural host and tissue tropism. No transfer of genetic material between the GMO and other organisms is predicted.

The transfer of genetic material is therefore limited to the theoretical genetic exchange of DNA by homologous recombination with wild type AAV which could only occur if human cells were simultaneously infected with both wild type AAV and AAV8-hFIX19, in the presence of a helper virus. In the case of AAV8-hFIX19, such recombination could only result in the exchange of the hFIX expression cassette with the *rep* and *cap* genes of the wild type virus. It is not possible for the AAV genome to contain both *rep/cap* genes and the transgene, as this is beyond the packaging limit of the virion.

Therefore the only mechanism by which the transgene could be mobilised is through a triple infection of the same cell by AAV8-hFIX19 (containing the transgene), wild type AAV (providing the rep and cap functions) and a helper virus. This scenario is expected to be a rare event, and would only result in the production of more wild type AAV and more AAV8-hFIX19 vector particles (which would still lack *rep* and *cap* genes and consequently could not be self-sustaining).

The final plasmid stocks used in the manufacture of AAV8-hFIX19 carry the gene for resistance to Kanamycin for selection purposes, and have been amplified and tested to confirm the correct DNA sequence.

Antibiotics are not used in the manufacturing process performed at CCMT for AAV8-hFIX19. 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.

The consequences of dissemination of DNA derived from the plasmids used to manufacture AAV8-hFIX19 are theoretically uptake and integration into microbial genomes, although infection of microbes by AAV is not described to our knowledge and considered unlikely. These DNA sequences are not capable of replication independently. Following administration to a patient, residual DNA sequences will be degraded via normal routes. It is possible they will persist in the patient cells if the sequences are encapsidated, but again they will be unable to replicate independently. Therefore the likely route of contamination of the environment would be through exposure of the product itself to microorganisms. Such an event could result in uptake and transient expression of a plasmid gene, but the genes present are unlikely to confer any selective advantage and consequently be quickly lost. The possible exception is the transfer of the gene for Kanamycin resistance, which would confer a selective advantage in certain environments, though likely not in sewerage and water systems.

4.   **LIKELIHOOD OF POSTRELEASE SELECTION LEADING TO THE EXPRESSION OF UNEXPECTED AND/OR UNDESIRABLE TRAITS IN THE MODIFIED ORGANISM**

The selective pressure on AAV8-hFIX19 will be towards reversion to wild-type, since both gene deletions (*rep* and *cap*) are required for rescue and replication of the organism in its host species.

The likelihood of this reversion is considered low, since it would require genetic exchange by homologous recombination with wild-type AAV which could only occur if human cells were simultaneously infected with both wild type AAV, AAV8-hFIX19 and a helper virus (e.g. adenovirus).

The transgene (human coagulation Factor IX) is not expected to confer any advantage to the GMO in terms of survival and selective pressure.

5.   **MEASURES EMPLOYED TO ENSURE AND TO VERIFY GENETIC STABILITY. DESCRIPTION OF GENETIC TRAITS WHICH MAY PREVENT OR MINIMISE DISPERSAL OF GENETIC MATERIAL. METHODS TO VERIFY GENETIC STABILITY**

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 infection leading to replication of the GMO (and therefore potential for dispersal) is not possible under normal circumstances.

AAV8-hFIX19 is expected to be genetically stable in isolation (see Section II.C.2 (c)).

In general, DNA viruses have greater genetic stability than RNA viruses. Firstly, DNA is more thermodynamically stable than RNA; secondly, replication of DNA is a much less error-prone process than the replication of RNA; and thirdly, more mechanisms exist in the host cell for repairing errors in DNA than in RNA.

Evolution of AAV viruses (like all viruses) is directed by spontaneous mutation or homologous recombination with other viruses of the same species, where such genetic modification confers a selective advantage.

Homologous genomic recombination may occur spontaneously in nature between the viral genomes of AAV strains only under circumstances where a cell of the host organism is infected simultaneously by two different strains of AAV and a helper virus which is permissive in that species (triple-infection). In the case of AAV8-hFIX19, such recombination could only result in the exchange of the hFIX expression cassette with the *rep* and *cap* genes of the wild type virus. It is not possible for the AAV genome to contain both *rep/cap* genes and the transgene, as this is beyond the packaging limit of the virion.

Therefore the only mechanism by which the transgene could be mobilised is through a triple infection of the same cell by AAV8-hFIX19 (containing the transgene), wild type AAV (providing the rep and cap functions) and a helper virus. This scenario is expected to be a rare event, and would only result in the production of more wild type AAV and more AAV8-hFIX19 vector particles (which would still lack *rep* and *cap* genes and consequently could not be self-sustaining).

6. ROUTES OF BIOLOGICAL DISPERSAL, KNOWN OR POTENTIAL MODES OF INTERACTION WITH THE DISSEMINATING AGENT, INCLUDING INHALATION, INGESTION, SURFACE CONTACT, BURROWING, ETC.

Outside of the host, non-lipid enveloped viruses such as AAV are resistant to low level disinfectants, survive well outside of the laboratory environment and can be easily transmitted via fomites. AAV particles are resistant to a wide pH range (pH 3-9) and can resist heating at 56°C for 1 hour (Berns and Bohenzky, 1987). AAV does not form survival structures but can remain infectious for at least a month at room temperature following simple desiccation or lyophilization.

Dispersal (dissemination) of AAV is not documented definitively, but is likely through inhalation of aerosolized droplets, mucous membrane contact, parenteral injection, or ingestion.

AAV8-hFIX19 is a replication-incompetent virus derived from AAV2/8. The genetic modifications do not affect its survival outside the host or probable mode of dissemination. However, the lack of replicative ability prevents multiplication and therefore severely limits its ability to disseminate.

7.   DESCRIPTION OF ECOSYSTEMS TO WHICH THE GMOS COULD BE DISSEMINATED

Dissemination of AAV8-hFIX19 would most likely only occur between human beings, since it is derived from AAV2/8. However no replication is expected in normal cells of treated individuals exposed to the replication-deficient virus, or from exposure of uninfected people to treated individuals.

8.   POTENTIAL FOR EXCESSIVE POPULATION INCREASE IN THE ENVIRONMENT

The potential for excessive population increase of AAV8-hFIX19 in the environment is extremely low, due to:

- Attenuation of the GMO rendering it even less replication competent than the parental virus (AAV2/8), by deletion of the replication genes (see Section II.C.2 (a))

- Intravenous administration to eligible patients by medical professionals in a medical facility.

- Limited host and tissue tropism (human/primate) of the parental virus (AAV2/8) (see Sections II.A.7 and II.A.8)

- Low and transient incidence of shedding of infective virus from treated individuals (see Section II.C.2 (i) 3).

- High levels of existing adaptive immunity in the human population

9.   COMPETITIVE ADVANTAGE OF THE GMOS IN RELATION TO THE UNMODIFIED RECIPIENT OR PARENTAL ORGANISM(S)

AAV8-hFIX19 is a replication-incompetent virus derived from AAV2/8 and is therefore at a competitive disadvantage when compared to its parent strain / wild type AAV. The transgene (human coagulation Factor IX) is not expected to confer any advantage to the GMO in terms of survival and selective pressure.

10.   IDENTIFICATION AND DESCRIPTION OF THE TARGET ORGANISMS IF APPLICABLE

The target organism is humans, specifically eligible, consenting adult males with X-linked severe Haemophilia B.

11.   ANTICIPATED MECHANISM AND RESULT OF INTERACTION BETWEEN THE RELEASED GMOS AND THE TARGET ORGANISM(S) IF APPLICABLE

In treated subjects, AAV8-hFIX19 is expected to preferentially localise to the liver (dictated by the tissue tropism of the capsid, derived from AAV8). Following infection of hepatocytes, the vector is expected to persist for months or years, primarily in the

episome but potentially by integration into the host cell genome (see Section II.A.10 (b)).

The presence of the codon-optimised human coagulation factor IX gene under the transcriptional control of the liver-specific human alpha 1 antitrypsin (hAAT) promoter, Apolipoprotein E (ApoE) Enhancer and Hepatic Control Region and the bovine growth hormone polyadenylation sequence is expected to result in expression of functional hFIX and its excretion into the circulation at levels which result in clinically meaningful increases in clotting function.

12. IDENTIFICATION AND DESCRIPTION OF NON-TARGET ORGANISMS WHICH MAY BE ADVERSELY AFFECTED BY THE RELEASE OF THE GMO, AND THE ANTICIPATED MECHANISMS OF ANY IDENTIFIED ADVERSE INTERACTION

AAV8-hFIX19 is derived from the non-pathogenic primate AAV2/8.

AAV8-hFIX19 is disabled by deletion of the *rep* and *cap* genes rendering it unable to replicate, even in the presence of a helper virus.

Therefore infection leading to replication of the GMO (and therefore potential for dispersal) is not possible under normal circumstances.

AAV8-hFIX19 will only be administered as an Investigational Medicinal Product to eligible, consenting adult males with X-linked severe Haemophilia B.

AAV shows some species specificity, but can replicate in cells of a different species when infected with AAV *in vitro*, provided it is in the presence of a helper virus to which that species is permissive (e.g. human AAV may be replicated in canine cells if co-infected with a canine adenovirus) (Berns and Bohenzky, 1987). It is not known whether zoonosis occurs in nature, nor whether other species can act as carriers or vectors under natural conditions However, given the inability to replicate and site of administration, the possibility of exposure of AAV8-hFIX19 to non-humans is considered negligible.

The non-target organisms which could conceivably be affected are unintended human recipients (healthcare workers and close contacts of the patient).

It is not expected that transmission would lead to adverse effects in healthy humans since neither wild type AAV nor AAV8-hFIX19 are known to be pathogenic. In the unlikely event that transmission to a healthy unintended human recipient occurs it is likely that the safety profile in healthy subjects would be at worst similar to those expected in patients (see Section II.C.2 (i) 1).

13. LIKELIHOOD OF POSTRELEASE SHIFTS IN BIOLOGICAL INTERACTIONS OR IN HOST RANGE

The likelihood of post-release shifts in biological interactions or host range is negligible.

AAV enters cells by interaction of specific viral capsid epitopes with cell surface receptors. The inserted gene in AAV8-hFIX19 is hFIX, a human clotting factor that is packaged in viral capsid proteins derived from AAV8, and therefore would not be expected to alter the host range or cell tropism of the virus. The gene deletions in AAV8-hFIX19 prevent the ability of the virus to replicate independently, but do not affect the packaging viral capsid proteins so would not be expected to have any effect on host range or cell tropism. In summary, the host range and tropism of AAV8-hFIX19 (or any variant) is expected to be identical to wild type AAV.

14. **KNOWN OR PREDICTED INTERACTIONS WITH NON-TARGET ORGANISMS IN THE ENVIRONMENT, INCLUDING COMPETITORS, PREYS, HOSTS, SYMBIONTS, PREDATORS, PARASITES AND PATHOGENS**

None known or predicted.

15. **KNOWN OR PREDICTED INVOLVEMENT IN BIOGEOCHEMICAL PROCESSES**

None known or predicted.

16. **OTHER POTENTIAL INTERACTIONS WITH THE ENVIRONMENT**

None known or predicted.

V. INFORMATION ON MONITORING, CONTROL, WASTE TREATMENT AND EMERGENCY RESPONSE PLANS

A. MONITORING TECHNIQUES

1. METHODS FOR TRACING THE GMOS, AND FOR MONITORING THEIR EFFECTS

1.1. Monitoring during treatment of patients

Patients will be monitored throughout treatment by the Principal Investigator and delegates. An independent Data Safety Monitoring Board (DSMB) will oversee data and safety monitoring, with interval meetings based on DSMB guidelines. Independent Contract Research Organisations (CROs) will be used for study monitoring and data management activities. Any serious adverse event will be reported in the appropriate time-frame to the Sponsor, and as required to each of the national regulatory agencies according to pharmaceutical legislation.

After vector administration, vital signs will be monitored hourly for 6 hours and then every 2 hours for 6 hours and then at 4-hour intervals. Patients will remain in-hospital for approximately 24 hours of observation to monitor for adverse effects related to the procedure.

A comprehensive battery of laboratory evaluations, comprising primarily of blood tests, will be conducted at regular intervals to assess safety throughout the first year following administration. Safety tests will include PCR testing for vector shedding in saliva, blood and urine at each weekly visit until week 12 (or until 2 consecutive samples are negative). PCR testing of semen will be performed at each monthly visit until 2 consecutive samples are negative.

Factor IX activity/antigen will be monitored weekly until 12 weeks, and monthly thereafter until Month 12.

1.2. Follow-up of patients after treatment

Patients will be subject to long term follow-up as described below:

In Years 2 to 5 following vector administration, an annual physical exam will be performed with complete history and a subset of laboratory evaluations for safety and Factor IX activity (as a surrogate test to indicate vector persistence).

The subject's body fluids will be monitored only during the first year of the study for persistent vector sequences. It is not anticipated, based on non-clinical studies and prior clinical experience, that testing for vector sequences will be necessary in the long-term follow-up period.

Subjects will be encouraged to monitor themselves and to assist in reporting adverse events; they will be provided with laminated wallet-sized cards with investigator contact information. Additionally, health care professionals from the subject's home

haemophilia centre, who are not otherwise associated with the clinical trial, will be notified to provide prompt reports of adverse events to the investigators.

Investigators will maintain in the case history records of exposures to mutagenic agents and other medicinal products along with subjects' adverse event profiles.

Clinical information will focus on information pertaining to new malignancies, new incidence or exacerbation of a pre-existing neurological disorder, new incidence or exacerbation of a prior rheumatologic or other autoimmune disorder, and new incidence of a hematologic disorder.

For the subsequent ten years, subjects will be contacted at a minimum of once per year. A clinical questionnaire, administered by telephone call or at the subject's home haemophilia treatment centre, will focus on information pertaining to new malignancies, new incidence or exacerbation of a pre-existing neurological disorder, new incidence or exacerbation of a prior rheumatologic or other autoimmune disorder, and new incidence of a hematologic disorder. It will ask subjects to describe any adverse events, including any unexpected illness and/or hospitalization, and provide a description of exposures to mutagenic agents and other medicinal products. Additionally, ultrasound of the target organ, the liver, will be performed every three years for the fifteen year long-term follow-up period.

1.3. Monitoring of unintended recipients

No monitoring of unintended recipients is planned or considered necessary.

2. SPECIFICITY (TO IDENTIFY THE GMOS, AND TO DISTINGUISH THEM FROM THE DONOR, RECIPIENT OR, WHERE APPROPRIATE THE PARENTAL ORGANISMS), SENSITIVITY AND RELIABILITY OF THE MONITORING TECHNIQUES

The vector genome contains unique sequences that are not expected to be found in clinical samples not exposed to the vector, namely the codon optimized hFIX DNA sequence, and the expression cassette sequence in proximity with AAV2 inverted terminal repeats. Hence polymerase chain reaction based methods using vector genome specific primers can be used to detect GMO genetic elements with high sensitivity (see Sections II.C.2 (f) and II.C.2 (g)).

3. TECHNIQUES FOR DETECTING TRANSFER OF THE DONATED GENETIC MATERIAL TO OTHER ORGANISMS

No plans for detecting transfer of genetic material to other organisms are considered necessary.

4. DURATION AND FREQUENCY OF THE MONITORING

Each subject's participation will last approximately one year for the active phase of the study. A companion long-term follow-up (LTFU) protocol, recommended by the US Food and Drug Administration (FDA), will be conducted with a duration of up to 15

years after vector administration (see Section V.A.1.2 for details of the frequency of evaluations).

B. CONTROL OF THE RELEASE

1. **METHODS AND PROCEDURES TO AVOID AND/OR MINIMISE THE SPREAD OF THE GMOS BEYOND THE SITE OF RELEASE OR THE DESIGNATED AREA FOR USE**

Distribution and supply:

Following the approval of the Clinical Trial Authorisation for AAV8-hFIX19, it is planned to make the product available to clinical trial site(s) in Ireland for use in accordance with the Clinical Trial Protocol [Protocol # AAV8-hFIX19-101]:

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.

The product label includes: Investigational product name (AAV8-hFIX19); manufacturer (CCMT, Children's Hospital of Philadelphia PA, USA); specific lot number; date of manufacture; vial number; storage instructions (< -60°C); Clinical Trial Protocol number (Protocol # AAV8-hFIX19-101) and investigational product warning (For Clinical Trial Use Only).

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.

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, preparation (dilution and combination of components) on the day of administration and disposal.

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.

Dose preparation and administration:

A qualified pharmacist with specific training on the Clinical Trial Protocol will be responsible for preparation on the day of infusion.

AAV8-hFIX19 will be administered to the patient by a medical professional in a medical facility.

Precautions to be adhered to during dose preparation and administration are outlined in Section III.A.8 of this document, and in the Investigational Drug Data Sheet (IDDS) and Handling Instructions for AAV8-hFIX19.

Spills:

In the context of clinical practice, spills will not constitute a volume greater than 200mL (ie. the entire contents of a prepared infusion bag). Any spill is likely to be of considerably smaller volume, and most likely occur during dose preparation (within a BioSafety Cabinet). Spills should be treated as outlined in Section III.A.10 of this document and in the Handling Instructions for AAV8-hFIX19.

Disposal:

AAV8-hFIX19 is sensitive to inactivation by commonly available physical and chemical methods (see Section IV.A.3). Waste disposal is described in Section III.A.10 and Section V.C of this document, and in the Handling Instructions for AAV8-hFIX19.

2. METHODS AND PROCEDURES TO PROTECT THE SITE FROM INTRUSION BY UNAUTHORISED INDIVIDUALS

AAV8-hFIX19 will be administered to the patient by a medical professional in a medical facility. The product itself will be stored prior to administration in a secure environment (pharmacy).

Intrusion by unauthorized individuals is therefore considered adequately controlled.

3. METHODS AND PROCEDURES TO PREVENT OTHER ORGANISMS FROM ENTERING THE SITE

No other procedures are considered necessary to prevent other organisms from entering the site, since AAV8-hFIX19 is a replication-incompetent version of wild-type AAV2/8. Within a medical facility, general pest control and cleaning procedures will be in place as dictated by site specific procedures for general hygiene.

<div align="center">**C. WASTE TREATMENT**</div>

1. TYPE OF WASTE GENERATED

AAV8-hFIX19 will be administered by a single peripheral intravenous infusion into eligible, consenting adult males with X-linked severe Haemophilia B.

Waste generated from the preparation and infusion of AAV8-hFIX19 will be limited to:

- Used vials of the Investigational Medicinal Product
- Used vials of empty capsid (containing no DNA)
- Used preparation equipment in the pharmacy; syringes, needles, vials
- Used Infusion bags and infusion kits
- Bags used to transport potentially contaminated equipment to and from the pharmacy
- Used swabs and items used to clean injected area
- Personal Protective Equipment used during dose preparation and administration

2. EXPECTED AMOUNT OF WASTE

AAV8-hFIX19 vector is supplied as a frozen liquid containing $>0.5 \times 10^{13}$ viral genomes (vg) in a volume of 1 mL in a 1.5 mL polypropylene sterile cryogenic screw cap vial. The actual concentration of vector will vary between batches; the concentration of the currently available batch (Lot A8FP1-1109C) is 1.79×10^{13} vg/ml.

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.

The maximum total dose for an 80 kg individual patient is therefore approximately 22 mL as a single treatment (approximately 40×10^{13} vg).

The proposed clinical trial aims to recruit 15 evaluable patients across 4 sites in the US, Australia and Ireland. It is therefore reasonable to assume that approximately 4-8 patients will be treated in Ireland.

Each administration will result in the waste identified above.

3. DESCRIPTION OF TREATMENT ENVISAGED

AAV8-hFIX19 is a replication-deficient non-pathogenic virus which is considered to present a much lower hazard to human health than other human biological waste which is frequently disposed of in medical facilities.

AAV8-hFIX19 is sensitive to inactivation by a variety of commonly available physical and chemical methods (see Section IV.A.3).

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. These will then be returned to the sponsor or destroyed according to sponsor instructions (see the Investigational Drug Data Sheet (IDDS)/ Pharmacy manual and Handling Instructions for AAV8-hFIX19).

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 (see Section III.A.10).

D. EMERGENCY RESPONSE PLANS

1. **METHODS AND PROCEDURES FOR CONTROLLING THE GMOS IN CASE OF UNEXPECTED SPREAD**

There are no specific procedures planned for controlling the GMO in the case of unexpected spread.

Wild type AAV is a non-pathogenic single-stranded DNA *Dependoviru*s, requiring helper DNA virus for replication. AAV8-hFIX19 is derived from wild type AAV, but encodes no replication genes in the expression cassette and is incapable of independently replicating its genome.

The potential for unexpected spread of AAV8-hFIX19 in the environment is extremely low, due to:

- Attenuation of the GMO rendering it even less replication competent than the parental virus (AAV2/8), by deletion of the replication genes (see Section II.C.2 (a))

- Intravenous administration to eligible patients by medical professionals in a medical facility.

- Limited host and tissue tropism (human/primate) of the parental virus (AAV2/8) (see Sections II.A.7 and II.A.8)

- Low and transient incidence of shedding of infective virus from treated individuals (see Section II.C.2 (i) 3).

- High levels of existing adaptive immunity in the human population

Any spread of AAV8-hFIX19 to unintended human recipients is therefore highly unlikely, and would be isolated to single cases in discrete geographical locations. The risk of widespread infection is considered negligible.

In the theoretical event that wild type AAV, supplying the requisite replication gene products, were to co-infect a hepatocyte, along with a helper DNA virus such as adenovirus or herpes simplex virus and the AAV8-hFIX19 vector (a triple co-infection), it is possible that vector replication could occur.

However, even if this rare event were to occur, the resulting virologic outcome would be increased synthesis of vector and wild type AAV, both intrinsically non-pathogenic viruses. It is therefore unlikely that such an event would present clinical symptoms and is therefore unlikely to become apparent.

If such spread were detected, the individual could be isolated pending further investigation, and consultation with IMB and the Environmental Protection Agency in Ireland.

2. **METHODS FOR DECONTAMINATION OF THE AREAS AFFECTED, FOR EXAMPLE ERADICATION OF THE GMOS**

There are no specific procedures planned for decontaminating areas in the case of unexpected spread, since the risk of spread is considered negligible.

In the unlikely event that transmission to an unintended human recipient occurred, this would likely be a local occurrence affecting a healthcare professional or close contact of a treated individual.

Decontamination of areas in which a recently treated patient had frequented (their home and or examination room at a medical facility) could be implemented by applying standard detergents (see Section IV.A.3) to areas of likely contact (for example, frequent touch-points such as handles, door knobs, hard surfaces, railings and hand-holds, washing facilities and lavatories). Fomites could be autoclaved or incinerated.

3. **METHODS FOR DISPOSAL OR SANITATION OF PLANTS, ANIMALS, SOILS, ETC., THAT WERE EXPOSED DURING OR AFTER THE SPREAD**

The predicted habitat of AAV8-hFIX19 is humans where it is expected to persist in a lysogenic state. AAV8-hFIX19 is a disabled version of a non-pathogenic wild-type primate (human) AAV, modified by deletion of the *rep* and *cap* genes rendering it unable to replicate, even in the presence of a helper virus.

Decontamination of plants, (non-human) animals and soils will not be required.

4. **METHODS FOR THE ISOLATION OF THE AREA AFFECTED BY THE SPREAD**

There are no specific plans for isolation of an area should horizontal transfer occur between a patient receiving AAV8-hFIX19 and an unintended human recipient.

In the extremely unlikely event that spread of the vector was detected, the individual could be isolated pending further investigation, and consultation with IMB and the Environmental Protection Agency in Ireland. Measures outlined in Section V.D.1 may be implemented to prevent further spread.

5. **PLANS FOR PROTECTING HUMAN HEALTH AND THE ENVIRONMENT IN CASE OF THE OCCURRENCE OF AN UNDESIRABLE EFFECT**

AAV8-hFIX19 will be regulated under medicines legislation in Ireland, requiring stringent pharmacovigilance overseen by the Competent Authority (Irish Medicines Board; IMB). Information will be collected regarding all individual adverse events and submitted to the IMB if they fulfil the criteria for a Serious Unexpected Suspected Adverse Reaction (SUSAR) as defined in the Clinical Trial Protocol. Development Safety Update Reports will be submitted to IMB on an annual basis while the trial is active. Procedures are in place at both IMB and CCMT/CHOP to monitor, review and act on urgent safety information relating to medicinal products so that human health is protected.

Information relating to trial-related monitoring activities is provided in Section V.A.

In the extremely unlikely event that spread of the vector to an unintended human recipient was detected, the individual could be isolated pending further investigation, and consultation with IMB and the Environmental Protection Agency in Ireland. Measures outlined in Section V.D.1 and Section V.D.2 may be implemented to prevent further spread.

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APPENDICES

Clinical Trial Protocol synopsis

INFORMATION DELETED FOR CONFIDENTIALITY PURPOSES

Investigational Drug Data Sheet (IDDS)/ Pharmacy Instructions

INFORMATION DELETED FOR CONFIDENTIALITY PURPOSES

Patient Information

INFORMATION DELETED FOR CONFIDENTIALITY PURPOSES

Handling Instructions

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The Children's Hospital of Philadelphia, 3615 Civic Center Boulevard, Philadelphia, PA 19104

Handling Instructions for Recombinant AAV-based Investigational Products

1. Scope

1.1. This document provides general Instructions for handling recombinant AAV-based investigational products.

1.2. The instructions provided herein should be used in conjunction with specific instructions provided in the product-specific Investigational Drug Data Sheet (IDDS) and Pharmacy Instructions provided with each product.

2. Responsibilities

A qualified pharmacist, trained on IDDS and other relevant product-specific protocols/instructions, is responsible for the Investigational Drug product receipt from the Sponsor (CCMT at CHOP), product storage, documentation of product traceability at the investigational site, and preparation of the administration device and dose in accordance with Pharmacy Instructions.

3. Handling procedures required to ensure personnel safety and appropriate product containment

3.1. Biosafety level 2 practices should be followed while handling recombinant AAV products (Ref 6.1). Universal precautions and appropriate personal protective equipment (gloves and lab gowning) should be used by investigational pharmacy staff and clinical personnel during investigational product preparation and administration;

3.2. Dose preparation should be performed in a BioSafety Cabinet to reduce the risks posed by the possibility of generation and inhalation of aerosols and to protect product from contamination;

3.3. Once prepared, the dose (administration device) is labeled according to protocol, double bagged and taken to the drug administration area in a designated container with a biohazard symbol;

3.4. Following administration of the investigational product to the trial participant, used and/or partially used vials of product and excipient (if applicable), syringes, and other items used in dose preparation, including infusion bags and infusion sets, should be retained. For each subject treated, these items should be placed into a labeled biohazard bag in the pharmacy and stored at < -60C for the period specified in the IDDS and Pharmacy Instructions. Afterwards these materials are returned to the sponsor or destroyed according to sponsor instructions;

3.5. Any other disposable instruments and materials used during the dose preparation and administration procedures should be disposed of in a manner consistent with standard practices for biohazardous materials and in compliance with institutional safety policies;

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The Children's Hospital of Philadelphia, 3615 Civic Center Boulevard, Philadelphia, PA 19104

Handling Instructions for Recombinant AAV-based Investigational Products

4. Instructions for disinfection and disposal of recombinant AAV based product contact biohazardous waste

4.1. *Routine Surface Disinfection before and after handling:*

a. Surfaces used for handling of the investigational product, including the surfaces of the biosafety cabinet used for product preparation, and those used during subject administration should be appropriately sanitized prior to use;

b. After pharmacy and subject administration procedures, surfaces should be wiped with an EPA-approved disinfectant /detergent or an approved germicidal wipe.

4.2. *Disposal of liquid waste:*

a. Fluids in quantities greater than 20 milliliters must be segregated in sealed, leak proof biohazard labeled containers. These containers can then be placed into the laboratory infectious waste receptacle.

4.3. *Disposal of needles, pipette tips, serological pipettes, tubes and other sharp items:*

a. Place sharps directly into impervious, right and puncture resistant purpose designed containers;

b. Do not recap needles prior to disposal.

4.4. *Clean up after an unintended spill:*

a. Remove staff from the immediate area;

b. Remove any contaminated clothing and place it in an autoclave bag. Wash contaminated skin thoroughly with disinfectant soap and water;

c. Use a disposable fluid-resistant gown, gloves, safety goggles, surgical mask;

d. Apply a suitable disinfectant (10% household bleach recommended) to perimeter of spill and over the spill itself. Apply absorbent material (e.g. paper towels) to absorb material and bleach. Allow 20 min contact time;

e. Dispose of absorbed materials and contaminated protective equipment in the infectious waste container;

f. Wash hands thoroughly.

5. Occupational Exposure

In the event of personnel contamination the following applicable procedures must be followed:

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Clinical Vector Core, Center for Cellular and Molecular Therapeutics
The Children's Hospital of Philadelphia, 3615 Civic Center Boulevard, Philadelphia, PA 19104

Handling Instructions for Recombinant AAV-based Investigational Products

5.1. *Eye exposure:*

Isotonic saline for irrigation shall be available in the preparation area for eye and face wash in the event of accidental exposure. The affected area shall be flushed for a minimum of 15 minutes. Seek prompt medical attention;

5.2. *Needle puncture:*

Immediately rinse the affected area with water for several minutes. Manage bleeding as necessary. Seek prompt medical attention;

5.3. *Skin exposure:*

Immediately rinse the affected area with water for at least 15 minutes. Then wash area with soap and water. Seek medication attention if necessary;

5.4. *Protective clothing contamination:*

Remove contaminated clothing. Attempt to avoid spreading contamination to other clothing or body surfaces. Dispose of contaminated materials in the infectious waste receptacle. If any body surface exposure occurs refer to the appropriate section above.

6. References

6.1. U.S. Department of Health and Human Services Centers for Disease control and Prevention and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories (BMBL). Current Edition. Section IV. Laboratory Biosafety Level Criteria. Biosafety level 2 requirements;

6.2. Material Safety Data Sheet: Adeno-associated Viral vectors;

6.3. CHOP Policy No. 04.28.20: Handling of gene transfer products.

7. Document History

Version	Change Control Record	Effective Date
01	CC-13010	30Jan2013

Material Safety Data Sheet (MSDS)

Material Safety Data Sheet

ADENO-ASSOCIATED VIRAL VECTORS

1. General description

Adeno-associated virus (AAV): AAV belongs to the family Parvoviridae. AAV is a non-enveloped, single- strand DNA containing virus that can replicate only in the presence of a helper virus. Known helper viruses are Adenovirus (Ad), Herpes virus, and Vaccinia virus. Wild-type AAV may integrate into the host-cell genome and remain latent until a infection that provides helper virus supplies the necessary genes for replication.

AAV vector: AAV vectors consist of recombinant transgene sequences (e.g., human gene or other sequence of interest) flanked by the AAV inverted terminal repeats. AAV vector cloning capacity is limited to the size of wild-type AAV genome. Recombinant AAV vectors can infect a wide range of mammalian cell types.
AAV vectors are normally provided as a suspension of purified viral particles in phosphate buffered saline. The material is stored frozen at $< - 60\ ^{0}$C.

2. Physical Data
Liquid or frozen particle suspensions

3. Health Hazards
Hazardous ingredients: None

Fire and Explosion: None

Reactivity: Stable. Potential to enter many types of mammalian cells following exposure.
In the presence of helper viruses such as adenovirus AAV may replicate. Recombinant AAV vector DNA forms a stable extra chromosomal expression cassette in transduced cells. At very low frequency AAV vectors may integrate into host cell DNA.

Pathogenicity: None.
AAV is not known to cause any diseases in humans or animals. AAV viruses are not associated with any human disease; however, there is evidence of AAV infection in the human embryo and an association of AAV with male infertility.
A significant correlation was found between the presence of AAV DNA in amnion fluids and premature amniorrhexis (rupture of the amnion) and premature labor.
Recombinant AAV vectors lose site specific integration into chromosome 19, thereby raising the theoretical concern of insertional mutagenesis, though frequency of genome integration very low.

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MSDS AAV.01   Effective Date: 30Apr2012   Page 1 of 3

Modes of transmission: AAV may be transmitted through direct contact with an infected individual or through indirect contact with the contaminated environment. Transmission routes include respiratory, gastrointestinal and possibly sexual transmission. A concern for vertical transmission from mother to fetus also exists. Most adults (85-90% in US) are seropositive for AAV, with approx 30% have neutralizing antibodies.

There is a theoretical risk of infection from exposure to laboratory cultures of wild-type adeno-associated virus or recombinant viruses. Transmission of AAV can occur through ingestion, inhalation of aerosolized droplets, mucous membrane contact and accidental injection (for example, as the result of a needlestick).

4.   Recommended precautions

Containment requirements: Biosafety level 2 practices and containment facilities for all activities involving the virus and potentially infectious body fluids or tissues

Personal protection: Laboratory coat, gloves, safety glasses recommended

Other precautions: None

5.   Handling information

Laboratory Practices

- Handle as biohazardous material under Biosafety Level 2 containment
- Use a biological safety cabinet (BSC) (a.k.a. tissue culture hood) for manipulations that can generate aerosols, such as pipetting, harvesting, infecting cells, filling tubes/containers, and opening sealed centrifuge canisters. If a procedure cannot be done in a BSC but only on an open bench, use a plastic shield to prevent exposure through inhalation or splashing.
- Use aerosol containment devices when centrifuging. These include sealed canisters that fit in the centrifuge bucket, covers for the centrifuge bucket, heat sealed tubes, or sealed centrifuge rotors. Rotors should be removed and opened inside a BSC. Centrifuge tubes should be filled and opened in a BSC.
- Protect vacuum lines with liquid disinfectant traps and micron filters.
- Biohazard signs and labels must be displayed in areas and on equipment where AAV is used and stored.

Personal Protective Equipment

Personal protective equipment (PPE) includes, but is not limited to:

- Disposable gloves (nitrile, latex, etc.).
- Lab coat when working in the area. Remove when leaving the laboratory.
- Goggles for splash protection.

Employee Exposure

Eye Exposure – Rinse eyes in an eyewash for at least 15 minutes.
Skin Exposure – Rinse skin with soap and water.
Accidental Needlestick Injury – Scrub contaminated skin with soap and water.

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MSDS AAV.01   Effective Date: 30Apr2012   Page 2 of 3

Due to the possibility of adenovirus contamination, recommended treatment is the same for exposure to adenovirus.

Disposal and decontamination procedures

AAV contaminated materials must be disposed of as biohazardous waste.

<u>Viral stock:</u> Dispose by autoclaving at 121°C for 30-45 minutes

<u>Cell culture medium and other liquids containing AAVvector:</u> Dispose by decontamination with chlorine bleach (10% f.c.) for at least 20 minutes and then dispose of in sink.

Infected animal carcasses or tissues: Dispose by incineration.

<u>Spills outside a biological safety cabinet</u>: Leave the area while holding your breath. Once outside the area, wash hands and face with soap and water. Do not allow anyone inside the area or room where the spill occurred. Allow 30 minutes for the aerosols to settle. Enter the room wearing requiredPPE, cover the spill with paper towels, and apply disinfectant starting at the perimeter and working towards the center. Allow the disinfectant to remain on the spill for at least 20 minutes before initiating spill cleanup. After initial clean up, disinfect the area a second time.

<u>Spills inside a biological safety cabinet</u>: Cover the spill with paper towels or wipes. Gently pour disinfectant over the spill area. Let the disinfectant soak for 20 minutes before cleaning up the spill. After initial clean up, disinfect the area a second time. Decontaminate adjacent surfaces with a bleach solution.

Disinfectants

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.

Alcohol is not an effective disinfectant against AAV.

6.   References

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MSDS AAV.01   **Effective Date: 30Apr2012**   Page 3 of 3