AMT-061
AAV5-hFIXco-Padua (serotype 5 adeno-associated viral vector containing a codon optimised human factor IX Padua gene)

Ireland

Biosafety Dossier

In accordance with S.I. No. 500/2003 - Genetically Modified Organisms (Deliberate Release) Regulations 2003

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1. CONTENTS

2. LIST OF ABBREVIATIONS

AAV	Adeno-associated viral vector
AAV-n	Adeno-associated viral vector serotype number (1, 2, etc)
AAV5-hFIXco-Padua	Serotype 5 adeno-associated viral vector containing a codon optimised human factor IX gene; the gene is codon-optimised to enhance expression and the product is a hyperactive variant of factor IX carrying an R338L mutation (referred to as “FIX-Padua”)
BEVS	Baculovirus expression vector system
DNA	Deoxyribonucleic acid
FIX	Factor IX
GMO	Genetically modified organisms
HCR	Hepatic control region
hFIXco	Codon optimised human Factor IX
IMP	Investigational Medicinal Product
ITR’s	Inverted Terminal Repeats
Kg	Kilogram
L	Litre
LOD	Limit of detection
LOQ	Limit of quantitation
μg	Microgram
μL	Microlitre
mL	Millilitre
ng	Nanogram
ORF	Open reading frame
PCR	Polymerase Chain Reaction
ppm	Parts per million
QPCR	Quantitative Polymerase Chain Reaction
rAAV	Recombinant adeno-associated viral vector
rcAAV	Replication competent adeno-associated viral vector
SV40	Simian virus 40
wt	Wild type

3. INFORMATION RELATING TO THE GMO

The Investigational Medicinal Product (IMP), AAV5-hFIXco-Padua, is a recombinant serotype 5 adeno-associated viral vector containing a codon optimised human factor IX gene; the gene is codon-optimised to enhance expression. The product is a gain-of-function variant of factor IX carrying an R338L mutation ("FIX-Padua") which results in increased activity.

The aim of the work is to further establish an AAV-based liver directed gene therapy for the treatment of haemophilia B.

Treatment is designed to deliver the sequence encoding human FIX to the liver, and thus enable sustained levels of plasma FIX activity in adult patients aged 18 years or older suffering from severe or moderately severe haemophilia B.

The Sponsor has introduced a design modification to its previous generation FIX product, AMT-060, resulting in AAV-hFIXco-Padua (AMT-061). In an attempt to achieve higher circulating FIX activity levels with the goal of alleviating the need for all exogenous therapy the Sponsor introduced the design modification to the wild type FIX coding sequence within the AMT-060 vector genome in order to encode the naturally occurring Padua FIX gain-of-function variant.

The AMT-060 and AMT-061 based gene therapy vectors are identical except for a two-nucleotide substitution in the coding sequence for FIX (See Figure 1). The coding genes, vector sequences in the vector and in the DNA inserted into the vector are described in Section 3.5.1.

Figure 1A: Structure of AMT-061. Compared with the previous generation product (AMT-060; see Figure 1B) AMT-061 has a two-nucleotide substitution resulting in a single codon change (AGG to CTG as indicated) in the sequence for FIX. This substitution results in an arginine to leucine substitution in the translated protein, at position 338 (R338L). The hFIXco expression cassette is flanked by two ITRs (hairpin structures) and consists of the LP1 promoter, SV40 intron, hFIXco coding sequence, and polyA signal, in that order. A schematic representation of the translated protein is also provided where PP is the pre-pro-peptide and AP is the activation peptide

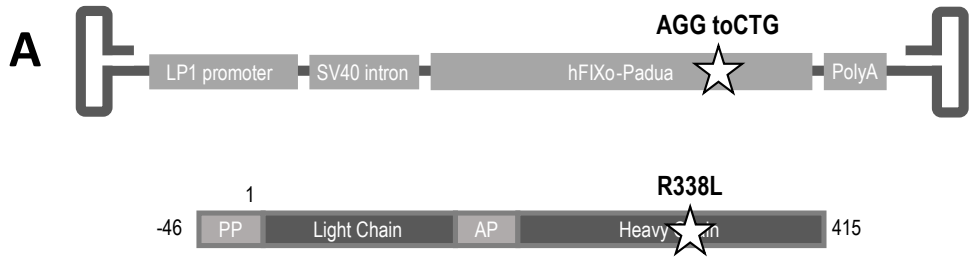
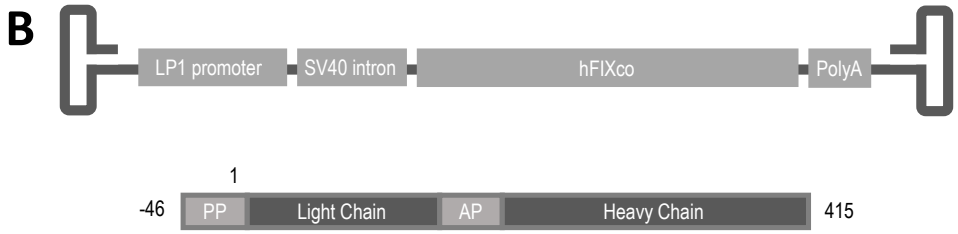


Figure 1B: Structure of the previous generation AMT-060 vector genome and derived wild type human FIX protein.



3.1 Parental Organism

The parental virus is adeno-associated virus (AAV). Adeno associated viruses are small (approximately 25 nm in diameter) non-enveloped, non-pathogenic parvoviruses. AAV infect cells through a receptor mediated process, after which the viral DNA is transported to the nucleus. AAV needs a helper virus, such as adenovirus or herpes virus, to replicate. The parental virus consists of a single-stranded DNA vector genome (derived from AAV2) which is encapsidated by an icosahedral protein capsid (derived from AAV5). Both parental AAV's, AAV-2 and AAV-5, are AAV serotypes that naturally occur in the human population. The vector genome used contains two DNA sequences (inverted terminal repeats: ITRs) that are derived from the viral genome of AAV serotype 2 (AAV-2). The capsid proteins are derived from AAV serotype 5 (AAV-5). The vector genome is packaged into capsids composed of the three viral proteins, VP1, VP2, and VP3. Each capsid consists of 60 VP proteins in total, which are arranged in icosahedral symmetry.

3.2 Parental Organism Host Range

Dependoviruses with similarity to AAV have been identified from other species; the AAV serotypes found in humans seem to be restricted to primates (Arbetman et al, 2005). Both humans and primates may show pre-existing (neutralising) antibody titres against AAV. Infections with AAV occur frequently and are world-wide. The prevalence of neutralising antibodies against AAV is found in the adult European population to be for AAV5 (3.2%) followed by AAV8 (19%) and is highest for AAV2 (59%) and AAV1 (50.5%) (Boutin *et al*., 2010; Calcedo *et al*., 2009). AAV infections are non-pathogenic, *i.e.* not associated with disease manifestations.

Tissue/cell tropism is determined by serotype, i.e. the capsid moiety. In the case of AMT-061, the capsid is AAV-5 derived. Preclinical studies in non-human primates (including the Sponsor's own studies in non-human primates) have shown that, following administration, AAV-5 displays strong liver-directed tropism, with vector DNA sequence also detected in the spleen and adrenal glands (Nathwani *et al*., 2011).

3.3 Parental Organism Origin - Wild type AAV Vector

Wild-type AAV is comprised of a linear single-stranded DNA genome that can only replicate in the presence of a helper virus. The wild type AAV genome carries only two genes; the Cap gene, which encodes the proteins forming the capsid, and the Rep gene, which encodes for proteins that replicate the viral genome and package it into the capsids. These elements are flanked by two inverted terminal repeats (ITRs), which serve as substrates for the Rep proteins

during replication and packaging of the vector genome. The vector genome may consist of a positive (+) or a negative (-) strand.

3.4 Host for GMO Expression - Baculovirus Expression Vector System

Replication-competent AAV are frequently detected in AAV preparations that were produced using conventional, mammalian cell-based production platforms; as an example, Allay *et al*. reported approximately 1 rcAAV per million particles (Allay *et al*., 2011). The preference for the expression system chosen includes the increased safety profile of baculovirus system-produced AAV resides in the fact that the Rep and Cap genes are under control of an insect promoter and hence, in humans, do not mediate functional expression of these genes.

The GMO AMT-061, is not constructed from a parental virus or vector in the classical sense. Rather, AMT-061 is assembled from individual molecular components expressed using a baculovirus expression vector system (BEVS). BEVS is characterised by its excellent safety characteristics. First, baculoviruses are insect-specific viruses that are not capable of replicating in vertebrates (Airenne *et al*., 2013). Second, they can easily be cleared from recombinant AAV preparations, because in contrast to AAV they are large enveloped viruses which are susceptible to inactivation by surfactants and to removal by nano-filtration.

The BEVS for recombinant AAV is based on the fact that the three required elements for the generation of functional AAV particles, being the Rep protein(s), the Cap protein(s) and the vector genome, can be provided in trans, i.e. the vector genome does not need to contain these sequences as long as the proteins they encode are provided alongside it. In practice this means that each can be provided by a different baculovirus vector. As such, the genome of a recombinant AAV-based vector can be gutted to contain little more than the transgene expression cassette encoding the therapeutic protein of interest. The resulting baculovirus construct thus contains the two AAV-2-derived Inverted Terminal Repeats (ITR's), which flank the transgene expression cassette. The ITRs that are present in the vector genome of the GMO AMT-061 were cloned from the viral genome of wild type AAV-2 (Samulski *et al*., 1987). The gutting of the vector genome renders the resulting recombinant AAV-based vector completely replication defective, i.e. even in the presence of a helper virus, because the Rep and Cap genes are missing. Intracellular expression of Rep and Cap, in the presence of the AAV vector genome, therefore results in (1) replication of the AAV vector genome by Rep protein, (2) assembly of capsid proteins to capsid particles, and (3) packaging of vector genome DNA into the capsid particles by Rep, in this case resulting in AMT-061.

To survive outside the host, wild type baculoviruses organise into so called occlusion bodies, to which the polyhedrin protein is an essential component (Rohrmann, 2013). Due to the absence of the polyhedrin gene, and the resultant absence of polyhedron protein, recombinant baculovirus cannot form occlusion bodies and are not stable outside of the culturing system.

Baculoviruses are incapable of replication in mammalian cells. They have a limited range of hosts and are typically restricted to a range of closely related insect species (Airenne *et al*., 2013).

The parental (baculovirus) vectors used in the manufacture of the GMO AMT-061 are able to infect and replicate in the insect cell line used for the production.

Regarding pathogenicity, baculoviruses are not harmful to humans (Berns K & Parrish CR., 2007).

3.5 Inserted Genetic Material - AMT-061 Vector

The Rep sequences used for the production of AMT-061 was cloned from the vector genome of wild type AAV-2. The Cap gene used for the production of AMT-061 was cloned from the vector genome of wild type AAV-5. The three components necessary for production of AMT-061 in insect cells are offered in the form of three recombinant baculoviruses, containing a Rep gene, a Cap gene, and the AAV vector genome (ITRs + transgene expression cassette). These three sequences were each derived (cloned) from wild type (parental) AAV. The DNA sequences were generated *in vitro* using molecular biological techniques. As such, the DNA sequences used to produce AMT-061 are synthetically derived and they do not have a true physical origin in AAV.

The FIX coding sequence is under the control of the LP1 promoter (Nathwani et al., 2006). Between the LP1 promoter and the FIX coding sequence is a SV40 intron. The entire expression cassette is flanked by intact inverted terminal repeats (ITRs) from AAV2 (Samulski et al., 1987).

These sequences, cloned from the viral genome of wild type AAV-2 (Samulski *et al*., 1987), contain self-complementary sequences forming hairpin structures. During production of the GMO, the ITRs are required for packaging of the viral genome into the particles. After transduction of the target cells, the ITRs are required for stabilisation of the viral genome. The ITRs initiate complementation of the (unstable) single stranded genome DNA into (stable) double stranded DNA by host cell polymerases. Alternatively, because the ITRs are palindromic, multiple viral genomes can assemble ITR to ITR to form larger double stranded DNA structures known as concatemers. These concatemers remain transcriptionally active and stable episomal structures (Schnepp *et al*., 2005). The ITRs do not contain any open reading frames for protein expression.

The LP1 enhancer/promoter consists of consecutive segments of the human apolipoprotein hepatic control region (HCR) and the human alpha-1-antitrypsin (hAAT) gene promoter. The enhancer/promoter is followed by a modified SV40 small t antigen intron, to enhance expression (Nathwani *et al*., 2006). The LP1 enhancer/promoter mediates robust and liver-specific expression of the therapeutic transgene (Nathwani *et al*., 2011).

The nucleotide sequence in AMT-061 was codon optimised to enhance FIX protein expression. The codon optimised hFIX-Padua sequence was designed based on a naturally occurring FIX-Padua variant.

Codon optimisation was performed by replacing the codons in the natural FIX-Padua sequence which are less frequently found in highly expressed human genes with the (synonymous) codons that are most frequently found in highly expressed eukaryotic genes, using a previously described algorithm (Haas *et al*., 1996). The optimised sequence was synthesised as oligonucleotides and subsequently assembled by ligation of these oligonucleotides. This strategy of codon optimisation does not affect the amino acid sequence of the protein. The protein translated from codon optimised messenger RNA is therefore the same as the naturally occurring protein. By consequence it does not have any effect on the normal functioning of the protein and the immune response.

Expression of this transgene in liver cells yields functional human clotting FIX-Padua which is secreted into the circulation. Hence, the FIX transgene is the therapeutic payload of AMT-061.

The SV40 polyA sequence serves to stabilise the messenger RNA.

The nucleotide sequences between the abovementioned functional sequences mainly consist of sequences that enable/d molecular engineering of the construct (e.g. multiple cloning sites). The vector genome contains 5 cloning/joining sites. These sites are extremely short in length, at 18, 4, 12, 5, and 23 nucleotides. Because of this, the possibility that coding or functional sequences in the cloning/joining sites can reasonably be excluded.

3.6 Quality testing

Quality control tests are performed to confirm the quality of the final investigational medicinal product. The quality tests relevant for environmental risk analysis are summarised in Table 1. Test parameters were considered to be relevant for environmental risk assessment when they (1) were representative of a GMO other than the intended vector, or (2) were representative of a nucleic acid sequence derived of such a GMO. The tests are discussed in detail below. All other quality testing is performed on test parameters not relevant for environmental risk assessment.

Table 1: Quality tests assessing environmental risk-related parameters

Test	**Method**
Replication competent-AAV (rcAAV)	Bio-assay
Residual infectious baculovirus	Bio-assay
Residual baculovirus DNA	Q-PCR
Rep full-length sequences	Q-PCR

Residual infectious baculovirus and replication competent AAV are the most relevant for environmental risk assessment. These impurities are controlled using the sensitive tests in Table 1.

3.6.1 Absence of residual infectious baculovirus

Substantial amounts of recombinant baculovirus are used during the production process. As baculovirus is an enveloped virus of a much larger size than AAV particles, it is susceptible to inactivation by detergents and can be cleared by virus filtration. Furthermore, many process steps have been designed to maximise baculovirus clearance. In the final product, absence of residual infectious baculovirus is confirmed by bio-assay monitoring utilising baculovirus-permissive insect cells. The assay can detect single infectious particles and the sensitivity is only restricted by the tested volume. The bioassay has a detection limit of 6.8 infectious units (i.e. infectious particles) per mL. No residual infectious baculovirus has ever been detected in any of the batches of AMT-061 that were thus far produced, nor in any of the other recombinant AAV-based product that were thus far produced. These results confirm that the viral clearance capacity of the process suffices for complete clearance of recombinant baculoviruses used in the production of AMT-061.

3.6.2 Residual baculovirus DNA

AMT-061 is also assessed for residual baculovirus DNA by means of a QPCR-based method. The test was qualified for use with a limit of quantitation well below the levels detected in the IMP.

3.6.3 Absence of replication competent-AAV (rcAAV)

The co-existence of Rep and Cap sequences with recombinant vector genome during fermentation could theoretically result in recombination events that yield replication-competent AAV (rcAAV). AMT-061 is routinely assessed for the presence of rcAAV by a sensitive limit test. In this test, vector preparations are plated onto AAV- and adenovirus permissive cells, in the presence of adenovirus. Thus far, no replication-competent AAV has ever been detected in any batch of AMT-061, nor in any of the other recombinant AAV-based product that have thus far been produced. The absence of detectable amounts of replication-competent AAV confirms that recombination of sequences yielding infectious, replication-competent particles is extremely unlikely.

Replication competent AAV (rcAAV) is defined as any AAV which is capable of replicating in the presence of helper virus (as stated elsewhere, AMT-061 is not able to replicate, even in the presence of helper virus). Generation of rcAAV during manufacturing is a theoretical possibility with an extremely low likelihood. AMT-061 is controlled for the presence of rcAAV by bio-assay able to detect 10 rcAAV amidst 2 x 10^{10} genome-containing AMT-061 particles. To date, no rcAAV has been found in any of the batches produced in the baculovirus production system.

3.6.4 Rep full-length sequences

AMT-061 is assessed for full length Rep levels by means of long-amplicon QPCR. The associated drug substance release criteria are a ratio of Rep full length sequences relative to genome copies which is well above the levels detected in AMT-061

DNA impurities

Regarding the potential for interactions between cellular DNA and parental (baculovirus) vectors: high throughput sequencing of the GMO AMT-061 vector preparations have shown that more than 99% of the DNA present in the IMP represents the anticipated vector genome. Small amounts (0.2% of total) of baculovirus derived DNA and traces of insect DNA were detected. These DNA impurities are controlled during quality testing as described above. For previous vectors produced using the baculovirus expression system it was shown that these fragments do not harbour coding sequences.

The traces of insect DNA detected in AMT-061 represented short sequences randomly scattered across the insect genome. These results strongly suggest that the baculovirus and insect derived DNA impurities present in AMT-061 are not the result of recombination events but due to promiscuity of the Rep packaging protein.

Since the Rep and Cap sequences are provided in trans during the AMT-061 production process, theoretically, recombination could occur resulting in replication-competent (albeit still helper-virus dependent) vector particles. Therefore, a quality control assay for the presence of replication-competent AAV particles in AMT-061 preparations is in place. To date, no replication-competent particles have been observed in any of the AMT-061 preparations tested, confirming that the formation of replication competent particles is a hypothetical risk and not a commonly occurring phenomenon.

The activities in the clinical trial relating to the environment potentially receiving the GMO is described below. An assessment of the potential risks posed and containment

4. ENVIRONMENTAL RISK ASSESSMENT

An environmental risk assessment was performed with respect to the potential risks from AMT 060/AMT 061 and is summarized in this section. Baculovirus (used as a system for AAV vector production) is not in scope of this ERA since the manufacturing process provides assurance that the risk from the presence of baculovirus in the final product is negligible.

4.1 Identification of Characteristics which may cause Adverse Effects and Potential Consequences

Characteristics outlined in this section include:

- Host range, tissue specificity, and tropism
- Transmission and replication
- Pathogenicity
- Stability
- Shedding
- Recombination
- Infection
- Immunogenicity
- Thrombogenic potential
- hFIX overexpression

4.1.1 Host range, tissue specificity, and tropism

The host range, tissue specificity, and tropism of AAV particles are determined by the capsid. The capsid of AMT-061 (as with AMT-060) is composed of the exact same proteins that make up wild type AAV-5. The host range and tropism of AMT-061, AMT-060 and wild type AAV-5 are therefore not different.

4.1.2 Transmission and replication

Wild type AAV likely spreads via the respiratory or gastro-intestinal route (Berns K & Parrish C.R., 2007). Infections with wild type AAV occur frequently and are world-wide. For example, up to 59% of the human population is seropositive for AAV-2 (Boutin *et al*., 2010).

Wild type AAV is dependent on a helper virus for replication, e.g. adenovirus or herpes virus. During an active helper virus infection, newly formed wild type AAV particles can be spread together with the helper virus. The spread of wild type AAV therefore will depend on whether a helper virus is present, and whether it is present in the same compartment, i.e. at the site of active infection (Berns, 2005). Pathogenicity

The AMT-061 vector genome is a gutted wild type genome, as it does not contain the Rep and Cap genes. As a result, AMT-061 is replication-defective, even in the presence of a helper virus. AMT-061 is expected to harbour lower pathogenicity as compared to the parental AAV2 and AAV5 viruses that were used for its construction. It should be noted that wild type AAV are considered to be non-pathogenic, and therefore the pathogenic potential

AAV are generally accepted to fall into the Risk Group Category 1 (WHO Laboratory Biosafety Manual, 3rd Ed (2004); NIH Recombinant DNA Guidelines (USA, 2011) Appendix B-I.;

Canadian Laboratory Safety Guidelines (2004) Standard AS/NZS 2243.3:2010; Safety in laboratories Part 3: Microbiological safety and containment standards).

Wild type AAV-2 and AAV-5, elements which form the basis of the GMO, are considered to be non-pathogenic. Infection with wild type AAV is asymptomatic and AAV is not known to cause any noticeable pathology (Berns K & Parrish C.R., 2007). Given that the structure and thus the potential to infect will not have changed, it is expected that the safety profile of the parental wild type AAV and the GMO AMT-061 are similar. In addition, it should be considered that AMT-061 lacks the Rep and Cap genes present in wild type AAV2 or wild type AAV5 (i.e. the parental strains). Due to the lack of these two genes, the vector is replication-defective. Even in the presence of helper virus, the vector genome of AMT-061 will therefore not be replicated, nor will capsids be formed. As such, two processes associated with wild type AAV infection (i.e. replication and capsid formation) will not take place after infection with AMT-061. In a strict sense, the safety profile of AMT-061 is therefore theoretically more favourable as compared to the safety profile of wild type AAV.

Regarding containment: it should be noted that these parental strains are not in fact used to generate the GMO. They are merely the origin, from which molecular components have been isolated and used to ultimately generate the GMO. As actual handling of wild type AAV strains does not take place, further (physical) containment is therefore not applicable.

Regarding attenuation: wild type AAV per se is dependent on the presence of a helper virus (adenovirus or herpes virus) to allow successful replication (Berns K. & Parrish C.R., 2007). Wild type AAV can infect host cells and release its genome to the host cell nucleus. However, in the absence of a helper virus, it remains dormant.

Since infection with wild type AAV is asymptomatic and AAV is not known to cause any noticeable pathology (Berns, 2005), treatment methods against the GMO are therefore not considered relevant. It should be noted that the GMO itself is an attenuated (recombinant) AAV: by design it does not contain any of the AAV genes that are essential for replication of viral DNA, the formation of viral particles, or the packaging of viral DNA into these particles.

4.1.3 Stability

Wild type AAV is a small, non-enveloped virus with a very stable capsid. Exposure to heat, UV radiation, or extreme pH can inactivate (recombinant) AAV (non-published data).

Wild type AAV and recombinant AAV show a similar structure and thus it may be expected that they demonstrate a similar stability, and similar susceptibility to inactivation/degradation under certain environmental or storage conditions. In a natural environment, AAV may also be subject to biological degradation (e.g. by micro-organisms).

4.1.4 Shedding

Shedding in non-clinical studies was assessed using a QPCR based method. Serum, saliva, urine and faeces were collected at several time points after dosing. Shedding of the predecessor product, AMT-060 (which in essence resembles AMT-061) was assessed in cynomolgous macaque. Clearance curves in saliva and urine mainly followed the clearance from the serum with the vector DNA concentrations about 2 – 4 logs lower. Serum cleared between weeks 12 and 26. Saliva was cleared between weeks 8 – 12. Vector DNA levels in urine were low and reached the limit of detection around week 8. The shed material will mainly include DNA fragments tested positive using the QPCR method. A high level of shedding of infectious vector particles has not been observed.

Non-clinical biodistribution and shedding studies with AMT-061 confirmed the earlier observations with the highly similar AMT-060 vector and demonstrated distribution and shedding in plasma urine and tissues. For urine AMT-061 vector DNA was not detectable 3 months after administration of the highest dose of 9×10^{13} gc/kg.

Shedding of AMT-060 was evaluated in clinical Phase I/II studies. Samples of whole blood, saliva, nasal secretions, urine, semen and faeces were tested. Vector DNA disappeared from whole blood at week 27 for 1 patient in cohort 1, however remained to be detectable for all other cohort 1 patients as well as all patients in cohort 2 until last assessment (Cohort 1, 78 weeks; Cohort 2, 52 weeks). Vector DNA disappeared from faeces between week 6-16 in Cohort 1 and week 16-20 in cohort 2 (2 patients still positive at last assessment at week 52), from nasal secretions between week 5-18 in Cohort 1 and week 7-12 in Cohort 2 (2 patients still positive at last assessment at week 52), from saliva between week 6-20 in Cohort 1 and between week 9-16 in Cohort 2 (2 patients still positive at last assessment at week 52), from semen between week 9-48 in Cohort 1 (1 patient still positive at last assessment at week 78) and in week 12-22 in Cohort 2 (3 patients still positive at last assessment at week 52).

In a scenario where infectious AAV is shed into the environment the amount of shed infectious particles is likely to be extremely low. This is based on observations that in most cases only vector DNA material could be observed through PCR, however, infectious particles are restricted to the plasma compartment and cleared from the circulation within 48 to 72 hours after infusion (Favre et al., 2001). In the unlikely event that infectious particles will be shed, these will still be replication-deficient due to the vector design and manufacturing strategy. Shedding of vector material may lead to exposure of third parties which theoretically may result in transmission to these third parties. However, as shedding of infectious vector particles is already considered unlikely the likelihood of transmission to- and infection of- third parties is also considered to be a highly unlikely event.

4.1.5 Recombination

AMT-061 itself is replication deficient. Nonetheless, scenarios in which it would replicate *in vivo*, or even revert to a replication-competent AAV, are theoretically conceivable. In any case, such scenarios would require Rep and Cap sequences to be present in the same cell and hence would require co-infection with wtAAV. The presence of wtAAV would however still not circumvent the dependence on a helper virus infection. Hence, replication or revertance of the GMO would require simultaneous infection of one-and-the-same cell with (1) AMT-061 (2) wtAAV and (3) a helper virus. This scenario of triple infection is in itself unlikely. Nonetheless, the conceivable scenarios upon triple infection are outlined below.

Without any recombination taking place, triple infection will result in replication of the AMT-061 vector genome by the Rep protein provided by wild type AAV. Consequently, the triple-infected cell would in this case produce replication-deficient AAV particles containing the AMT-061 vector genome and wild type AAV particles. In any case, no replication-competent AMT-061 particles would be formed regardless of the presence or absence of helper virus.

Homologous recombination would only involve the ITRs of AMT-061 and wild type AAV, as only these sequences share homology. Essentially, homologous recombination most likely would yield the AMT-061 expression cassette flanked by wild type AAV-derived ITRs, and the wild type AAV-derived genome flanked by AMT-061-derived ITRs. The likelihood of homologous recombination would depend on the degree of homology between the AMT-061-derived ITRs (derived from wtAAV2) and the wild type AAV-derived ITRs, which logically depends on the serotype of the wild type AAV in question. In the case of triple infection, both

recombinants would be subject to intracellular replication by the Rep protein provided by wild type AAV. As a result, the triple-infected cell would in this case produce replication-defective AAV particles containing the AMT-061 vector genome, wild type AAV particles, and AAV particles containing either of the recombined genomes. Also, in this scenario, no replication-competent AMT-061 particles would be formed regardless of the presence or absence of helper virus.

Non-homologous recombination could theoretically produce a hybrid sequence containing wild type AAV-derived Rep and Cap sequences as well as the hFIX expression cassette. The likelihood of such recombination events is intrinsically much smaller than the likelihood of homologous recombination. Depending on its primary structure (i.e. depending on whether it is flanked by ITR sequences or not) such hybrid genomes may or may not be valid substrates for Rep protein. In the case of triple infection, hybrids representing valid Rep substrates would be subject to intracellular replication by the Rep protein provided by wild type AAV. Because of their expected size however, such hybrids cannot be packaged into the wild type AAV capsids. The maximum packaging capacity of AAV capsids is approximately 5kb, and Rep- and Cap- and ITR sequences already make up for 4.7 kb (Daya *et al*., 2008). The triple-infected cell would in this case only produce AAV particles containing the AMT-061 vector genome and wild type AAV particles. No replication-competent AMT-061 particles would be formed regardless of the presence or absence of helper virus.

Most importantly, in any of the scenarios described above, immunological responses would have been mounted against helper virus- and/or wild type AAV-derived proteins. Therefore, adaptive immunity would silence any extracellular manifestation that could result from triple infection, and eliminate triple-infected cells, just as it would in the case of a naturally occurring co-infection with wild type AAV and helper virus. The risk of spread to other cells (which would, also in these cells, require the event of triple infection) is therefore extremely small. It should be noted that cells that are infected with AMT-061 only will not be affected by these immune responses as they do not express AAV-derived proteins. Hence, none of these responses would compromise therapeutic efficacy.

Taken together, the likelihood of recombination is small as it depends on the existence of a particular constellation of events at a brief moment in time. Replication-competent AMT-061 would not be formed in any case. Recombinant replication-deficient particles could theoretically be formed but none of these would have different characteristics as compared to AMT-061 or wild type AAV, respectively. In any case these particles would not have any significant systemic consequences, as they would be neutralised by the immune system as soon as they leave the intracellular compartment. None of these events would affect cells transduced with the therapeutic vector alone. The risk associated with recombination is therefore considered to be negligible.

Even in the very unlikely event of recombination, the products of the outlined recombination events would not increase the likelihood of shedding and transmission based on the shedding and transmission scenarios outlined above. It is therefore concluded that the environmental impact will not be affected by any of the theoretical and unlikely recombination events.

4.1.5.1SV40 recombination

The AMT-061 vector contains an SV40 intron and an SV40 polyA sequence (described in Section 3.5.1). The possible recombination of these viral sequences with wild-type viruses is discussed below.

Recombination of the SV40-derived sequences present in the AMT-061 vector genome with other virus-derived sequences depends on two likelihoods, being (1) the likelihood that homologous sequences are present, and (2) the likelihood that in such case recombination actually occurs (the likelihood of non-homologous recombination was taken to be negligible).

The simian SV40 virus can infect humans, as approximately 1 in every 5 individuals may be seropositive for this virus (Taronna *et al*., 2013; Corallini *et al*., 2012; Mazzoni *et al*., 2014). Infection in any case remains subclinical (Garcea *et al*., 2003). Persistence of SV40 DNA in the normal population is a matter of debate. A review article summarised that half of the available screening studies suggested SV40 is not present in the normal population, while the other half suggested that it is at frequencies of 5 to 25% (Paracchini *et al*. 2006). It is not unthinkable that SV40 sequences are present in patients during AMT-061 administration but the likelihood is low. Using BLAST search engines no human viruses were found that displayed significant homology with the SV40 sequences present in the AMT-061 vector genome [http://blast.ncbi.nlm.nih.gov], suggesting that in humans the presence of virus-derived sequences (SV40 or other) with significant homology is not a likely event.

In the hypothetical case that homologous sequences are present, the likelihood of homologous recombination depends on the extent of homology. In a study on this exact relation, the recombination frequency of SV40 DNA in monkey cells sharply dropped when the length of the homologous sequences was less than 200 base pairs (Rubnitz et al., 1984). The SV40 intron in the AMT-061 vector genome is 94 base pairs in length, the SV40 polyA signal sequence is 133 bp in length. As such, even in the presence of homologous sequences recombination is an unlikely event.

4.1.6 Infection

It should be noted that the GMO AMT-061 itself is an attenuated (recombinant) AAV: genes essential for DNA replication and DNA packaging into an AAV particle have been removed.

Infection with wild type AAV is asymptomatic; AAV is not known to cause any noticeable pathology. Given that the structure and thus the potential to infect will not have changed, it is expected that the safety profile of the parental wild type AAV and the GMO AMT-061 are similar. In addition, it should be considered that AMT-061 lacks the Rep- and Cap genes present in wild type AAV2 or wild type AAV5 (i.e. the parental strains). Due to the lack of these two genes, the vector is replication-defective. Even in the presence of helper virus, the vector genome of AMT-061 will therefore not be replicated, nor will capsids be formed. As such, two processes associated with wild type AAV infection (i.e. replication and capsid formation) will not take place after infection with AMT-061. In a strict sense, the safety profile of AMT-061 is therefore theoretically more favourable as compared to the safety profile of wild type AAV.

Following administration in humans, the GMO AMT-061 is expected to home to the liver, where it will infect and transduce liver tissue. Such GMO administration will elicit an immune response very similar to natural infection with (wild type) AAV.

Following liver transduction, the therapeutic transgene is transcribed and translated, and the human FIX protein is produced by the liver cells. The liver is a natural source of FIX protein, i.e. the transgene is expressed in a natural environment. Local responses to GMO-mediated FIX expression are therefore not expected. The GMO-derived FIX protein is a human protein and immunological responses against this FIX protein are not expected to occur. The codon

optimised Padua-FIX sequence resembles a naturally occurring FIX protein with normal function of the protein although with a higher specific activity.

Altogether as infection with wild type AAV already does not result in any noticeable pathology and FIX is expressed in its natural environment (the liver), in practice, the safety profiles of AMT-061 and the parental strains will be similar, in that neither will mediate any noticeable pathological effects.

4.1.7 Immunogenicity

A potential risk by introducing a Padua-FIX containing a variant sequence distinct from wild type FIX is the onset of an immune response to the neotransgene product. The risk on immunogenicity of Padua-FIX has been investigated in haemophilia B dogs treated by AAV gene therapy (Finn *et al*. 2012). These authors report the absence of formation of inhibitory antibodies to the Padua-FIX protein demonstrated on multiple challenges with wild type FIX protein (even > 1 year after stopping immunosuppression). These observations were supported by the lack of IFN-γ secretion by T-cells after exposure to wild type FIX protein peptides spanning the 338 residue with either the wild type FIX or Padua FIX amino acid sequence. Finn *et al* concluded that no detectable immunogenicity to Padua-FIX could be observed. These conclusions are aligned with the result of in-silico analysis performed by uniQure. The full length wild type human FIX sequence as well as the Padua mutation were evaluated for their immunogenic potential by use of an in-silico platform for epitope identification and prediction (EpiMatrix system developed by Epivax, Inc) for both Class I (all nucleated cells) and Class II (antigen presenting cells) HLA. The accuracy of the EpiMatrix system has been thoroughly documented (Koren *et al*., 2007). The Padua mutation does not result in a significant change in EpiMatrix hits restricted by Class I or Class II HLA, with minimal observed changes in EpiMatrix score. Altogether it is concluded that the immunogenic difference between the wild type FIX and the Padua-FIX is insignificant.

4.1.8 Thrombogenic potential

The only risk associated with the Padua-FIX modification would relate to unintended achievement of supraphysiological levels of circulating FIX activity, either as the result of intended or unintended exposure. It has been reported that only in patients with these supraphysiological levels of Padua-FIX (>700% of normal) thrombosis may be observed (Simioni *et al*., 2009).

For the case of intended exposure, i.e. in patients, the scenario of reaching extreme levels of circulating FIX activity is unlikely given that Padua FIX will display at most tenfold increased specific activity as compared to wild type FIX, and FIX protein levels will be similar for AMT-061 and AMT-060. It is therefore concluded that the risk of thrombosis following supraphysiological Padua-FIX levels is negligible.

4.1.9 hFIX overexpression

The intended purpose of the GMO AMT-061 is to transduce hepatocytes of haemophilia B patients with a gene encoding a normal human clotting FIX.

The two effects of intended exposure are (1) expression of normal human clotting FIX (i.e. the normal human condition), and (2) occurrence of an immune response to AAV5-hFIX capsid proteins (not to the expressed transgene). The immune response against AAV5 capsid proteins will be asymptomatic, in the same way that a naturally occurring infection with AAV elicits an asymptomatic immune response.

Unintended exposure of humans to the GMO AMT-061 will have the same two effects as intended exposure. The chance that these effects result in any adverse effects or even noticeable effects is however negligible.

Overexpression of (h)FIX in third parties, or in patients, has never been described. As such, the consequences of overexpression are not known. Slight overexpression may be considered normal, as in healthy individuals hFIX levels can range from 50% to 200% of the (normal) reference value of 1 international unit/mL (Khachidze *et al*., 2006). As a transgene for gene therapy, FIX has a broad therapeutic window. In haemophilia patients, levels as low as 2% are expected to result in therapeutic benefit. Only extreme overexpression is associated with risk of thrombosis. Subjects will however be required to use a condom during sexual intercourse in the period from IMP administration until the AAV5 has been cleared from their semen, as evidenced from negative results for at least three consecutive semen samples (this criterion will also be applicable for subjects who are surgically sterilised); this will be one of the inclusion criteria for study participation. Extreme overexpression of hFIX after FIX gene transfer has been established in the Sponsors pivotal safety study in mice where infusion of 2.3×10^{14} gc/kg (more than 10 times the high dose of the clinical Phase I/II study with AMT-060) resulted in a 70-fold overexpression, i.e. 70 times the level found in the normal human population. No adverse effects were associated with this immense overexpression. The absence of adverse events was not due to impaired or lacking functionality of hFIX, as hFIX expressed in mice displays normal functionality and was shown to revert the clotting deficiency in FIX-deficient mice FIX (Nathwani *et al*., 2006). These preclinical results suggest that overexpression of hFIX is not necessarily associated with adverse effects.

In any case, the likelihood of overexpression of hFIX in any human, resulting from unintentional exposure or intentional exposure, is extremely small. This is in part because the target hFIX level in gene therapy for haemophilia B is very low. Establishment of more than 1% of normal levels is already expected to revert a severe phenotype into a moderate phenotype, and establishment of more than 5% is expected to revert a moderate phenotype into a mild phenotype.

Indeed, in the recent clinical study on the AAV8-based vector that carried the hFIX expression cassette, transgenic FIX levels reached 1% to 12% of normal in all treated patients and these levels mediated significant clinical benefit (Nathwani *et al*., 2011). This was also confirmed in the Sponsors Phase I/II study for AMT-060, circulating FIX activity levels reached up to 12% of normal human levels, demonstrating that at the intended doses the scenario of achieving extreme overexpression was not realistic. In non-human primates, infusion of AMT-060 at the intended clinical dose resulted in 1% to 10% of normal human levels. This intended clinical dose corresponds to approximately 25 to 100mL of vector preparation per 50kg body weight, infused intravenously in order to reach the liver.

Other than potency, all quality attributes of AMT-060 and AMT-061 are expected to be similar, and AMT-061 is expected to mediate identical FIX protein expression levels as compared to AMT-060. The modification is therefore expected to return the same the safety profile as AMT-060. The only risk associated with the Padua-FIX modification would relate to unintended achievement of supraphysiological levels of circulating FIX activity, either as the result of intended or unintended exposure. For the case of intended exposure, i.e. in patients, the scenario of reaching extreme levels of circulating FIX activity is unlikely given that Padua FIX will display at most tenfold increased specific activity as compared to wild type FIX, and FIX protein levels will be similar for AMT-061 and AMT-060.

The probability of unintended exposure to significant amounts of AMT-061 in such a way that the vector will be able to transduce hepatocytes and mediate detectable hFIX expression is extremely low. It would entail unintended intravenous infusion of 25 mL vector preparation or more. In addition, the probability that such unintended exposure would result in overexpression of hFIX expression levels is extremely low, as the target levels for intended exposure are close to 5% of normal. Finally, the probability that overexpression of hFIX would have any clinical consequence for a third party is low, as already in the normal population there is considerable 'over' expression in otherwise healthy individuals, and non-clinical studies suggest that even extreme overexpression holds negligible biological consequence. The overall risk that overexpression of hFIX in third parties due to unintentional exposure will result in observable effects is therefore negligible.

4.2 Effects of Exposure of the Environment to AMT-061

AAV infection is naturally restricted to primates. Exposure of the environment to the replication- deficient AMT-061 is therefore not considered to have any potentially harmful effects.

Both humans and primates may show pre-existing (neutralising) antibody titres against AAV. Consequently, the likelihood and effect of shedding will be reduced. Infections with AAV occur frequently and are world-wide. The prevalence of neutralising antibodies against AAV5 is found in the adult European population to be 3.2% (Boutin *et al*., 2010; Calcedo *et al*., 2009). Also, AAV infections being non-pathogenic (not associated with disease manifestations) reduce the likelihood of a negative effect following infection.

4.3 Scenario for Environmental Exposure to the GMO

Overall the exposure of the environment to AMT-061 is considered limited. The most likely groups being exposed to the GMO are:

- Healthcare professionals involved in preparation and administration of the GMO and obtaining clinical samples.
- Laboratory professionals involved in sample preparation and analysis.
- Close contacts of the patients who potentially may be exposed to shed vector.

The likelihood for exposure of these groups will be different and depending on different scenarios and is described further in the sections below.

Spread of infectious GMO into the environment through nasal secretions, saliva, urine or faeces is considered negligible. Spread of infectious GMO through blood is conceivable, as blood samples are drawn during the first 3 days after administration of the GMO. Blood samples will nevertheless be assessed for determination of vector DNA levels. However, following the worst-case scenarios described in the section: Exposure at the administration site, even the risk that AMT-061 will spread into the environment through blood and thus lead to a GMO related risk is considered negligible.

It is not known whether recombinant AAV shed in semen is infectious or, like AAV shed in the other fluids, represents non-infectious vector DNA. In either case, the risk of horizontal or vertical transmission cannot be excluded. Although it is not anticipated that this will relate to a risk it is considered an unwanted effect as such any potential risk is addressed by requiring the use of a condom during the trial in the period from administration of the AMT-061 until the AAV5 vector has been cleared from semen, as evidenced by negative analysis results for AAV5 vector for at least three consecutively collected semen samples.

Assessment of vector DNA in semen will be performed using a validated method. The likelihood that the GMO will spread through semen is low and controlled.

Exposure at the administration site

Due to the well-controlled administration conditions the potential contact of the product with healthcare professionals at the administration site environment is extremely limited. The only possibilities for a contact of the administration site environment with the product would be accidental spillage of the product solution to surfaces; accidental self-administration (needle-stick injury), (mucous) skin contact with the product or inhalation of aerosolised product by the administering health care professional. As the vector is replication deficient, the only effect could be a marginal increase in FIX levels if any and an immune response to the AAV5 capsid.

Laboratory professionals working with clinical samples may also be exposed to the GMO. However, analysis of patient samples is not within the scope of the current application as these activities will be performed within laboratories which are covered through different licenses.

Level of risk that spread will actually occur

As outlined above, shed vector DNA does not equal infectious vector particles. It has been described that infectious particles can only be found in blood during the first 3 days after vector infusion (Favre *et al*., 2001). After this period all infectious particles are likely to have infected test subject cells or to have been rendered non-infectious through other mechanisms (e.g. degradation by test subject effector mechanisms). The level of risk that spread will actually occur is therefore dependent of the scenario, i.e. fluid or excrement type.

Due to the above, spread of infectious GMO into the environment through nasal secretions, saliva, urine or faeces is considered negligible. Spread of infectious GMO through blood is conceivable as blood samples are drawn during the first 3 days after administration of the GMO. However, following the worst-case scenarios described above, even through blood, the risk that AMT-061 will spread into the environment and lead to a GMO related risk is considered to be negligible.

It is not known whether recombinant AAV shed in semen is infectious or, like AAV shed in the other fluids, represents non-infectious vector DNA. In either case, the risk of horizontal or vertical transmission cannot be excluded. Although it is not anticipated that this will relate to a risk it is considered an unwanted effect as such any potential risk is addressed by requiring the use of a condom during the trial in the period from administration of the AMT-061 until the AAV5 vector has been cleared from semen, as evidenced by negative analysis results for AAV5 vector for at least three consecutively collected semen samples.

Assessment of vector DNA in semen will be performed using a validated method. The likelihood that the GMO AMT-061 will spread through semen is therefore considered to be low and controlled.

Influence of the number of test subjects and/or the dosage to be administered on the risks

Due to the negligible risk even when the highly unlikely spreading and transmission to third parties would occur the number of test subjects nor the anticipated doses, are expected to

influence the conclusion on the negligibility of the environmental risk of gene therapy using AMT-061.

Risk likelihood consideration

As outlined above, shedding of AMT-061 derived DNA is expected but shedding of infectious vector particles is considered highly unlikely. Shedding studies using applicable dose levels demonstrate that the amount of shed vector DNA represents a minute fraction of the dose and corresponds to an even lower amount of infectious vector, if at all.

As a result, the absolute amount of infectious particles that could practically spread and thus be transmitted is negligible. The chance that the unintended exposure and potential adverse effects as described in Table 2 could actually occur is therefore considered to be negligible as well.

Risk estimation

There are no significant risks associated with the application of the vector.

The overall risk of the GMO has been evaluated by summing the potential risks and consequences with respect to the likelihood, as detailed in Table 2.

Table 2: Estimation of the risk posed by each identified characteristics of the GMO

Adverse effect	Type of exposure*	Magnitude	Likelihood	Risk
Toxic effects to humans	self-inoculation	negligible	low	negligible
	exposure	negligible	negligible	negligible
Pathogenicity to humans	self-inoculation	negligible	low	negligible
	exposure	negligible	negligible	negligible
Immunogenicity to Padua FIX	self-inoculation	negligible	negligible	negligible
	exposure	negligible	negligible	negligible
Tumorigenicity to humans	self-inoculation	negligible	low	negligible
	exposure	negligible	negligible	negligible
Thrombosis following supraphysiological Padua FIX activity	self-inoculation	negligible	low	negligible
	exposure	negligible	negligible	negligible
Germ-line transmission	self-inoculation	low	negligible	negligible
	exposure	negligible	negligible	negligible
Genome integration in humans	self-inoculation	low	negligible	negligible
	exposure	negligible	negligible	negligible
Disease or any other adverse effect to animals or plants	exposure	negligible	negligible	negligible
Population dynamics and genetic diversity of populations	exposure	negligible	negligible	negligible
Facilitating the dissemination of infectious diseases	exposure	negligible	negligible	negligible
Compromising prophylactic or therapeutic treatment	exposure	negligible	negligible	negligible
Disturbance of environmental biogeochemistry	exposure	negligible	negligible	negligible

* Accidental self-inoculation by a healthcare professional, exposure = due to incidental spillage or shedding.

The overall risk of AMT-061 to people and the environment can be considered negligible.

Therefore, no specific risk management measures are deemed necessary. Nevertheless, standard biosafety measures (outlined below) are implemented which relate to general hospital procedures which by no means relate to any risk management to reduce the already negligible risk related to the gene therapy with AMT-061.

4.4 Risk Management

Measures in place to minimise the impact on the environment are addressed. The following activities with AMT-061 are within the scope of the GMO application:

- Receipt and storage
- IMP preparation
- Transportation within the hospital

- Administration and observation of patients
- Patient sampling, sample processing, analysis, and storage
- Waste management and disposal of unused materials

The scope of the application excludes production of AMT-061, transport of AMT-061 to and from the hospital, and transport of biological samples from the hospital to a central laboratory since these activities are covered through different licenses. The activities that will be carried out with AMT-061 are described below.

4.4.1 GMO receipt and storage

The GMO, AMT-061, is manufactured by uniQure Inc. in compliance with current Good Manufacturing Practices. Manufacturing facilities are located in Lexington USA. The manufacturing is not within the scope of the GMO application. The hospital pharmacy, or equivalent, will receive the product and store in accordance with GMO legislation and/or local hospital procedures. Storage will be in a facility with restricted access using equipment that is labeled in accordance with GMO legislation and/or local hospital procedures.

4.4.2 GMO dose preparation

In the hospital pharmacy, or equivalent, the AMT-061 will be pulled from the supplied glass vials into an infusion bag in a Class II Biological Safety Cabinet.

Preparation in hospital pharmacy or equivalent

The standard precautions that are applied in the hospital pharmacy, or equivalent setting, to control exposure to hazardous substances such as cytotoxic agents are appropriate and will be applied during the preparation of AMT-061. Therefore, no additional measures have been taken. In the hospital pharmacy, the AMT-061 infusion bag will be prepared in a Class II Biological Safety Cabinet. During the preparation of AMT-061 personnel will wear protective clothing and gloves.

4.4.3 Transportation within the hospital

The AMT-061 infusion bag will be transported to the treatment room in accordance with GMO requirements and/or local hospital procedures.

4.4.4 Administration in the clinic

The standard precautions that are applied to mitigate spillage and/ or aerosol formation of hazardous substances, such as cytotoxic agents, are appropriate and will be applied during the preparation and administration of AMT-061. Therefore, no additional measures have been taken. Accidental exposure and/or spilling of AMT-061 are mitigated by the following standard precautions:

- Personnel administering AMT-061 will wear protective clothing and additional protective measures like safety glasses, gloves and mouth-nose mask.
- The hospital pharmacy will provide the infusion bag connected to pre-filled tubing with sterile diluent. Should spillage and/or aerosol formation occur, this will be only 0.9% sodium chloride diluent.
- The pre-filled tubing of the infusion bag will be connected to the main infusion tubing, which has been primed with sterile 0.9% sodium chloride. When removing the intravenous catheter after completion of the infusion, there is an increased risk of

spillage and/or aerosol formation. This risk is mitigated by flushing the infusion tubing with 0.9% sodium chloride, before removing the intravenous catheter.

- The catheter, tubing, infusion bag and other ancillary items used are all disposables and are disposed of as biohazard waste in accordance with local hospital procedures and biohazard standards. Non-disposable materials (tools, devices) are cleaned with a disinfectant with virucidal activity, e.g. a chlorine releasing disinfectant like hypochlorite containing 0.1% available chlorine (1000 ppm) after usage and then autoclaved, if possible. Contact surfaces are disinfected with a similar disinfectant.

These standard precautions will be described in the Investigational Medicinal Product handling manual which will be provided to all clinical staff involved in the preparation and administration of the product.

Furthermore, after administration the treatment room will be decontaminated with a disinfectant. A 250-ppm chlorine solution will be used for regular disinfection on used surfaces. In case of a spill the surface will be treated with 1000 ppm chlorine solution.

4.4.5 Observation of patients

Observation of patients will be by means of post-administration surveillance for up to 24 hours. Note that the post administration surveillance is not related to any anticipated environmental risk. Subjects will be followed with respect to safety and efficacy parameters for at least 5 years.

4.4.6 Sampling and analysis

Sampling and transportation as well as processing and/or storage of these samples within the hospital will be done per GMO legislation and/or local hospital procedures. For all human samples routine precautions will be taken, designed to contain pathogens in blood and to protect the hospital staff from pathogens in blood. These precautions include the use of gloves and a laboratory coat, gown or uniform. After sampling the waste will be processed as hospital specific waste.

Samples that are not analysed at the hospital will be transported to the central laboratory per the GMO requirements.

4.4.7 Waste treatment and disposal of unused material

The nature of the waste generated includes (partially) used vials, ancillary components used for preparation and administration (e.g. tubing, needles, syringes, infusion bag, personal protective equipment, etc.) and components used for collecting body fluids samples after administration.

Following handling and administration, the unused AMT-061 remaining in the opened vials and all the materials that have been in contact with AMT-061 (such as gloves, syringes, needles, tubing and infusion bag) will be disposed per GMO legislation and/or local hospital procedures.

Unused (non-opened) vials of AMT-061 will be returned to the Sponsor or disposed as per study procedures, in accordance with GMO legislation and/ or local hospital procedures.

Following handling and administration of AMT-061 the unused product remaining in the vials, and ancillary materials, will be disposed of per GMO legislation and/or local hospital procedures.

4.4.8 Safety measures - Spillage

The spill procedures for handling AMT-061 are similar to those already in place for handling hazardous substances such as cytotoxics. A spill kit will be provided to the clinical sites and pharmacy. The spill kit should be available during all the steps with AMT-061, but minimally during the preparation and administration of AMT-061. The spill kit will contain at least:

- Disinfectant sachets
- Absorbent paper towels
- Disposal forceps
- Biohazard incineration bags
- Emergency contact number
- Copy of the spillage procedure

Connecting the infusion bag to the main infusion tubing in the clinic, creates an increased likelihood of spillage and/or aerosol formation. For AMT-061 administration, the possibility of spillage and /or aerosol formation is reduced as the hospital pharmacy, or equivalent, will prepare and provide the infusion bag connected to a pre-filled tubing with an appropriate, suitable and sterile diluent (e.g. WFI or sterile saline solution). In the exceptional case, should spillage and/or aerosol formation occur, this will only be the diluent.

The spill procedure will be addressed in the IMP handling manual.

4.4.9 Accidental exposure to AMT-061

Accidental exposure of health care professionals to AMT-061 should be treated according to the measures listed in Table 3. These are standard measures for which it should be indicated that they are not needed to reduce the already negligible risk.

Table 3: Measures for occupational exposure with AMT-061

Type of exposure	Measure
Needle stick	Encourage bleeding of the wound. Wash injection area well with soap and water. Obtain medical attention.
Eye contact	Immediately flush eyes with water for at least 15 minutes. Assure adequate flushing by separating the eyelids with fingers. Obtain medical attention.
Inhalation	When inhaled, move person into fresh air. Obtain medical attention.
Ingestion	Rinse mouth with water. Obtain medical attention.
Skin contact	Wash-off with a gauze soaked in a virucidal disinfectant* (not alcohol solution) and subsequently wash with soap and plenty of water. Obtain medical attention.

Inclusion and exclusion criteria and environmental safety

From the risk assessment it is concluded that there is negligible risk related to the AMT-061 gene therapy. As such no inclusion or exclusion criteria are applied that relate to the safety of third parties or the environment at large.

After administration patient contact

As AMT-061 is not infectious after being shed the risk to the environment is considered negligible. Standard hospital hygiene measures are sufficient and no additional measures will be taken for the period after IMP administration.

Procedures requiring changes in the risk management

Since the environmental risk is considered negligible patients will not be kept in isolation at any point in time. Therefore, no additional measures will be required in hypothetical situations where medical care may require interventions or treatments in another physical location.

Aftercare when test subject prematurely ends participation in the study

The investigator will make all reasonable attempts to retain the test subjects in the trial to allow long-term follow-up on patient safety. The test subject will continue to receive the standard of care, which will not be affected by withdrawal from the trial. As AMT-061 is not infectious after being shed and therefore the risk to the environment is considered negligible, no additional measures will be taken for the period after administration.

4.5 Conclusions of the Possible Environmental Effects

4.5.1 Likelihood of the GMO to become persistent and invasive

The GMO could spread from the recipient into natural habitats via blood, urine, faeces, saliva, nasal secretions and semen.

As also noted previously, AAV-based GMO found in body fluids is not infectious (Favre *et al*., 2001), except for the blood compartment where infectious AAV-based GMO was found to be infectious up to 3 days after administration. Therefore, persistence through infection is unlikely when the GMO spreads into natural habitats via body fluids since true shedding will only occur through body fluids such as urine, faeces, and saliva.

Persistence through replication and/or integration is dependent on infection of a (natural) host. As stated above infection of a host following spread via body fluids of the recipient is unlikely. In addition, the GMO has been rendered replication defective, by removal of Rep and Cap sequence from the genome. Integration occurs at a low frequency and at random even when administered IV at a high dose (Paneda *et al*., 2013).

Taken together the likelihood of persistence of the GMO into natural habitats is considered extremely unlikely.

4.5.2 Advantages or disadvantages conferred to the GMO

No selective advantage has been conferred to the GMO. In contrast, the GMO has been rendered replication-defective through removal of Rep and Cap sequences. Therefore, a selective disadvantage has been conferred to the GMO (vs for example wild type AAV), which is likely to be realised under the conditions of the proposed release (by IV injection). In addition, spread of infectious GMO following release is limited by the fact that the GMO shows poor potential for infection once shed via body fluids, as shed material will predominantly contain only DNA fragments of the GMO and is unlikely to contain infectious particles.

4.5.3 Potential for gene transfer to other species

The likelihood of gene transfer to species other than humans and (some) primates is low, given the host preference of AAV.

As far as (unintentional) gene transfer to humans and primates is concerned, the likelihood is low given the selective disadvantage conferred to the GMO under conditions of proposed release (as explained above).

4.5.4 Immediate and/or delayed environmental impact

No immediate and/or delayed environmental impact is expected, again given the selective disadvantage conferred to the GMO under conditions of proposed release (as explained above).

4.5.5 Immediate and/or delayed effects on human health

Under conditions of release, persons working with the GMO could potentially come into contact with the GMO prior to or during administration, when the GMO is handled as distributed, at high concentration and fully functional.

Standard procedures to mitigate spillage and/or aerosol formation will be applied. As explained above, blood samples taken shortly after administration of the GMO should be regarded as containing active (infectious) GMO.

Should persons working with the GMO come into direct contact with the GMO (through inhalation or accidental injection during administration, or via blood samples taken shortly after administration), no immediate and/or delayed effects different from those expected for the recipients (test subjects) are expected: a (dose-dependent) immune response to the GMO could occur that will not affect the subject's general well-being.

It should be noted that humans are natural hosts for AAV, infections are asymptomatic and AAV is not known to cause any noticeable pathology. Similarly, dose-dependent administration of AAV/based GMO´s to humans has been shown to be safe. As noted above, a dose-dependent immune response does occur in a recipient and is without clinical consequence.

Overall, no immediate and/or delayed effects on human health are expected for persons working with the GMO or coming into contact with or in the vicinity of the GMO as it is released.

4.5.6 Immediate and/or delayed effects on animal health

As explained above, under conditions of release, shedding into the environment can occur via blood, urine, faeces, saliva and semen i.e. all body fluids. However, concentrations of the GMO in body fluids is low and shed GMO is not infectious. The exception is blood, up to 3 days after release.

The greatest risk for shedding into the environment lies with urine, faeces, and saliva. As noted previously, GMO present within these body fluids is non-infectious. Therefore, should the GMO be consumed following shedding, a low amount of a non-infectious GMO is consumed. Therefore, no immediate and or delayed effects on animal health or consequences for the feed or food chain are expected.

4.5.7 Change in the current medical practice

AMT-061 has been designed to deliver the human coagulation FIX gene to the liver of patients suffering from haemophilia B, enabling the restoration of stable expression of coagulation FIX thus ameliorating the bleeding phenotype and improving the quality of life of these patients.

5. MONITORING PLAN TO IDENTIFY THE EFFECTS ON HUMAN HEALTH AND THE ENVIRONMENT

There is no monitoring plan to identify the effects on human health and the environment. Due to the negligible risk presented by AMT-061 to the environment, as described in the sections above, no further monitoring is considered necessary.

6. DESCRIPTION OF THE INTENDED MONITORING MEASURES AND PARTICULARS OF ANY RESIDUAL SUBSTANCE AND THE TREATMENT THEREOF AND ALSO ANY EMERGENCY RESPONSE PLANS

Due to the negligible risk presented by AMT-061 to the environment no further monitoring is considered necessary.

In case of spillage the affected area, lineated with absorbing material, will be decontaminated using appropriate disinfectants with virucidal activity. A spill kit will be available at all times during the administration procedure. Details will be provided in the IMP Handling Manual. Should persons working with the GMO come into direct contact with the GMO (through inhalation or accidental injection during administration, or via blood samples taken shortly after administration), no immediate and/or delayed effects different from those expected for the recipients (test subjects) are expected: a (dose-dependent) immune response to the GMO could occur that will not affect subjects' general well-being. For splashes to the eye of the GMO, the eye will be rinsed with eyewash for 15 minutes and the patient will then report to the hospital emergency room for evaluation. In case of accidental injection of material containing the GMO, bleeding of the wound will be encouraged, the area will be washed well with soap and water and the patient will report to the hospital emergency room for evaluation.

7. REFERENCES

Regulatory Guidance

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Safety in laboratories (Part 3) Microbiological safety and containment. Standards

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