# COMMON APPLICATION FORM FOR INVESTIGATIONAL MEDICINAL PRODUCTS FOR HUMAN USE THAT CONTAIN OR CONSIST OF AAV VECTORS

**This application form implements the requirements of the Directive 2009/41/EC and of the Directive 2001/18/EC, as adapted to the specific characteristics of adeno-associated viral vectors ("AAVs") contained in investigational medicinal products for human use.**

**Investigational Product:** SERPINA1(human)-AAV8-1

**Sponsor:**
Intellia Therapeutics, Inc.
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# LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

| Abbreviation | Definition |
|---|---|
| AAT | alpha-1 antitrypsin |
| A1AT | alpha-1 antitrypsin |
| AATD | alpha-1 antitrypsin deficiency |
| AAV | adeno-associated virus |
| AAV8 | adeno-associated virus serotype 8 |
| BSE | bovine spongiform encephalopathy |
| Cas9 | clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein 9 |
| CRISPR | clustered regularly interspaced short palindromic repeats |
| cGMP | current Good Manufacturing Practice |
| DNA | deoxyribonucleic acid |
| DP | drug product |
| DS | drug substance |
| EMA | European Medicines Agency |
| FDA | Food and Drug Administration (US) |
| FIH | first-in-human |
| GTMP | gene therapy medicinal product |
| hA1AT | human alpha-1 antitrypsin |
| HEK | human embryonic kidney |
| ITRs | inverted terminal repeats |
| IV | intravenous |
| LNP | lipid nanoparticle |
| LNP1265 | an LNP encapsulating the 2 active CRISPR/Cas9 components targeting the insertion point in the human albumin gene |
| LTFU | long-term follow-up |
| MCB | master cell bank |
| mRNA | messenger RNA |
| NGS | next generation sequencing |
| NIH | National Institutes of Health |
| NHP | nonhuman primates |
| PAM | protospacer adjacent motif |
| PI | principal investigator |
| PiZZ | *SERPINA1* ZZ genotype |
| PK | pharmacokinetic(s) |
| qPCR | quantitative polymerase chain reaction |
| rAAV | recombinant adeno-associated virus |
| RG1 | Risk Group 1 |
| RNA | ribonucleic acid |
| SCR | Screening |
| sgRNA | single-guide RNA |
| SoA | Schedule of Activities |
| TSE | transmissible spongiform encephalopathies |

# 1 SECTION 1 – ADMINISTRATIVE INFORMATION

## Identification of the applicant

| | |
|---|---|
| Organisation Name: | Medpace UK Ltd |
| Address Details: | Vintners Place, 68 Upper Thames Street, London, EC4V 3BJ |

## Identification of the sponsor (to the extent that is different from the applicant)

| | |
|---|---|
| Organisation Name: | Intellia Therapeutics, Inc. |
| Address Details: | 40 Erie Street, Cambridge, MA 02139, USA |

## Identification of the manufacturer of the clinical vector

| | |
|---|---|
| Organisation Name: | FUJIFILM Diosynth Biotechnologies Texas, LLC |
| Manufacturing location: | 3939 Fujifilm Way<br>College Station, Texas (TX) 77845, United States (USA) |

**CONFIDENTIAL INFORMATION IS PRESENTED IN:**

- **ANNEX 1 ADMINISTRATIVE INFORMATION**

# 2 SECTION 2 – INFORMATION RELATING TO THE INVESTIGATIONAL MEDICINAL PRODUCT

The Sponsor is developing an *in vivo* CRISPR/Cas9-based genome editing investigational therapeutic, **NTLA-3001**, for the treatment of adults with Alpha-1 Antitrypsin Deficiency (AATD)-associated lung disease.

NTLA-3001 is a one-time administered investigational transgene insertion product for the treatment of AATD-associated lung disease. It is composed of 2 drug products (DPs), which are intended to be sequentially administered. **SERPINA1(human)-AAV8-1** (referred to as AAV8-1) is a liquid suspension of recombinant, non-replicating adeno-associated virus serotype 8 (rAAV8) vector that displays liver tropism. It is packaged with a DNA template comprising a promoterless *SERPINA1* gene cassette with inverted terminal repeats (ITRs) and splice acceptor site. Given that AAV8-1 is a bioengineered viral vector containing recombinant nucleic acids intended to incorporate into the host genome, this component of the therapeutic medicinal product does fall within the scope of Deliberate Release Directive 2009/41/EC and Contained Use Directive 2001/18/EC and is the focus of this application.

AAV8-1 is one of two components of the therapeutic investigational gene therapy medicinal product (GTMP) called NTLA-3001; the other component of the GTMP is LNP1265, which is a liquid suspension (dispersion) of lipid nanoparticles (LNP1265) containing a single guide RNA (sgRNA), and a messenger RNA (mRNA) encoding Cas9 protein. The single guide RNA is chemically synthesized and the mRNA is synthesized through an in vitro transcription reaction. As LNP1265 is not a living organism, this portion of the therapeutic medicinal product is outside the scope of the Deliberate Release Directive 2009/41/EC and Contained Use Directive 2001/18/EC. Therefore, this application focusses on SERPINA1(human)-AAV8-1 only.

AAV8-1 and LNP1265 are formulated independently and administered to the patient systemically and sequentially as consecutive intravenous (IV) infusions.

The Sponsor confirms:

- The absence of replication competent virus in the final DP,

AND

- The transgene is not expected to be harmful.

## 2.1 Description of the production system

### 2.1.1 Introduction

**Indication**

Alpha-1 Antitrypsin Deficiency (AATD) is a rare, debilitating, and potentially fatal inherited autosomal co-dominant disorder arising from mutations in the *SERPINA1* gene, which encodes the alpha-1 antitrypsin (A1AT) protein, a protease inhibitor of the proteolytic enzyme elastase. AATD is primarily characterized by progressive lung disease in adults, as well as liver disease in a smaller subset of pediatric and adult patients.

**Adeno-associated virus**

Adeno-associated virus (AAV) belongs to the Parvoviridae family and is a small, non-pathogenic virus with an average diameter of 20 - 25 nm. The genome of wild-type AAV is ~4.7 kb long flanked by two T-shaped inverted terminal repeats (ITRs) that largely serve as the viral origins of replication and the packaging signal. In nature, the virus is replication defective and is dependent on the presence of helper virus replication elements to complete its replication cycle. At least 12 natural serotypes of this virus have been isolated and characterized to date. These serotypes differ in their tropism (the types of cells they infect), making AAV a very useful system for preferentially transducing specific cell types. The ability of AAV to package and express transgenes in a variety of tissue types makes this virus a powerful tool for therapeutic gene delivery.

**Investigational therapeutic**

In contrast to conventional recombinant AAV gene therapies, NTLA-3001 harnesses a CRISPR-mediated double-stranded DNA break to facilitate insertion of the transgene into a safe harbour locus to leverage an endogenous genomic promoter for transcription and protein expression, mitigating potential loss of transgene expression in dividing cells and obviating the need for an exogenous promoter to stably express human alpha-1 antitrypsin (hA1AT) protein.

Expression of the *SERPINA1* gene in hepatocytes results in synthesis of the human A1AT (hA1AT) protein to remediate lung diseases caused by a lack of functional A1AT in patients.

### 2.1.2 AAV8-1 Production and Cell Line

**SERPINA1(human)-AAV8-1** is a purified recombinant AAV8 (rAAV8) vector, manufactured using transient transfection of human embryonic kidney (HEK) 293 cells with three plasmids:

- a gene of interest plasmid - contains the *SERPINA1* DNA template;
- a rep/cap plasmid - includes the rep gene and cap genes that are necessary for assembly of the protein capsid, processing the DNA encoding the AAV transgenic genome into single-stranded DNA, and packaging this ssDNA transgenic genome into the capsid; and
- a helper plasmid - encodes the necessary adenoviral proteins for the AAV producer cells to successfully express rep and cap proteins from the AAV rep/cap plasmid.

Information about the three plasmids, including maps of the vectors used, are provided in **confidential** Annex 2.1 as this is confidential information which is not in the public domain or publicly available, and disclosure may undermine the legitimate economic interest of the Sponsor.

**CONFIDENTIAL INFORMATION IS PRESENTED IN:**

- **ANNEX 2.1 PLASMID MANUFACTURING**
- **SERPINA1 GOI PLASMID**
- **REPCAP PLASMID**
- **HELPER PLASMID**

### 2.1.3 HEK293 cell line and master cell bank (MCB)

AAV8-1 is produced in an HEK293 host cell line which has been adapted for growth in serum-free suspension culture.

The HEK293 cell line was derived from human embryonic kidney (HEK) cells in 1973 and derivatives of this cell line are broadly used in biomedical research for viral packaging and biopharmaceutical production. HEK293 was developed as a single clone cell line after being transformed with sheared Adenovirus 5 DNA in 1973. Characterization of the cell line identified a 4-kbp adenoviral genome fragment integrated in chromosome 19 which encodes for the E1A/E1B proteins. These proteins, required for AAV vector production, are known to interfere with the cell cycle control pathways and counteract apoptosis. The HEK293 cells were gradually adapted into suspension growth in chemically defined, serum-free medium formulations and were single-cell cloned prior to establishing a current Good Manufacturing Practice (cGMP) master cell bank for virus production.

The AAV8-1 MCB was tested for various viruses according to the ICH Guideline on viral safety evaluation of biotechnology products derived from cell lines of human or animal origin (ICH Q5A). There are no known viruses, virus-like particles, or animal-derived components present in the cell line or materials for AAV8-1 manufacturing. The manufacturing process includes virus inactivation/removal steps, in addition, no adventitious agents were detected during routine testing in manufacturing campaigns. Thus, contamination of the producer cell line with viruses that could serve as a helper virus for AAV8-1 or introduction of any viruses during manufacturing that could potentially recombine with the vector genome or complement the function of deleted viral genes is highly unlikely.

### 2.1.4 Testing of AAV8-1 Lots

The control of contaminants begins at incoming raw materials for the production of AAV8-1 DS. All materials of biological origin are controlled for adventitious agent contamination, certified by the vendor to be transmissible spongiform encephalopathies / bovine spongiform encephalopathy (TSE/BSE) free, and all buffer components are filtered (0.22 μm) prior to addition into the product stream. All product contact equipment is single use.

Comprehensive release testing panels for the plasmids, drug substance, and drug product were developed to ensure the identity, strength, quality, and purity of the product and to control its physical, chemical, and biological characteristics. Additionally, a packaged single-stranded vector genome isolated from a batch of AAV8-1 DS was analyzed with long-read Next Generation Sequencing (NGS) to provide characterization of the AAV8-1 genome population.

## 2.2 Demonstration of absence of formation of replication-competent virus

The AAV8-1 vector genome is a single stranded DNA with inverted terminal repeats (ITR) at each end. The non-coding ITRs flank the two copies of the CpG minimized, codon-optimized *SERPINA1* genes without promoters and start codons and are the only wild-type viral genomic elements present in AAV8-1.

In the absence of viral genes such as the rep and cap genes and helper viruses that are required in wild-type AAVs to drive viral replication, AAV8-1 is replication defective. On its own, AAV8-1

is also inert due to the promoterless design and lack of start codons. It cannot stably infect humans or animals as no new particles can be produced.

The required rep and cap genes for AAV8-1 manufacturing are supplied in a separate plasmid that doesn't contain packaging signals. To mitigate the remote possibility of recombination events between ITR-containing and RepCap-containing plasmids during AAV8-1 manufacturing, each production lot is tested for the presence of rcAAV using a sensitive PCR-based assay, and a confirmed positive result would preclude the use of the lot in clinical studies. Information regarding the testing method, its specificity and sensitivity are provided in Annex 1.3 as this is confidential information which is not in the public domain or publicly available, and disclosure may undermine the legitimate economic interest of the Sponsor.

The generation of rcAAV in patients after administration of AAV8-1 is also very unlikely because it would require the triple infection of the same cell by AAV8-1, a naturally occurring AAV, and a helper virus. Even in that case, emerging rcAAV could not contain the DNA template and the rep and cap genes due to the limited packaging capacity of AAVs. Finally, the potential for a recombination event to occur between AAV8-1 and a naturally occurring AAV is further limited by the fact that the only viral regions of homology are the viral ITRs.

## 2.3 Provide a diagram ('map') of the clinical vector

The clinical vector comprises two unique copies of the *SERPINA1* gene, coding sequence for hA1AT, with a splice acceptor site encoded upstream of each copy and a polyadenylation (polyA) signal sequence placed after the stop codon of each *SERPINA1* gene. The transgene of interest is flanked by ITRs.

A diagram of the clinical vector genome, as well as its sequence, are provided in **confidential** Annex 2.3, as this is confidential information which is not in the public domain or publicly available, and where disclosure may undermine the legitimate economic interest of the Sponsor.

**CONFIDENTIAL INFORMATION IS PRESENTED IN:**

- **ANNEX 2.3 ANNOTATIONS OF AAV8-1 TRANSGENE DS GENOME STRUCTURE**

## 2.4 Molecular characterisation of the clinical vector

AAV8-1 is generated by transfection of HEK293 cells using fully characterized, sequenced plasmids.

AAV8-1 is generally considered to be genetically stable, for several reasons:

- Single-stranded DNA: AAVs carry a single-stranded DNA genome, which is less prone to recombination events compared to double-stranded DNA or RNA viruses. This reduces the chances of generating unwanted genetic variants.
- Non-replicative nature: AAV8-1 is replication-defective, even in the presence of a helper virus, due to the absence of the viral rep and cap genes that are required in wild-type AAVs to drive viral replication. This limits its ability to mutate and evolve.

- Packaging capacity: AAV8-1 has a limited packaging capacity (about 4.7 kilobases), which restricts the size of the genetic material it can carry and further limits the potential for genetic instability.

An annotated sequence of the AAV8-1 vector genome is provided in **confidential** Annex 2.3 as this is confidential information which is not in the public domain or publicly available, and where disclosure may undermine the legitimate economic interest of the Sponsor.

**CONFIDENTIAL INFORMATION IS PRESENTED IN:**
- **ANNEX 2.3 ANNOTATIONS OF AAV8-1 TRANSGENE DS GENOME STRUCTURE**

## 2.5 Description of the insert

The *SERPINA1* expression cassette comprises two ITRs that flank the hA1AT coding sequence, with a splice acceptor and polyA sequence. Of note, the DNA template does not include a promoter or start codon. Moreover, human protein can only be expressed once the cassette has stably integrated at a specific site into the genome of the hepatocytes as guided by the material delivered by LNP1265 which is sequentially administered.

The hA1AT is not expected to be toxic or otherwise harmful to humans, including the clinical trial patients. Neither wild type AAV nor the experimental vector AAV8-1 is known to be pathogenic to humans.

AAV8-1 is unable to replicate independently, even in the presence of a helper virus, because it lacks the *rep* and *cap* genes required for rescue/packaging. The transgene is not expected to confer any advantage in terms of survival and selective pressure.

## 2.6 Biodistribution and shedding

### 2.6.1 Biodistribution

The biodistribution of NTLA-3001 was evaluated across 2 nonclinical studies. Biodistribution was assessed in cynomolgus monkey (nonhuman primates (NHP)), hepatic and extra-hepatic tissues, after sequential administration of SERPINA1(human)-AAV8-1 and LNP1265. Quantitative polymerase chain reaction (qPCR) was utilized for the analysis of residual tissue distribution of the SERPINA1(human)-AAV8-1 vector genome. The tissues collected and analyzed in both studies were done according to the ICH S12 guideline for nonclinical biodistribution considerations for gene therapy (March 2023) and historical characterization of the LNP delivery system. The results showed that SERPINA1(human)-AAV8-1 biodistributed across all tissues examined, with the highest concentration observed in liver (target organ) followed by the spleen. In general, levels appeared to be dose-dependent and decreased over the study period, remaining detectable at the last timepoint evaluated in most tissues. Further information regarding biodistribution is provided in **confidential** Annex 5 as this is confidential information which is not in the public domain or publicly available, and disclosure may undermine the legitimate economic interest of the Sponsor.

**CONFIDENTIAL INFORMATION IS PRESENTED IN:**
- **ANNEX 5 INVESTIGATOR'S BROCHURE**

### 2.6.2 Vector Shedding

Given this is a replication incompetent vector, per FDA guidance (FDA 2015), template vector shedding will be assessed later in development (during the Phase 2 portion of the human clinical study of NTLA-3001) at a clinically relevant efficacious dose.

Vector transgene shedding to unintended recipients is a potential risk. There is currently no human shedding data for AAV8-1. However, the environmental impact is considered low given that:

1. AAV8-1 is a replication defective recombinant AAV8 vector;

2. AAV8-1 has a promoterless cargo without a start codon; and

3. AAV vectors are based on viruses that are non-pathogenic in humans, and the vectors themselves are not known to cause any diseases in humans or animals (Directive 2000/54/EC, NIH 2019)

Viral shedding involves the release of vectors that did not infect the target cells and were cleared from the body via bodily fluids (e.g., blood, saliva, feces, semen, urine). In human studies with IV-administered AAV2 or AAV8, the vector was detectable in urine for a few days to 4 weeks, serum for a couple of weeks to 14 weeks, and peripheral blood mononuclear cells and semen for several weeks (Manno et al 2006 and Nathawani 2014). However, with other AAV serotypes, shedding in semen has plateaued after as long as 48 weeks (Miesbach et al 2018) and in blood after 36 weeks (Rangarajan et al 2017). Data have shown that the amount of vector that is shed is lower than the amount required for successful transduction of the gene, and that successful transduction needs systemic or localized delivery (Dismuke et al 2013).

# 3 SECTION 3 – INFORMATION RELATING TO THE CLINICAL TRIAL

## 3.1 General information about the clinical trial.

Study ITL-3001-CL-101 is a Phase 1/2, multicentre open-label study to evaluate the safety, tolerability, PK, and PD of NTLA-3001 in adult participants ages 18 to 75 with AATD associated lung disease. Approximately 30 participants will be enrolled to study intervention.

Enrolled participants will take part in the study for a total of approximately 162 weeks:

- Screening period: 6 weeks
- Study intervention administration: on Day 1
- Postdose follow up: 156 weeks

The study design consists of 2 portions: Phase 1 and Phase 2.

- The **Phase 1** portion of this study is an open-label, single arm ascending dose design of up to 18 dose-limiting toxicity (DLT)-evaluable AATD participants with evidence of pulmonary emphysema and FEV1 compromise to characterize safety, tolerability, PK, and PD. Analysis and review of the Phase 1 data will be used to identify a safe and potentially efficacious dose that will be further evaluated in a single arm Phase 2 open-label expansion.

Three dose levels (cohorts 1-3) are planned for Phase 1. Each dose level will include a minimum of 3 participants, with up to 6 total participants treated at a given dose.

- In **Phase 2**, approximately 12 AATD participants will be assessed to further characterize safety, tolerability, PK, and PD of NTLA-3001 at the dose level selected for expansion. Phase 2 will also provide an initial assessment of the effect of NTLA-3001 on clinical measures of pulmonary function.

Together, the 2 portions of this study will be used to identify a safe and potentially efficacious dose for future studies.

| | |
|---|---|
| **EU CT-number:** | 2023-508138-33-00 |
| **Deliberate release reference number:** | GMOB-2024-29313 |
| **Title of the clinical trial:** | Phase 1/2 Multicenter, Open-label Study to Evaluate Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of NTLA-3001 in Participants with Alpha-1 Antitrypsin Deficiency (AATD)-Associated Lung Disease |
| **Name of principal investigator:** | Information about the principal investigator is provided in **confidential** Annex 1. |
| **Objective of the study:** | The primary objective of this open-label Phase 1/2 study is to evaluate the safety and tolerability of NTLA-3001 following a single treatment in adult participants with AATD associated lung disease. The primary endpoint is safety, as demonstrated by treatment-emergent adverse events (TEAEs). The pharmacodynamic effect of NTLA-3001 will be assessed by measuring circulating AAT protein and activity. |
| **Intended start and end date:** | June 2024 –March 2030<br>Individual participants will take part in the study for up to approximately 162 weeks (Screening through final visit), including follow-up for safety and efficacy assessments up to 156 weeks after therapy. At the completion of this study, participants will be followed under a separate long-term follow-up study, which will include observation of participants for 15 years from the NTLA-3001 infusion. |
| **Number of trial subjects that will take part in the study:** | Approximately 30 subjects worldwide.<br>Approximately 5 subjects in Ireland. |
| **Indicate if an application related to the same investigational medicinal product has been submitted -or is planned to be submitted- to other EEA Member States. In the affirmative, identify the countries** | In the EEA: No.<br>Outside EEA: UK. |

## 3.2 Intended location(s) of the study

| | |
|---|---|
| **Organisation Name:** | Beaumont Hospital (Primary study site) |
| **Address Details:** | Clinical Research Centre, Smurfit Building, Beaumont Hospital, Beaumont Road, D09 YD60. |
| **Contact person:** | • PI: Professor Gerry McElvaney<br>• Study Coordinator: Ann Collins |
| **Telephone No:** | • PI: +353 (1) 809 3764<br>• Study Coordinator: +353 (1) 809 3864 |
| **Email Address:** | • PI: gmcelvaney@rcsi.ie<br>• Study Coordinator: annmcollins@rcsi.ie |
| **Planned activities:** | Collection and storage of samples from Day 21 (According to the Protocol - Section 1.3 Schedule of Activities - Table 1). |
| **Location where patient's samples that contain GMO are stored:** | Ground Floor laboratory, Smurfit Building, Beaumont Hospital, Beaumont Road, D09 YD60. |
| **Containment level:** | Containment Level 1. |
| **Name and contact details of the responsible person:** | • PI: Professor Gerry McElvaney<br>• e-mail: gmcelvaney@rcsi.ie<br>• Phone: +353 (1) 809 3764 |

| | |
|---|---|
| **Organisation Name:** | St James Hospital (Study Sub-Site) |
| **Address Details:** | The Wellcome - Health Research Board Clinical Research Facility at St James Hospital (SJH-CRF), H&H Level 2, St James's Hospital, St James's Street, Dublin 8 D08 A978 |
| **Contact person:** | • Sub-I: Professor Martina Hennessy<br>• Study Coordinator: Derval Reidy<br>• BSO: Edel O Dea |
| **Telephone No:** | • Sub-I: +353 1 410 3900<br>• Study Coordinator: +353 1 410 3919 |

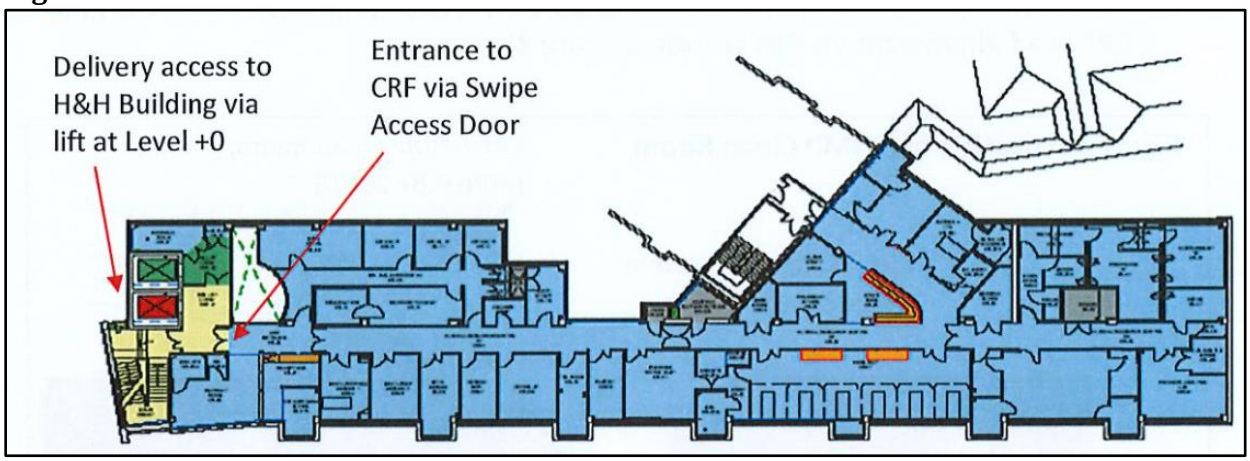
| | |
|---|---|
| | • BSO: +353 1 410 3931 |
| **Email Address:** | • Sub-I: mhenness@tcd.ie<br>• Study Coordinator: reidyde@tcd.ie<br>• BSO: edodea@tcd.ie |
| **Planned activities:** | All activities according to the study protocol ITL-3001-CL-101, the Pharmacy Manual and Laboratory Manual from screening, up until Day 14 post dose including storage, preparation and administration of IMP, support and rescue medications, as well as collection and storage of samples from D -42 until D14 post dosing (According to the Protocol - Section 1.3 Schedule of Activities - Table 1). |
| **Location where patient's samples that contain GMO are stored:** | Collection of samples from screening up until Day 14 will take place in Wellcome HRB Clinical Research Facility at St James's hospital. |
| **Containment level:** | Containment Level 1.<br>Site has capabilities/authorization to work under containment Level 3. |
| **Name and contact details of the responsible person:** | • Name: Edel O Dea<br>• e-mail: edodea@tcd.ie<br>• Phone: +353 1 410 3931 |

## 3.3 Storage of the clinical vector at the clinical site

The investigational medicinal product (IMP) will be stored at the Wellcome HRB Clinical Research Facility (CRF) only, the unit has its own dedicated pharmacy, and the IMP will be stored and prepared in a negatively pressurised isolator. The IMP will be prepared for dosing at the above-mentioned pharmacy.

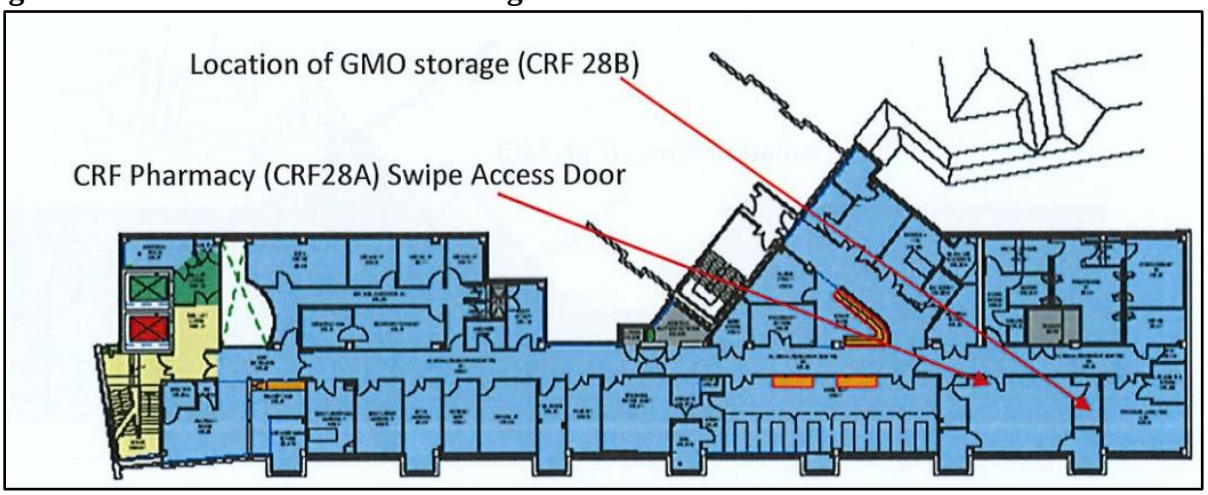
The IMP will be delivered directly to the Wellcome HRB Clinical Research Facility located in the Haematology and Hepatology Building (H&H Building) in St. James's Hospital via a service entrance accessed via the H&H building lifts (Figure 1). A member of CRF staff will meet the courier at the service entrance and accompany them to the CRF.

**Figure 1. CRF Entrance and Service Entrance**




The IMP will be stored in a ≤ -65°C freezer in room CRF 28B within the pharmacy in the Wellcome HRB Clinical Research Facility, which is located on Level 2 of the H & H building in St. James's Hospital (Figure 2). There is restricted access to the pharmacy through door CRF 28A with access granted by the chief pharmacist via St. James's Hospital security. Within the pharmacy the clinical vector will be stored in the Fridge/Freezer room (CRF 28B). This room is accessed by authorised personnel only.

**Figure 2. Location of GMO IMP Storage Area**




The IMP will be provided as a frozen (≤-65ºC) liquid in a single-use 10 mL glass vial with a 7.5 mL extractable volume. Vials will be shipped on dry ice (-80°C). Vials will be individually labelled with a label complying with local regulatory requirements. Vials will be packed into cartons; cartons will be labelled with a label complying with local regulatory requirements.

IMP shipments will be triggered via the Interactive Response Technology (IRT) upon patient screening. The IMP will be shipped in a qualified cold chain shipper to the investigative site once triggered by the IRT. A notification will be sent to the site once the shipment is triggered in the IRT. Receipt of IMP will be promptly handled upon arrival at the investigative site.

The IMP is expected to arrive at the site approximately 28 calendar days prior to the planned administration date. Routine storage of the IMP for more than 42 calendar days is not expected. In the event of unforeseen circumstances, the IP may be stored for a period up to 6 months.

The day of administration, the IMP will be thawed in room CRF-28A and prepared in the CRF Cleanroom Facility (room CRF-35) which is also within the CRF on Level 2 of the H&H building. Site will prepare the IMP infusion bag in advance of dosing and place it in a 2-8°C refrigerator located in room CRF 28A on Level 2 of H&H Building for up to 24 hours after the first vial was punctured until ready for infusion. When subject is ready for infusion, the bag(s) will be removed from the refrigerator and the time of removal from refrigerated storage will be noted on the Sponsor Preparation Administration Checklist.

## 3.4 Logistics for on-site transportation of the clinical vector

Administration will occur in a patient isolation room within the Clinical Research Facility (CRF-28C, CRF-28D) on Level 2 of the H&H building.

Both the CRF pharmacy (infusion bag storage area) and the CRF isolation rooms are on Level 2 of the H&H building separated by approximately 10 metres.

The infusion bags and saline flush bag will be transferred to the administration team by the pharmacy team. Each IP bag will be transferred separately immediately prior to administration. Each infusion bag will be contained in a labelled ziplock bag which will be placed in a tray. The tray will be wheeled on an instrument trolley to the patient bedside.

A fresh solution of Klorsept® 87 (5000ppm) will be used to disinfect the transfer trays.

## 3.5 Information about reconstitution, finished medicinal product and administration to patients

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|---|---|
| **Reconstitution (Where applicable, summarise reconstitution steps):** | AAV8-1 drug product will be supplied as a frozen liquid (7.5 mL extractable volume) in a single-use 10 mL vial. AAV8-1 DP is to be stored at ≤ -65°C. |
| **Pharmaceutical form and strength:** | Solution for IV infusion.<br>Further information regarding strength is provided in Annex 3 as this is confidential information which is not in the public domain or publicly available, and disclosure may undermine the legitimate economic interest of the Sponsor. |
| **Mode of administration:** | IV infusion |
| **Information on dosing and administration schedule (in case of repeated dosing):** | Not applicable. |
| **Information on concomitant medication that may affect the shedding of the clinical vector/ environmental risks** | Not applicable. |

**CONFIDENTIAL INFORMATION IS PRESENTED IN:**
- **ANNEX 3 PHARMACEUTICAL FORM AND STRENGTH**

## 3.6 Measures to prevent dissemination into the environment

### a) Control measures during reconstitution (if applicable), handling and administration

The IMP will be prepared in the CRF Cleanroom Facility (CRF 35) with controlled access to this facility through door CRF 33.

The IMP will be prepared by trained staff using aseptic technique in a negatively pressurised pharmaceutical isolator. All approved local procedures and both Protocol and Pharmacy Manual Instructions will be followed.

The IMP will be administered by trained and delegated personnel only.

### b) Personal protective equipment

Personal protective equipment (PPE) will be worn by staff at all times when handling the IMP. The PPE includes disposable protective gowns, double gloves, facemask, visor or face shield.

**c) Decontamination/cleaning measures after administration or in the case of accidental spilling (i.e. decontamination /cleaning measures of potentially contaminated materials, surfaces and areas). In addition, the disinfection procedures applied should be justified by providing evidence that the chosen method is sufficiently active against the clinical vector.**

A spill will be cleaned as per GMO Spill SOP using prepared spill-kits. The decontamination is achieved using Klericide Sporicidal Active Chlorine which is virucidal after one minute.

Transfer trays are cleaned using bleach (Klorsept 87®).

The pharmaceutical isolator will be cleaned prior to use using vaporized 35% hydrogen peroxide. The workspace will also be wiped with denatured ethanol 70/30.

The pharmaceutical isolator is cleaned post-production with Klericide Sporicidal Active Chlorine, followed by a clean with denatured ethanol 70/30. This is followed by a vapourised hydrogen peroxide 35% decontamination cycle.

If a spill occurs inside the pharmaceutical isolator the area will be decontaminated with Klericide Sporicidal Active Chlorine.

**d) Elimination or inactivation of left-overs of the finished product at the end of the clinical trial**

Remaining IMP will be disposed of as GMO waste. All remaining finished product will be placed in a GMO waste bin, marked as biohazard and a dedicated waste collection will be arranged. The waste transfer number will be recorded, and a certificate of destruction will be requested from the waste management company and filed for record.

St James Hospital does not incinerate biohazard waste in house. All the biohazard waste is handled by SRCL Limited.

**e) Waste treatment (including also –where applicable- decontamination and disposal of potentially contaminated waste that accumulates outside the clinical trial site). Where applicable, identify also the company responsible for waste management.**

All consumables used in the preparation of the IMP are single-use and will be disposed of as GMO waste after preparation. This includes all PPE, syringes, needles and infusion bags.

The waste will be placed in a GMO waste bin marked as Biohazard. These bins will be sealed on the day of administration and placed in the CRF Cleanroom Facility (CRF-35). Collection will be arranged for the day after administration. The bins will be collected by SRCL Limited, Unit 1A Allied Industrial Estate, Kylemore Road, Dublin 10. Stericycle Ireland, GMO register number G0163-02.

A certificate of destruction will be requested and retained on-site for each occasion that waste was disposed.

**f) Recommendations given to clinical trial subjects to prevent dissemination (where applicable).**

Not Applicable.

**g) Recommendations on donation of blood/cells/tissues/organs by the clinical trial subject.**

In order to minimize the potential risks of transmission, protocol guidance advice patients against donation of sperm and eggs. The protocol clarification letter dated 30 July 2024 clarifies restrictions on blood donation.

- Male participants should not donate sperm beginning at the signing of informed consent through at least 52 weeks post NTLA-3001 administration or per local guidance if longer.
- Female participants should not donate eggs (ova, oocytes) from the signing of informed consent through at least 52 weeks post NTLA-3001 administration or per local guidance if longer.
- The informed consent provides guidance advice against donation of blood by participants. Participants should not donate blood for at least 52 weeks post NTLA-3001 administration.

**h) Other measures (where applicable).**

Not Applicable.

## 3.7 **Sampling** and further analyses **of samples from study subjects**

**a) Describe how samples will be handled/stored/transported**

**Wellcome HRB Clinical Research Facility at St James's Hospital**

Samples collected from patients during their visit will be spun down in the CRF laboratory which is on the same floor that the patient will have their visit. A dedicated lab co-ordinator will be responsible for processing of laboratory samples. All samples will be processed in accordance with the Laboratory Manual and all personnel dealing with patient samples will be trained to handle the samples.

All samples will be processed using centrifuge (lids will be used) and separated out in the Class II BioSafety Cabinet.

Samples that require freezing will be frozen in the laboratory, equipped with -80°C and -20°C temperature mapped and alarmed freezers.

Ambient samples will be shipped on the day of collection, some refrigerated samples will be shipped on the day of collection (as per Laboratory Manual) and others will be shipped in the next frozen shipment (maximum of one week later).

**Beaumont site will collect samples from week 3 visit.**

Samples collected from patients during their visit will be spun down in the centrifuge (lids will be used) and separated out in the Class II BioSafety Cabinet.

All of this will take place in the Sample Processing area of the ground floor lab (beside CRC) and then the pertinent samples which require freezing, will be stored in an alarm monitored freezer (used only for this study) and as per Lab Manual requirements, before being sent away for analysis.

Ambient samples will be shipped on the day of collection, some refrigerated samples will be shipped on the day of collection (as per Lab manual) and others will be shipped in the next frozen shipment (maximum of one week later).

**b) Indicate whether and at which time points samples that may contain the administered clinical vector are taken from study subjects.**

Information regarding time points of sample collection is provided in **confidential** Annex 4 as this is confidential information which is not in the public domain or publicly available, and disclosure may undermine the legitimate economic interest of the Sponsor.

**CONFIDENTIAL INFORMATION IS PRESENTED IN:**
- **ANNEX 4 SCHEDULE OF ACTIVITIES**

**c) If samples are stored at the clinical site, describe storage location and storage conditions**

Please refer to section 3.7 (a).

**d) Explain if there is any non-routine[1] testing of the samples and indicate whether the clinical vector is generated de novo during the testing.**

Not applicable. No non-routine testing will occur.

[1] Standard clinical care tests as well as tests required to fulfil long-term follow-up of clinical trial subjects need not be mentioned.

# 4 SECTION 4 – OTHER DATA REQUIREMENTS

## 4.1 Plan of the site(s) concerned

**Figure 3. Wellcome HRB CRF at St James's Hospital: Cleanroom Facility floorplan**

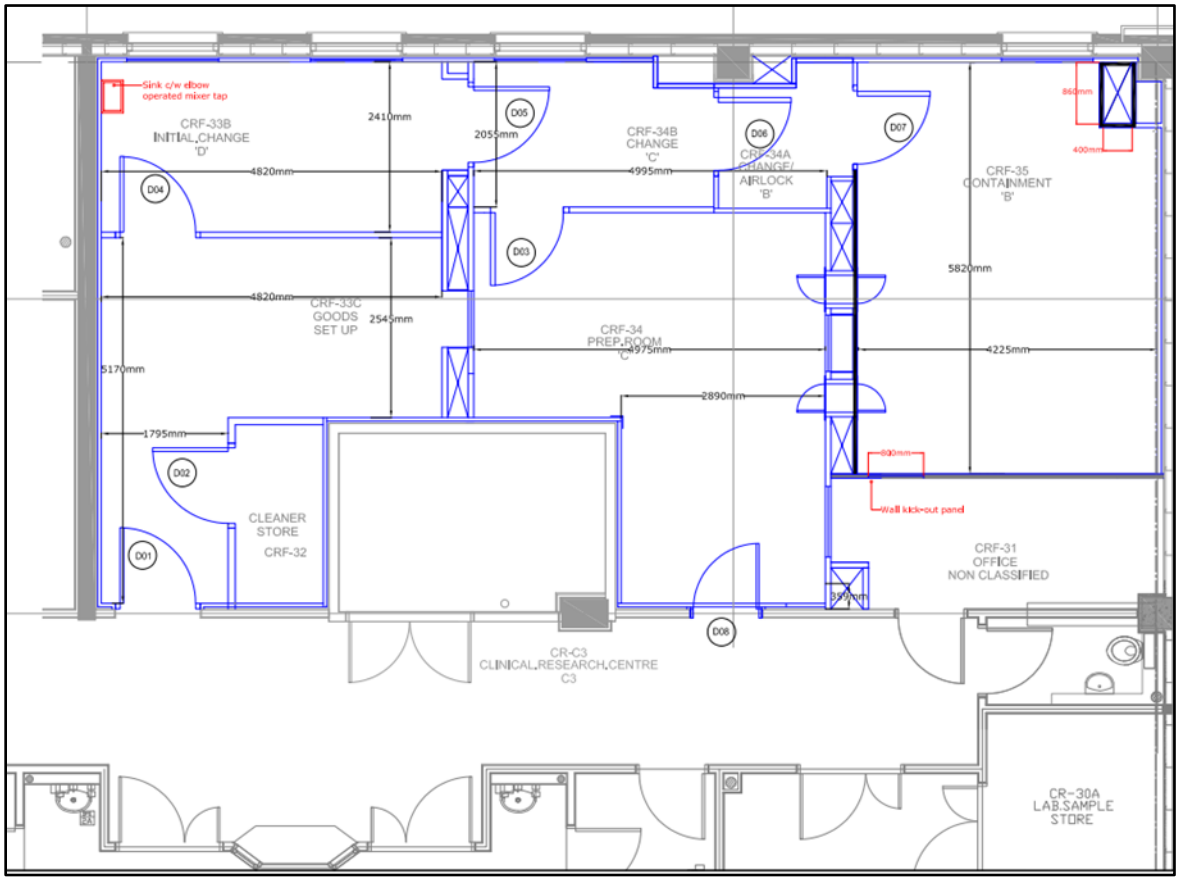
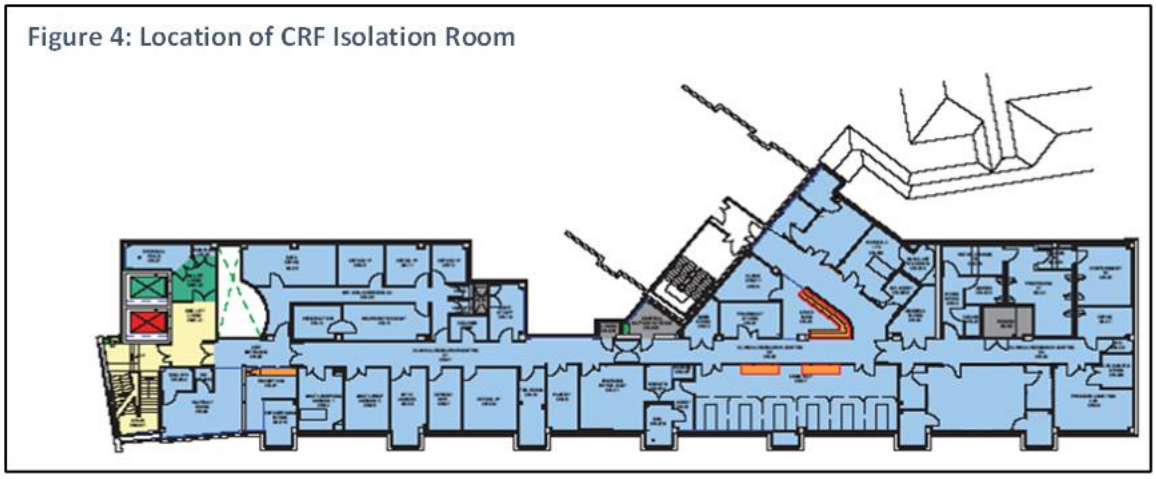



**Figure 4. Location of CRF Isolation Room**



# 5 SECTION 5 - ENVIRONMENTAL RISK ASSESSMENT

**Specific environmental risk assessment**

Considering the specific characteristics of the investigational medicinal product (as described in Section 2 of the application form), the applicant considers that the specific environmental risk assessment provided for in Section 2 of the Good Practice on the assessment of GMO related aspects in the context of clinical trials with AAV clinical vectors is applicable:

Yes: ☒

No: ☐

If the answer to the above is NO, the following information should be provided:

- For submissions made under Directive 2001/18/EC: an environmental risk assessment is required in accordance with Annex II thereof.
- For submissions made under Directive 2009/41/EC: an assessment of the risks to human health and the environment in accordance with Article 4 thereof.

# 6 REFERENCES

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