**Meeting Type**

[redacted]

**Product**

Adeno-Associated Viral 9/Sulfatase Modifying Factor 1 (AAV9/SUMF1)

**Indication**

Multiple Sulfatase Deficiency (MSD)

**Investigator/Contact**

[redacted]

**Date of Submission**

12/20/2024

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# 1. INTRODUCTION

## 1.1. BACKGROUND

AAV9/SUMF1 was developed as a gene therapy treatment for Multiple Sulfatase Deficiency (MSD). This treatment is planned as a one-time infusion of AAV9/SUMF1 via an injection into the cisterna magna.

The 9-exon SUMF1 gene encodes formylglycine-generating enzyme (FGE), which is required for post-translational modification and activation of sulfatase enzymes. As such pathogenic mutations in the gene impact the function of all 17 human sulfatase enzymes. FGE modifies a common active site cysteine residue into C-alpha-formylglycine. Without this post-translational modification, sulfatase activity is absent. Individuals affected by MSD exhibit a constellation of neurologic and somatic features that overlap with known inherited single sulfatase disorders (i.e., metachromatic leukodystrophy (MLD) and five mucopolysaccharidoses (MPS) subtypes, X-linked ichthyosis and X- linked chondrodysplasia punctata). In addition, all other sulfatases without known clinical correlation also contribute to the complex and variable phenotype found in individuals with MSD. For additional information concerning MSD prevalence and characteristics, we refer the Agency to our designated FDA Orphan Drug and Rare Pediatric Disease Designations, DRU-2024-10275 and RPD-2024-900, respectively.

There are currently no FDA approved treatments available for this disorder. Individuals affected by MSD are managed by supportive care, consultation with medical professionals from multiple disciplines, physical therapy, and pharmacological interventions to alleviate symptoms. Preclinical testing of AAV9/SUMF1 in a KO mouse model of MSD have shown substantial life-altering treatment benefits. Gene therapy may represent a reasonable and promising approach to provide a meaningful and long-term therapeutic benefit for this population in the near future.

A Type B pre-IND meeting was held with the FDA on September 18, 2018 (CRMTS #11357 and PTS # PS003895), where initial CMC guidance was provided.

The lead investigator, Dr. Steven Gray, from UT Southwestern Medical Center, is working in collaboration with the Bespoke Gene Therapy Consortium (BGTC) on the development of AAV9/SUMF1, one of eight investigational gene therapies in the BGTC portfolio.

The BGTC is managed by the Foundation for the National Institutes of Health (FNIH) and is a public–private partnership between the NIH, FDA, industry and nonprofit partners with experience in designing, developing and manufacturing AAV-based gene therapies. It was formed to address the challenge of developing AAV gene therapies for rare monogenic diseases. The BGTC's objectives are to streamline and accelerate clinical development, support first-in-human clinical trials for several rare diseases, and to increase knowledge about AAV transduction, production and gene expression through grants that support research. By reducing the cost and challenges for the development of AAV gene therapies, BGTC's efforts may change what diseases are of commercial interest, with rare diseases becoming more mainstream for investment by the pharmaceutical industry and other stakeholders[1].

The aim of the BGTC is to facilitate drug development for rare diseases using a platform based approach where appropriate. While this meeting request is specific to AAV9/SUMF1, some referenced approaches may be relevant across the BGTC portfolio. Agency feedback obtained through this meeting may be used to inform other BGTC gene therapies as the context is deemed scientifically appropriate. The BGTC and collaborating investigators may refer to the information contained in this briefing package along with Agency feedback obtained from this meeting in future BGTC meetings, applications or other correspondence.

### 1.2.PRODUCT NAME

| | |
|---|---|
| Product Name | Adeno-Associated Viral 9/Sulfatase Modifying Factor 1 (AAV9/SUMF1) |
| Vector Name | scAAV9/CBh-*hSUMF1opt*-SV40pA |
| AAV Serotype | AAV9 Serotype Capsid |
| Formulation | Delivered in 1x PBS with 5% D-sorbitol |
| Gene Insert | codon-optimized human SUMF1 transgene (Self-Complementary) |

| | | | | |
|---|---|---|---|---|
| AAV2 mutant ITR | Synthetic CBh promoter | Human SUMF1 gene | SV40 polyA | AAV2 ITR |

### 1.3. DESCRIPTION OF PRODUCT

AAV9/ is a recombinant serotype 9 adeno-associated virus (AAV) encoding a codon-optimized human SUMF1 transgene (*hSUMF1opt*). The final product consists of AAV9 capsids that are packaged with the self-complementary AAV genome comprising a mutant AAV2 inverted terminal repeat (ITR) with the D element deleted, the “CBh” promoter (796 kb CMV enhancer, chicken beta actin promoter, synthetic intron), codon-optimized human SUMF1 DNA coding sequence (1122 bp), the simian virus 40 polyadenylation signal (143 bp), and WT AAV2 ITR.

### 1.4. PROPOSED INDICATION

Treatment of patients with MSD, which is an autosomal recessively inherited neurometabolic disorder caused by loss-of-function or null mutations in the SUMF1 gene.

## 2. PRODUCT DEVELOPMENT SUMMARY

The AAV9/SUMF1 vector and treatment approach was initially developed by Dr. Steven Gray’s laboratory, which now resides at UT Southwestern Medical Center. Preclinical testing of the AAV9/SUMF1 vector was conducted in a mouse model of SUMF1 gene deficiency by Jackson Laboratories, in collaboration with the laboratories of Dr. Steven Gray and Dr. Rachel Bailey. These initial efforts were primarily funded and supported by the United MSD Foundation, which is a patient advocacy organization that continues to strongly support this work.

Following a 2018 Type B pre-IND meeting with the agency, the technology was licensed to a biotechnology company to conduct the product development. This initial company eventually dropped the MSD program due to financial considerations. A second company licensed the

technology with a goal to further develop the product, but likewise disengaged from the program due to financial considerations.

Starting in 2022, the United MSD Foundation worked to continue advancement of the program towards a clinical trial by initiating a GLP toxicology study in normal rats at Charles River Laboratories, in collaboration with Dr. Gray at UT Southwestern. The toxicology batch of AAV9/SUMF1 was manufactured by Catalent. This study followed the guidance from the 2018 pre-IND interaction with the FDA.

In May 2023, the AAV9/SUMF1 treatment for MSD was selected for inclusion in the FNIH Accelerating Medicines Program (AMP), the Bespoke Gene Therapy Consortium (BGTC). The BGTC is facilitating the AAV9/SUMF1 drug product manufacture at Henogen, and it is anticipated that an IND submission can occur in roughly Q4 2025 or Q1 2026, following completion of the GMP drug product manufacture.

A longer-term product development plan (i.e., towards a Phase III trial) has not been formulated and no commercial partner is currently identified. We anticipate further consideration on this following review of the outcomes observed in the Phase I/II trial and some of the questions provided in this briefing book are meant to foreshadow potential future commercial development.

## 3. PURPOSE OF MEETING

The sponsor wishes to clarify several details related to the manufacture and release of the AAV9/SUMF1 drug product, for use in the initial first-in-human Phase I/II clinical trial.

## 4. LIST OF SPECIFIC QUESTIONS

**Question 1:** Given the profound unmet need for MSD patients, it is our goal to initiate the Phase I/II clinical trial as quickly as possible. The pivotal GLP toxicology study is complete, and the only major barrier to the IND submission is the drug product manufacture and issuance of the COA for inclusion in the IND. To expedite the trial startup, we would like to request to submit the IND prior to completion of the GMP batch, based on the release specification. In this scenario, we would commit to not dosing patients until the IND is updated with the final COA for the clinical batch and at least 30 days have elapsed. Would the Agency be willing to review our IND without the final drug product COA?

**Question 2:** Similar to Question 1, to expedite our IND submission and trial startup, we'd like to request to submit the IND with stability data on an engineering batch using a process, materials, and container similar to the GMP drug product batch. We propose to seek IND approval and initiate patient dosing based on the release testing data for the drug product and the stability data on the engineering batch, while the stability testing of the GMP drug product batch is ongoing. The IND will be updated as stability data from the GMP drug product batch becomes available. Does the Agency agree with this plan?

**Question 3:** We would like to conduct some of our analytical method qualifications using the toxicology batch made previously at Catalent. Our position is that while the toxicology batch was produced under a different manufacturer and using a slightly different process, enough similarities exist to enable the analytical method work without a meaningful risk of any matrix effects that

would impact the reliability or reproducibility of the analytical methods. Similarly, we plan to only conduct product-specific assay qualification on the vector genome titering assay and the mRNA-based in vitro potency assay. The remaining release assays will rely on historical assay qualifications done with similar AAV-based products, and then confirmed for use as fit-for-purpose assays to release AAV9/SUMF1 for this initial Phase I/II study. Does the Agency agree with this plan?

**Question 4:** We recognize that we are using a different manufacturer and slightly different manufacturing process for the pivotal toxicology batch and the GMP drug product batch (See **Section 5.1** and **5.2**). Our position is that the process changes are not likely to adversely affect any critical quality attributes, but that this will need to be assessed through bridging analytical testing. Our approach will be to re-test the toxicology batch by the same methods as the GMP drug product batch, aiming for the GMP drug product batch to meet or exceed the purity of the toxicology batch for the critical quality attributes. This analytical bridging plan is outlined in **Section 5.4** (**Table 3**). In the event that any critical quality attribute in the GMP drug product does not exceed the purity of the toxicology bath, a risk assessment will be made to address the suitability of the GMP drug product for use in the Phase I/II clinical trial. Does the Agency agree with this analytical bridge testing strategy?

**Question 5:** The sponsor proposes to use the mRNA potency assay described in **Section 5.5.1** to release drug product for first-in-human clinical studies, which the Agency had indicated would be acceptable during the 2018 pre-IND meeting. Once validated, the investigator and BGTC believe that this potency assay described would be an adequate and reproducible measure of potency for release of Phase III and commercial product. To adequately prepare for the next phases in development of AAV9/SUMF1, the investigator and BGTC request Agency feedback on the plan for potency testing including any guidance on the use of these assays for commercial release of drug product.

## 5. SUMMARY OF SUPPORTING DATA

### 5.1. OVERVIEW OF AAV9/SUMF1 COMPLETED AND PLANNED BATCHES.

There are 2 previous batches of AAV9/SUMF1 that have been produced and used in preclinical studies. Two additional batches of AAV9/SUMF1 are planned: an engineering/pilot batch and the GMP drug product to be used in the planned Phase I/II study. The batches used in preclinical studies each used a separate manufacturer than is being used for the engineering batch and GMP batch. These 4 batches are summarized in **Table 1**.

**Table 1 Summary of AAV9/SUMF1 Batches**

| **Batch Use** | Nonclinical pharmacology: Pivotal Efficacy | Nonclinical GLP toxicology: Pivotal Safety | Engineering: Reference Standard and Stability | GMP Drug Product: Human Use |
|---|---|---|---|---|
| **Batch ID** | LAV46 | 114-0322-740 | *TBD* | *TBD* |
| **Manufacturer** | UNC Vector Core | Catalent | Thermo/ Henogen | Thermo/ Henogen |
| **Cell line** | Suspension HEK293 | Suspension HEK293 | Suspension HEK293 | Suspension HEK293 |
| **Production Scale** | 20L | 2x200L | Planned 200L | Planned 1000L |
| **Purification strategy** | Density gradient followed by ion exchange chromatography | Affinity chromatography followed by anion exchange chromatography | Affinity chromatography followed by anion exchange chromatography | Affinity chromatography followed by anion exchange chromatography |
| **Titer** | $1.36x10^{14}$ vg/mL[1] | $7.9x10^{13}$ vg/mL[2] | *TBD* | *TBD* |
| **% empty particles** | 13%[3] | 35%[4] | *TBD* | *TBD* |

[1] ITR-based qPCR titer
[2] ddPCR-based titer
[3] Quantified by transmission electron microscopy (TEM)
[4] Quantified by analytical ultracentrifugation (AUC)

## 5.2.OVERVIEW OF THE BIOLOGICAL PRODUCT AND MANUFACTURING COMPONENTS

### 5.2.1. Product Development, Manufacturing History, and Upcoming Plans

The AAV9/SUMF1 vector for the preclinical dose-finding efficacy studies was produced at the UNC Chapel Hill Vector Core, using production plasmids containing an Ampicillin backbone. The UNC Chapel Hill Vector Core utilized a manufacturing process involving triple plasmid transfection into suspension HEK293 cells, followed by low-speed centrifugation to clarify a cell lysate, iodixanol gradient purification, and finally anion exchange chromatography as a polishing step before fill/finish. The Ampicillin gene in these plasmids were subsequently replaced by Kanamycin, but were otherwise functionally identical. All subsequent manufacturing activities utilized these revised Kanamycin backbone plasmids.

An engineering/toxicology batch was manufactured by Catalent, and this AAV9/SUMF1 batch was used in the pivotal GLP rat toxicology study. The production process by Catalent for this batch involved triple plasmid transfection of plasmids into suspension HEK293 cells (two 200L bioreactors), followed by cell lysis and Benzonase treatment, depth filtration, affinity chromatography, and anion exchange chromatography. Fairly abundant retains are available from this batch.

Manufacturing activities for the GMP drug product have shifted to Thermo/Henogen, utilizing their typical manufacturing process for AAV9. The production process by Henogen will be similar

to the Catalent batch, but there will be minor process-related details as the process has been updated. These differences will be highlighted in the IND, but in brief the Henogene production process similarly involves triple plasmid transfection of plasmids into suspension HEK293 cells, followed by cell lysis and Benzonase treatment, depth filtration, affinity chromatography, anion exchange chromatography, tangential flow filtration, nanofiltration and final 0,2µm filtration. Our strategy is to mostly rely on analytical release testing results to determine the suitability of the Catalent toxicology batch to adequately predict the safety of the GMP drug product for human use. Henogen will manufacture a 200L pilot/engineering batch using comparable materials and a comparable process to the planned GMP drug product batch. This pilot/engineering batch will be used to initiate stability studies, serve as reference material, and conduct device compatibility studies. Some of this material may be available for analytical method development and qualification work, as needed.

It is noteworthy that the engineering/toxicology batch at Catalent, as well as the planned batches at Henogen, will be formulated in the same buffer (PBS containing 5% sorbitol and 0.001% pluronic F68) and are vialed in 2 mL CZ vials.

### 5.2.2. Biological Substance

The biological substance is a bulk purified AAV in chromatographic elution buffer that undergoes lot release testing prior to tangential flow filtration into final formulations buffer and sterile 0.2µm filtration to produce the biological product.

### 5.2.3. Biological Product

The biological product is an aqueous suspension of the AAV9/SUMF1 vector in PBS with 5% sorbitol and 0.001% pluronic 88. It will be stored in frozen aliquots at <-60°C. The expected concentration is at least $6.7\pm1 \times 10^{13}$ vector genomes (vg)/ml allowing the target dose of $1 \times 10^{15}$ vg to be delivered in a 15 ml volume or less.

## 5.3. DESCRIPTION OF COMPONENTS USED IN MANUFACTURING

### 5.3.1. Vector, Packaging, and Helper Plasmid DNA

Three plasmids are critical to the manufacturing of the AAV9/SUMF1 vector: pAAV9, pALD-X80, and the ITR plasmid containing the SUMF1 gene. The 3 plasmids used to make AAV9/SUMF1 are the same across all lots, with the exception that the initial LAV46 lot used pXX6-80 in place of pALD-X80, and the ITR-containing SUMF1 plasmid used an Ampicillin resistance gene instead of a Kanamycin resistance gene in the bacterial backbone.

### 5.3.2. Plasmid DNA Manufacturing, Testing, and Qualification

The plasmids used in the Catalent batch # 114-0322-740 and the proposed Henogen batches were all produced at Aldevron. The Catalent batch used research-grade plasmid, but the Henogen batches will utilize GMP-S plasmid. Plasmids were made by a process free of animal-derived reagents in single-use fermentation devices. Production will be fully documented in production records provided by the manufacturer or available for FDA review by cross-reference.

**Table 2 Lot release for plasmids for clinical grade AAV production (GMP-S)**

| Assay | Method | Specification |
|---|---|---|
| Appearance | Visual inspection | Clear Colorless Solution, free from visible particulate matter |
| Purity by OD 260/280 | UV Spectrophotometry | 1.80 – 2.00 |
| Nucleic Acid Concentration | UV A260 | 1.0 ± 0.1 mg/mL |
| Identity | Next generation sequencing | 100% identity with reference sequence |
| Endotoxin | USP <85>, Ph. Eur. 2.6.14 | ≤ 10 EU/mg |
| Residual Host Cell DNA | Quantitative PCR | ≤ 5% w/w gDNA |
| Residual Host Cell Protein | ELISA | ≤ 2% HCP w/w |
| Residual Host RNA | RP-HPLC | ≤ 5% w/w RNA |

## 5.4. QUALITY TESTING PROGRAM FOR THE AAV9/SUMF1 VECTOR

Each lot of AAV9/SUMF1 vector will be tested prior to product release. The proposed list of tests, methods, and release specifications are provided in the table below. For the tests with "report value" release specification, it is our expectation that with additional manufacturing runs and a stronger manufacturing history of AAV9/SUMF1 using these production methods, appropriate release criteria values can be set. Testing for mycoplasma, adventitious virus, and bioburden will be conducted in the unprocessed bulk harvest prior to lysis, treatment with nuclease, and clarification.

Importantly, the vector genome titering assay and the mRNA-based in vitro potency assay would undergo qualification specific to the AAV9/SUMF1 drug product. The remaining assays would rely on historical qualification data from similar AAV products, and confirmed for use with AAV9/SUMF1 as fit-for-purpose release assays suitable for use to release a first-in-human Phase I/II product.

**Table 3 Quality testing program for AAV9/SUMF1**

| Test | Method | Specification | Research Batch | Toxicology Batch | Engineering Batch | GMP Batch |
|---|---|---|---|---|---|---|
| Batch ID | | | LAV46 | 114-0322-740 | TBD | TBD |
| **Safety** | | | | | | |
| Sterility | USP<71> | No growth | | Not tested | | † |
| Bioburden | USP<61> | < 10 CFU / 100 mL | | TAMC < 1 CFU/mL<br>TYMC < 1 CFU/ mL | | † |
| Endotoxin | USP<85> | < 0.2 EU / mL | | 0.0273 EU/mL | † | † |
| Mycoplasma | USP<63> | None detected | | | | † |
| Adventitious Viruses | In vitro cell culture, 28-day test in 3 cell lines | None detected | | | | † |
| Container Closure Integrity* | USP <1207> or equivalent | pass | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Purity** | | | | | | |
| Residual Host Cell DNA | qPCR or ddPCR | Report Value | | w SAN: 10407.48 ng/mL, 1.32µg/1x$10^{13}$ vg copy<br><br>w/o SAN: 9149.43 ng/mL, 1.16µg/1x$10^{13}$ vg copy | † | † |
| Residual Host Cell Protein | ELISA | Report Value | | < 20 ng/mL | † | † |
| Residual affinity ligand | TBD | Report Value | | 277.25 ng/mL | † | † |
| Residual Plasmid | qPCR or ddPCR | Report Value | | w SAN: 4.51x$10^{12}$ copies/mL, 5.71x$10^{11}$ Kan/ 1x$10^{13}$ vg copy<br><br>w/o SAN: 4.64x$10^{12}$ copies/mL, 5.87x$10^{11}$ Kan/ 1x$10^{13}$ vg copy | † | † |
| Residual Benzonase | TBD | Report Value | | <0.62 ng/mL | † | † |
| Residual transfection reagent | TBD | Report Value | | | † | † |
| Vector Purity | SDS-PAGE gel and Silver Staining | Number and position of the bands conform to reference | pass | | | |
| | CE-SDS | Report value | | 93.9% VP1, VP2, and VP3 | † | † |
| Residual E1A gene | qPCR or ddPCR | Report Value | | w SAN: 2.80x$10^{7}$ copies/mL, 3.54x$10^{6}$ E1A/1x$10^{13}$ vg copy<br><br>w/o SAN: 3.21x$10^{7}$ copies/mL, 4.06x$10^{6}$ E1A/1x$10^{13}$ vg copy | † | † |
| **Strength / Dose** | | | | | | |
| Vector Genome Titer | Transgene-specific ddPCR | ≥6.7E$^{13}$ vg/ml | 1.36x$10^{14}$ vg/mL ** | 7.90x$10^{13}$ vg/mL ** | † | † |
| Activity / Potency | TCID50 | Report Value | | 6.33 x$10^{10}$ IU/mL 1249 vg/IU | | |
| Potency | mRNA transgene expression following Lec2 infection | Report Value | † | † | † | † |

| Identity / Characteristics | | | | | | |
|---|---|---|---|---|---|---|
| Genomic Identity | Sequencing of Vector Transgene | 100% match to reference sequence | | | † | † |
| Full:empty capsid ratio | AUC | Report Value (≤50% empty for GMP) | | 35% Empty<br>23% Full<br>33% Intermediate<br>6% LMW<br>4% HMW | † | † |
| | TEM | | 87% full (13% empty) | | | |
| Particle aggregates | SEC-UPLC | Report value | | 98.6% Monomer | † | † |
| Subvisible Particles | USP <788> or equivalent | • ≥ 10 µm: ≤ 6000 particles per vial<br>• ≥ 25 µm: ≤ 600 particles per vial<br>• ≥ 2 µm: Report Results<br>• ≥ 5 µm: Report Results | | | † | † |
| Appearance | Visual Exam | Clear colorless solution | | | † | † |
| pH | Potentiometric, USP<791> | 7.4 ± 0.5 | | | † | † |
| Osmolality | Freezing point depression, USP<785> | 500-800 mOsm/kg of $H_2O$ | | | † | † |
| Capsid Identity | ELISA | Report value | | $3.40x10^{14}$ cp/mL | † | † |

† Planned testing to occur
* CCIT will be done using formulation buffer vialed at the same time and in an identical fashion as the drug product.
** Lot will be re-tested using the method qualified for GMP drug product release

## 5.5. AAV9/SUMF1 STABILITY TESTING PLAN

Pilot stability testing was conducted out to 6 months for the engineering/toxicology batch of AAV9/SUMF1 produced by Catalent. The results of that stability testing are shown in **Table 4**, demonstrating acceptable stability of AAV9/SUMF1 in this formulation buffer, stored in 2 mL CZ vials, for at least 6 months. The formulation buffer, container, and storage conditions for the Catalent batch are identical to those planned for the Henogen batches.

To ensure stability for the duration of clinical testing, the drug product will be assessed periodically for 2 years or the duration of vector administration into human subjects, according to the plan outlined in **Table 5**. The same stability testing plan will be carried out on the engineering/pilot batch produced by Henogen for the 6 month time point only, to provide lead-in data on the stability of the AAV9/SUMF1 GMP drug product. We propose to submit the IND and initiate dosing of patients using the stability data obtained from the engineering/pilot batch, along with the "0

month" release testing values for the GMP drug product. The IND will be updated as stability data on the GMP drug product is obtained, and any results that are outside of the specifications will result in a halt to patient dosing while the results are investigated and a risk assessment conducted. Alternatively, per Question 1 we are requesting to submit the IND prior to completion of the GMP batch and without any stability testing results from batches manufactured at Henogen. In this case, we would not initiate dosing of a patient until the GMP drug product is fully released and we have 6 months of stability testing data available from the engineering/pilot batch.

**Table 4 Prior Stability Data from the Catalent Toxicology Batch (AAV9/SUMF1)**

| Test | Specification | Release | 1 Month | 3 Month | 6 Month |
|---|---|---|---|---|---|
| Titer by GOI ddPCR | Report results | $7.90x10^{13}$ vg/mL | $9.10x10^{13}$ vg/mL | $8.29x10^{13}$ vg/mL | $9.20x10^{13}$ vg/mL |
| Potency (TCID50) | Report results | 6.33 $x10^{10}$ IU/mL | 4.74 $x10^{10}$ IU/mL | 1.13 $x10^{11}$ IU/mL | 1.13 $x10^{11}$ IU/mL |
| Aggregation by SEC | Report results | 98.6% Monomer | 99.2% Monomer | 91.6% Monomer | 98.9% Monomer |
| Purity by CE-SDS | Report results | 93.9% | 92.1% | 91.6% | 93.4% |

**Table 5 Planned Stability Protocol for Final Drug Product (AAV9/SUMF1)**

| Test | Method | Specification | Timepoint (months)* |
|---|---|---|---|
| Titer | Transgene-specific ddPCR | ≥50% of the release titer | 0, 6, 12, 24 |
| CCIT** | USP <1207> or equivalent | Pass | 0, 24 |
| Potency | Lec2 cell transduction and transgene mRNA quantification | Report value | 0, 6, 12, 24 |
| Appearance | Visual Exam | Clear colorless solution | 0, 6, 12, 24 |
| Aggregation | SEC | TBD | 0, 6, 12, 24 |
| Subvisible Particles | USP <788> or equivalent | ≥ 10 µm: ≤ 6000 particles per vial<br>≥ 25 µm: ≤ 600 particles per vial<br>≥ 2 µm: Report Results<br>≥ 5 µm: Report Results | 0, 6, 12, 24 |
| pH | Potentiometric, USP<791> | ±0.4 pH from release | 0, 6, 12, 24 |
| Osmolality | Freezing point depression, USP<785> | 500-800 mOsm/kg of $H_2O$ | 0, 6, 12, 24 |
| **Release testing results would be used for the 0 month time point.*<br>***CCIT will be conducted on vials of formulation buffer (diluent) that are vialed at the same time and in the same manner as the drug product, as a surrogate.* | | | |

### 5.5.1. In-Vitro Potency Assay

A potency assay will be developed to quantify transgene expression following transduction of cells *in vitro*. This will involve infecting Chinese Hamster Ovary derived Lec2 cells (which are permissive to AAV9) with standardized quantities of the AAV9/SUMF1 vector, then measuring SUMF1 expression via mRNA levels by ddPCR.

The aspects of AAV vector biology that affect potency are the vector's ability to bind to cells, properly traffic to the nucleus, uncoat, and deliver the DNA cargo. Once the DNA has been faithfully delivered and mRNA is expressed, any downstream events such as translated protein function would be influenced solely by factors beyond the components of the drug product and thus only indirectly measure potency. We contend that measuring transgene mRNA expression following product application to cells *in vitro* would be a reliable, relevant, and reproducible method to compare lot-to-lot variability in product potency. As long as the same vector DNA sequence has been used across all preclinical studies (which it has for AAV9/SUMF1), if the sequence of the vector DNA is verified then it is reasonable to conclude that the expressed mRNA will exert the desired downstream therapeutic effect.

While this *in vitro* transgene mRNA-based potency assay will be initially developed as a fit-for-purpose assay suitable for this Phase I/II investigational product, we would like the Agency to consider the development of this approach as an acceptable potency assay for the further development of the AAV9/SUMF1 product through Phase III clinical trials and commercial release. Prior to initiation of Phase III studies, the assay would be fully qualified and implemented by a GMP-compliant testing facility. This assay would be coupled to a GMP-compliant Sanger sequencing based approach to verify that the sequence of the vector is correct, which is already part of our planned release testing plan.

### 5.5.2. Comparability to prior preclinical lots of AAV9/SUMF1

As outlined in **Table 3**, critical attributes of the vectors used in all preclinical studies (i.e., vector from academic cores and the toxicology lot) will be directly compared to the engineering and GMP drug product batches produced at Henogen, and with each other. Our acceptance criteria for analytical comparability will use a strategy that all critical quality attributes in the toxicology lot will need to be met or exceeded with the GMP drug product. Further, the potency of the lots will need to be shown equivalent. In the event that an impurity value for the GMP drug product batch exceeds that of the toxicology batch, a risk assessment will be conducted to determine the suitability of using the GMP drug product in the Phase I/II clinical trial without additional preclinical safety testing. The titer of all batches will be directly compared using the same qualified ddPCR-based titering assay, and the minimally effective dose and maximum tolerated dose will be calibrated to the GMP drug product titer.

## 5.6.MANUFACTURER INFORMATION

The University of North Carolina Vector Core is an academic AAV manufacturing facility that has been producing AAV vectors since 2000. It transitioned to the current production system that was used to make the AAV9/SUMF1 vector over 10 years ago[2].

Catalent is a Contract Development and Manufacturing Organization (CDMO) that performs manufacturing of a wide variety of personalized medicines, investigational drugs, and approved commercial drugs. Catalent helps accelerate over 1,000 partner programs and launch over 150 new products every year. Its flexible manufacturing platforms at over 50 global sites supply over 80 billion doses of nearly 8,000 products annually. They have deep experience with manufacturing biologicals, and specifically AAV. They are headquartered in Somerset, New Jersey.

Themo Fisher acquired Patheon / Henogen S.A. (Belgium) in 2021, combining the biological manufacturing capacity and expertise of both companies. Both Thermo Fisher and Patheon / Henogoen have extensive experience developing AAV purification processes and providing CDMO services. Patheon is considered a best-in-class viral vector CDMO focused on process development, clinical and commercial manufacturing of viral vectors for gene and cell therapies. Patheon's track record includes over 20 years of cGMP experience, support for 130+ products and manufacture of 500+ clinical and commercial lots. Patheon offers a wide range of technologies and services to support AAV, lentivirus, retrovirus, adenovirus, and other custom viral vectors. The Seneffe (SEN) Henogen site in Belgium (34,000 $ft^2$) supports both clinical and commercial manufacturing. This site has over 20 years experience in virus and viral vector manufacturing and has been previously approved by EMA for commercial vaccine production, FDA approved for a commercial vaccine product, and received Authorization of Foreign Manufacturer for Japan. A cross reference letter to Henogen's DMF will be provided for the purposes of the future IND.

[redacted]

## 9. REFERENCES


1 Brooks, P. J. et al. The Bespoke Gene Therapy Consortium: facilitating development of AAV gene therapies for rare diseases. Nat Rev Drug Discov 23, 157-158 (2024). https://doi.org/10.1038/d41573-024-00020-8

2 Grieger JC, Soltys SM, Samulski RJ. Production of Recombinant Adeno-associated Virus Vectors Using Suspension HEK293 Cells and Continuous Harvest of Vector From the Culture Media for GMP FIX and FLT1 Clinical Vector. *Mol Ther*. 2016 Feb;24(2):287-297. https://doi.org/10.1038/mt.2015.187