Mucolipidosis type II AAV9 gene therapy pilot study (M002): *In vivo* safety of over-expressing modified GlcNAc-1-phosphotransferase (S1S3) in wild-type mice

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Introduction

Mucolipidosis type II alpha/beta (MLII) is one of the most severe lysosomal diseases, caused by a deficiency of the key Golgi enzyme, GlcNAc-1-phosphotransferase (Ptase), responsible for the formation of a specific targeting signal, mannose 6-phosphate, on lysosomal hydrolases which is required for targeting proteins to the lysosome via the cation independent mannose 6-phosphate receptor (CI-MPR). Mutations in either subunit can lead to a loss of GlcNAc-1-phosphotransferase activity resulting in lysosomal proteins not being targeted efficiently to the lysosome.

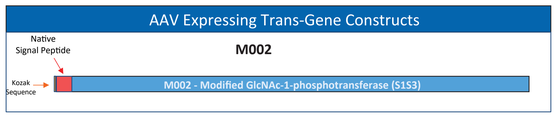
A novel platform has been developed allowing for a more efficient phosphorylation of lysosomal enzyme by a modified GlcNAc-1-phospohtransferase (S1S3). This modified Ptase is being evaluated as a potential transgene for AAV9 gene therapy (M002). However, there are potential concerns of over expressing such a key regulatory enzyme .

A long-term safety study was conducted in wild-type mice to establish the safety of an AAV9 delivered modified S1S3 GlcNAc-1-phosphotransferase transgene (M002) as a potential therapy for MLII. A six-month safety study in wild-type mice was conducted with evaluations at 2, 4 and 6 months to demonstrate that the constant expression of S1S3 would not lead to unexpected pathology. Animals were dosed with 2.5e13, 0.5e13, or 0.25e13 vc/kg at 5 wks via tail vein injection. During the in-life portion of this study, survival, weight gain and behavior between those animals treated with vehicle and M002 at three different doses were evaluated. In addition, At 2, 4 and 6-months animals were euthanized, and complete blood count (CBC) evaluation performed. Histological/path slides of key tissues were also prepared for evaluation of expression and pathology.

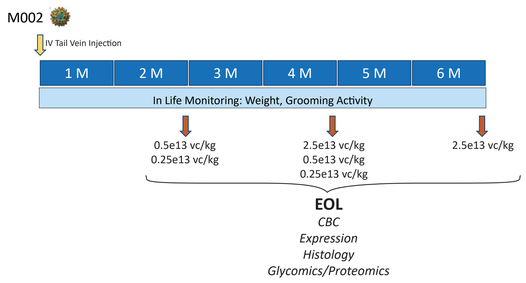
AAV Gene Therapy Initial Safety Preclinical Study Design

- AAV Transgene:
  - M002: Modified GlcNAc-1-phosphotransferase
- Animals:
  - Strain: Wild-type C57BL/6J (JAX 000664)
  - Gender: Mixed
  - Age: 5 wks
- Dose:
  - PBS
  - M002
    - 2.5e13 vc/kg (4 and 6 months)
    - 0.5e13 vc/kg (2 and 4 months)
    - 0.25e13 vc/kg (2 and 4 months)
- Route:
  - Tail Vein IV
- In Life:
  - Weight
  - Behavior/Health
- End of Life:
  - Complete Blood Count
  - DNA, RNA and protein expression
  - Histological Pathology
  - Serum glycomic/proteomics

AAV Expressing Trans-Gene Constructs



Study Design: 4 Week Animal Study



Tissues Collected: *Adrenal glands, Aorta, Bone marrow, Femur, Brain, Colon, Duodenum, Gallbladder, Heart, Ileum, Jejunum, Kidneys, Liver, Lung, Ovaries, Pancreas, Quadriceps, Spleen, Testes, Thyroid gland, Urethra, Bladder and Serum*

Methodology

In Life Evaluations

Animals were monitored weekly for overall well-being with parameters including appearance, natural behavior, body weight, hydration status, clinical signs, and provoked behavior. Animals were scored from 0 (normal) to 5 (distressed) in each category with a total score generated out of 25.

Gene Expression

Total RNA and cDNA was generated from brain, liver, heart, and kidney. The 2^-Delta-Delta Ct method was used to calculate relative gene expression of the human transcript for M002 normalized to Gapdh as the housekeeping control.

Transcript Detection (Basescope)

Slides containing brain and liver samples were labeled with a human specific AAV9.M002 probe (ACDBio Cat No 874031), which consisted of 1 double Z pair in a region of the S1S3 gene with little homology between mouse and human. Slides underwent labeling with the BaseScope Reagent v2- RED kit (ACDBio Catalog no 323910) with an alkaline phosphatase substrate which labels the target probe with a chromogenic substrate and counterstained with hematoxylin to label nuclei. Tissue sections were mounted on slides under coverslips using a permanent, non-aqueous mounting media (VectaMount, Vector Labs) and imaged and analyzed using an Aperio Digital Pathology Slide Scanner (VERSA) and associated software. Percent area of transcript was quantified using a threshold analysis in ImageJ.

Protein Expression (IF)

Slides containing liver samples were processed with standard immunofluorescence protocols. Primary antibodies included anti-V5 tag (Thermo Fisher, R960-25; 1:500). Secondary antibodies included anti-mouse (Thermo Fisher, A-21235; 1:1000). Sections were imaged on a Nikon NiE microscope equipped with a Photometrics CoolSNAP HQ2 camera at 20X. Images were extracted from the liver, with multiple images taken of each tissue from each animal. Percent area of immunoreactivity was quantified using a threshold analysis in ImageJ.

CBC Measurement

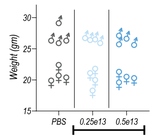
CBC readouts are performed on a Scilvet ABC Hematology Analyzer using mouse whole blood sample.

Histopathological Evaluation

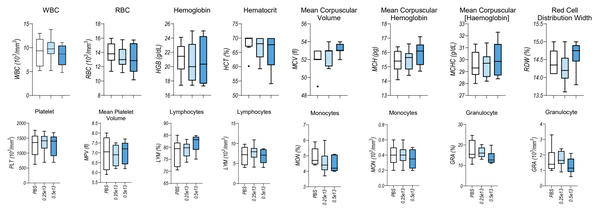
Hematoxylin and eosin (H&E) stained microscopic slides from 34 mice were transferred by Sanford Research to Greenfield Pathology Services, Inc. (GPS) for microscopic examination. Pristima® v7.4.3 was utilized by the Study Pathologist for recording of microscopic findings and table generation. Microscopic findings were given a severity grade based upon a scale of minimal (1), mild (2), moderate (3), marked (4), and severe (5).

M002 GTx has no effect on weight or CBC

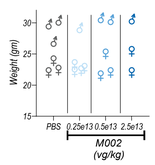
2M Weight



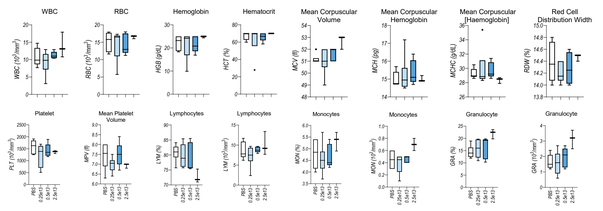
2M CBC Values



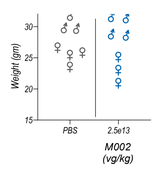
4M Weight



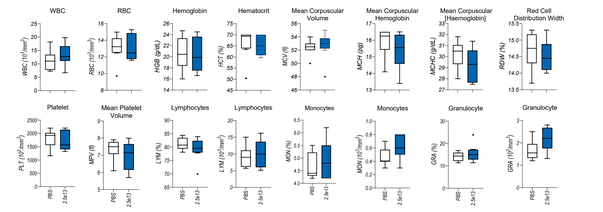
4M CBC Values



6M Weight



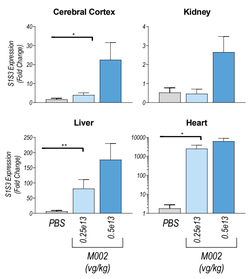
6M CBC Values



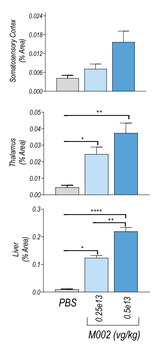
M002 GTx Resulted in S1S3 Phosphotransferase Expression

2M Data

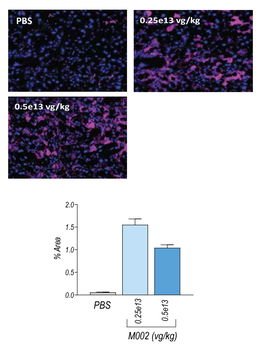
Gene Expression – qRT-PCR



Transcript Expression – BaseScope

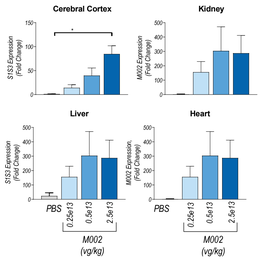


Liver Protein Expression - IHC

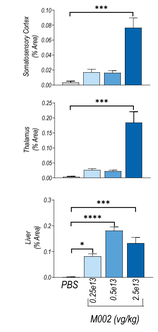


4M Data

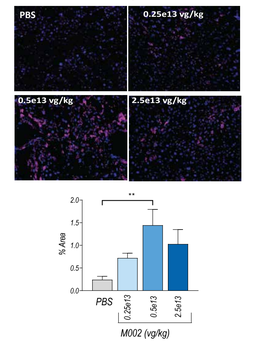
Gene Expression



Transcript Expression - BaseScope

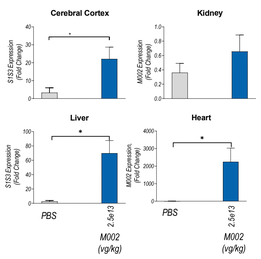


Liver Protein Expression - IHC

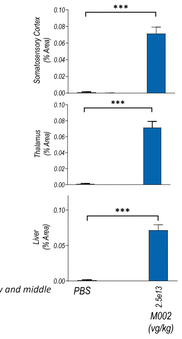


6M Data^

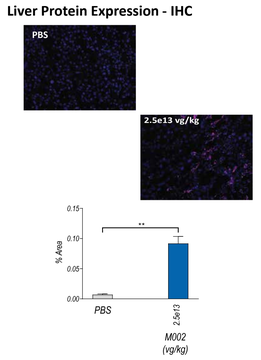
Gene Expression – qRT-PCR



Transcript Expression – BaseScope



Liver Protein Expression - IHC



^For the 6M time point, only the 2.5e13 dose was administered. The low and middle dose were not evaluated at this time point.

Histopathological Evaluation

- Macroscopic Observations

  No macroscopic findings were noted.

- Microscopic Observations

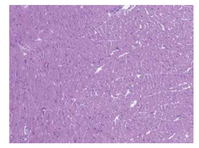
  No test article-related microscopic findings were noted at the 2 month necropsy in animals given 0.25e13 vg/kg or 0.5e13 vg/kg M002.

  At the 4 month necropsy, one male and one female mouse given 2.5e13 vg/kg M002 had mild fibrosis of the myocardium.

  At the 6 month necropsy, males and females given 2.5e13 vg/kg M002 had minimal to mild fibrosis of the myocardium.

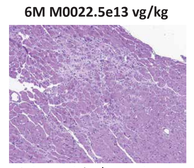
  One male given 2.5e13 vg/kg M002 had mild vacuolation of the pancreatic acinar cells at the 6 month necropsy.

6M PBS Treated



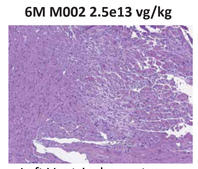
Left Ventricle
Normal. 10X objective

6M M0022.5e13 vg/kg



Interventricular septum
Fibrosis, mild. 10X objective

6M M002 2.5e13 vg/kg



Left Ventricular septum
Fibrosis, mild. 10X objective

Summary

- Expression of M002 in a wild-type mouse did not affect weight gain or CBC
- There was sustained expression of the membrane bound modified GlcNAc-1-phosphotransferase
- For tissues evaluated, the only consistent pathological finding was mild fibrosis in the heart at the highest dose (2.5e13 vg/kg)
- This data suggests continued development of M002 as a gene therapy for MLII is warranted

Next Steps

- Hypoglycemics and proteolytic evaluation of 2, 4 and 6 month serum samples
- Complete M002 efficacy evaluation in the MLII mouse model

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