Gene Transfer to the Central Nervous System for Treatment of GM2 Gangliosidosis

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PROGRAM SUMMARY

A key problem in treating central nervous system (CNS) diseases is the delivery of the therapy. The blood brain barrier (BBB) blocks most biologics and large molecules from freely entering the CNS. In this program, we are developing an *in vivo* gene therapy for GM2 gangliosidosis, a disease that affects tissue throughout the brain and spinal cord. Our program has engineered a gene transfer vector specifically designed for broad distribution within the CNS and has begun investigation on enhanced delivery methods utilizing convective transport of this vector.

Disease Overview:

- GM2 gangliosidosis is a rare, hereditary, neurodegenerative disease caused by a deficiency of a lysosomal enzyme, β-hexosaminidase (HexA), which is required for hydrolysis of anionic GM2 ganglioside.
- HexA deficiency results in lysosomal ganglioside storage and eventual cell death.
- Disease severity varies with the degree of enzyme deficiency. Severe deficiencies cause death in childhood; milder deficiencies present clinically in adulthood with ataxia, muscle atrophy, severe psychiatric events, and cognitive decline.
- There is no cure for the CNS aspects of any lysosomal storage disease.

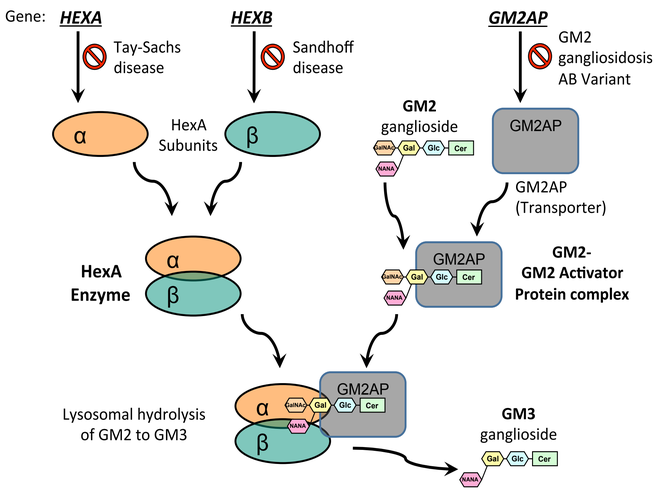


Figure 1: GM2 gangliosidosis background. GM2 ganglioside hydrolysis requires active HexA enzyme in conjunction with the GM2 Activator Protein. The most prevalent forms of the disease are caused by mutations of *HEXA* or *HEXB genes*, which encode the α- and β-subunits of the HexA heterodimer, and are commonly called Tay-Sachs and Sandhoff disease, respectively.

GENE VECTOR DESIGN

Requirements and Constraints:

- The small Adeno-associated virus (AAV) capsid size (~25 nm) is essential for transport within the narrow brain interstitial spaces.
- Self-complementary adeno-associated virus (scAAV) vectors have been shown to have high transduction efficiency in applications with low multiplicity of infection, as would occur with broad vector distribution. scAAV vectors have been shown to be 10 to 100 times more efficient than traditional single stranded AAV vectors.
- The total gene carrying capacity of AAV capsids is only ~4700 bases. Minus the three inverted terminal repeats (439 bases), the regulatory and transgene sequences must total less than ~2130 bases to allow scAAV packaging.
- The Hex α- and β-subunits have a combined transgene length of ~3300 bases, and therefore, only one Hex sub-unit transgene can fit within a scAAV package.
- The scAAV packaging of one Hex subunit requires the promoter, intron, and polyA be less than a total of 460 bases (i.e., 2130 minus *HEXB* transgene length, 1670).

Design of a New Hexosaminidase Subunit Capable of Forming Stable Homodimers that Hydrolyze GM2:

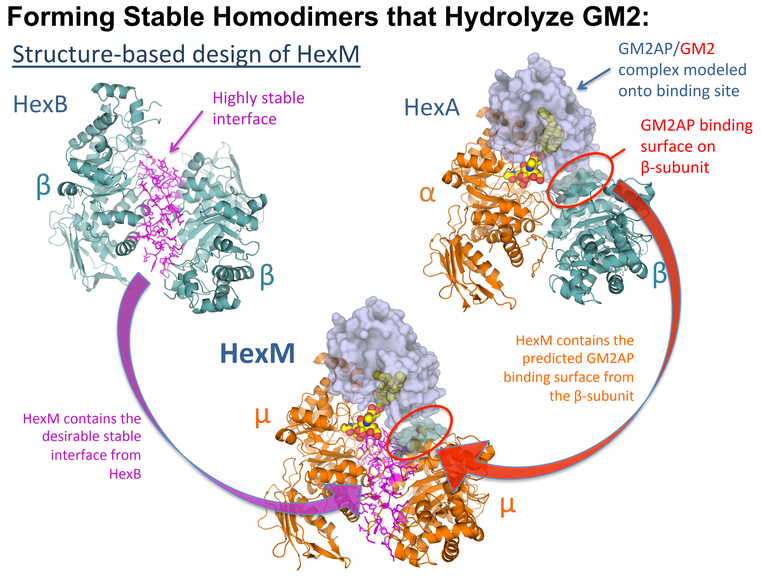
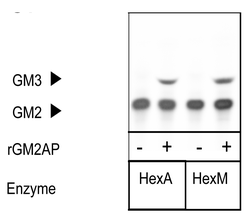


Figure 2: Model of engineered hexosaminidase homodimer (HexM, μ-subunit dimer, patent pending). Shown is the active HexM quaternary complex. The μ-subunit of HexM is derived from the α-subunit (orange) of human HexA, which was modified to include the stable homodimer interface (magenta) formed between the β-subunits (teal) of human HexB and a region from the β-subunit predicted to interact with GM2AP. The GM2AP (grey) is shown bound to GM2 (spheres). A total of 22 amino acids of the α-subunit were modified. Heat stability testing has demonstrated that HexM is as stable as HexB. (Data not shown.)

Figure 3: *In vitro* HPTLC assay of GM2 hydrolysis by human Hex isozymes: HexA and HexM; in the absence (-) or presence (+) of human rGM2AP.



Development of a Short Promoter Intron Combination:

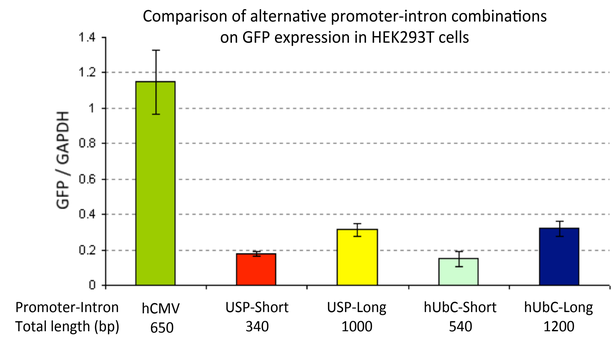


Figure 4: Comparison of Promoter-Intron combinations: A ubiquitous synthesized promoter (USP) plus short intron was developed (patent pending) to allow for scAAV packaging of Hex subunit transgenes. The chart shows the relative GFP expression level with this USP promoter with a short intron compared to the cytomegalovirus (CMV) promoter and the human ubiquitin C (hUbC) promoter with either a short or long intron. Relative GFP mRNA was assessed in HEK293T cells using real-time PCR normalized to levels of GAPDH.

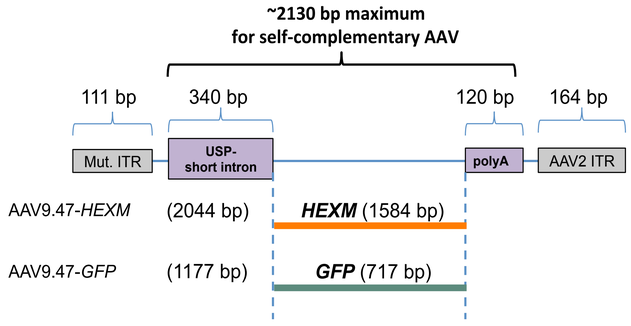


Figure 5: Design of *HEXM* and *GFP* self-complementary AAV9.47 vectors. AAV9.47 is a variant of AAV9 that de-targets the liver. The USP-short intron was used to keep the total length of the HexM and regulatory sequences less than the 2130 maximum.

IV VECTOR INJECTIONS IN NEONATAL SANDHOFF MICE

Study Design:

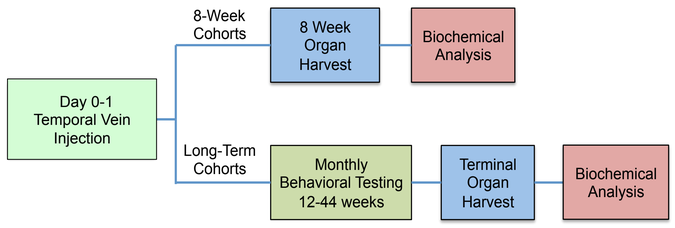


Figure 6: In-vivo study design. To determine the HexM functionality *in vivo*, neonatal Sandhoff mice were injected with 5.0E+10 vg/mouse and either terminated at 8 weeks or at the humane end-point.

Results:

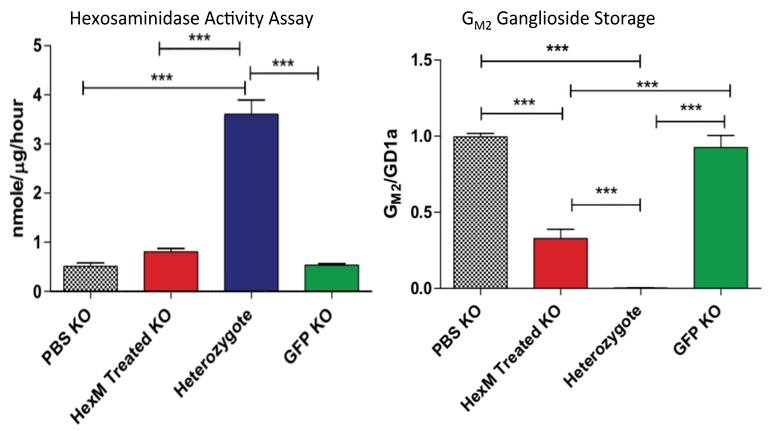


Figure 7: Biochemical Assays. Hexosaminidase (MUGS) activity increased (non-significant) and yet GM2 levels are significantly reduced in the mid-brains of Sandhoff mice 8 weeks after neonatal, intravenous injection of AAV9.47-*HEXM* vector. GM2 levels are expressed as a ratio of the densities of the GM2 versus GD1a spots from HPTLC plates (Y-axis). Mice injected with vehicle, AAV9.47-*HEXM*, and AAV9.47-*GFP* were compared to untreated heterozygous, *hexb*$^{-/+}$ mice (Het). N= 6; *** p< 0.001

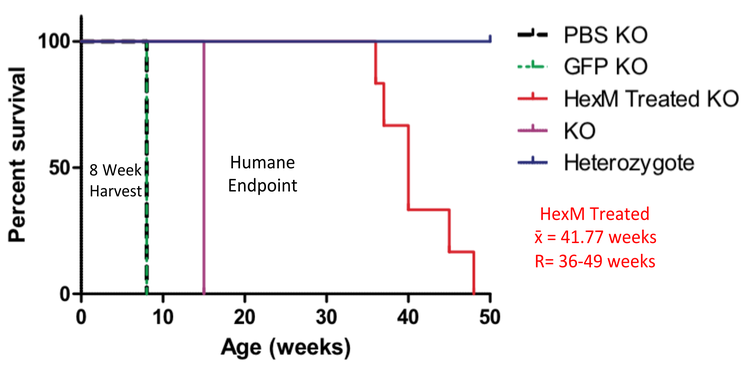


Figure 8: Survival. The survival of Sandhoff (β-subunit knock-out, KO) mice was significantly increased by the neonatal injection of AAV9.47-*HEXM*.

Neonatal Mice Study Limitations:

- The BBB does not mature in mice until several weeks after birth.
- Mice have an alternative pathway for hydrolyzing GM2 ganglioside by sequentially using sialidase and hexosaminidase enzymes. This alternative pathway is not present in humans. It is possible that the GM2 ganglioside reduction observed in mice injected with the *HEXM* vector was utilizing this alternative pathway.
- AAV particles (~25 nm diameter) have a low diffusion coefficient (Stokes-Einstein estimated $D \cong 27\ \mu m^2/s$) which has a calculated average diffusion distance of less than 2 mm during 6 hours in cerebrospinal fluid. Therefore, the distribution of the HEXM vectors in neonatal mice might not be applicable to large animals and humans due to the large differences in the transport characteristic length for the AAV capsid (i.e., differences in the Péclet number) between species.

NEXT STEPS

1. The transport of AAV vectors within the CNS of large animals is highly dependent on the cerebrospinal and interstitial fluid flow. To better understand and utilize this flow in obtaining broad distribution of AAV vectors within the CNS, conduct an analysis of cerebrospinal fluid and interstitial fluid flow using computer modeling techniques (Fig. 9) in order to optimize delivery methods in large animals and man.
2. Validate the modeled delivery methods using injections of AAV9-*GFP* vector in normal sheep.
3. Develop assays to determine the immunogenicity of the AAV9-HEXM vector and the expressed HexM protein, and if needed, explore methods for inducing immune tolerance.
4. Conduct efficacy studies of AAV9-*HEXM* in a sheep model of GM2 gangliosidosis (Hex α-subunit deficient Jacobs sheep).

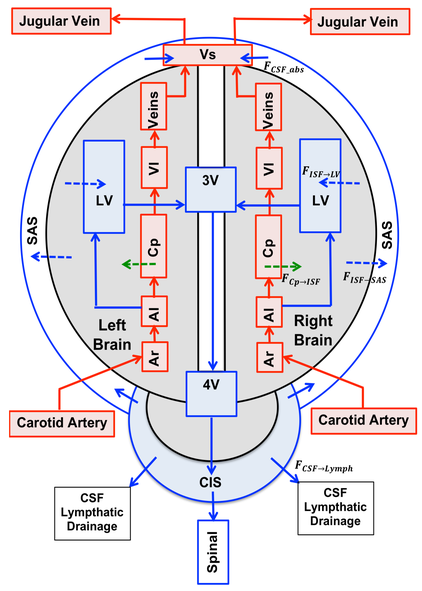


Figure 9: A multi-compartment model of fluid flow within the cranium and spinal column. The grey compartments represent the two phases (cells and ISF) of the parenchyma including the right and left brain, cerebellum, and spinal cord. Compartments outlined in blue represent CSF compartments: Lv – lateral ventricle, 3V – third ventricle, 4V – fourth ventricle, CIS – cisterna magna and basal cisterns, SAS – subarachnoid space, Spinal – spinal canal. The compartments with the blue background are assumed to be well-mixed. Blue arrows depict CSF and ISF flow between compartments. Dashed arrows represent flow that is distributed across a tissue surface. Compartments outlined in red are lumped blood vaculature compartments distributed within the right and left hemispheres: Carotid Artery, Ar – arteries, Al – arterioles, Cp – capillaries, Vl – veinules, Veins, Vs – venus sinus, and Jugular Vein. Red arrows depict the lumped flow between compartments. The green dashed arrows represent flow across the BBB. The directions of the arrows depict positive flow.

RESEARCH SUMMARY

- A new β-hexosaminidase subunit has been developed capable of forming a stable homodimer, HexM (μμ), and hydrolyzing GM2 ganglioside in conjunction with the GM2 activator protein, GM2AP.
- A new short promoter-intron combination has been developed that allows the construction of self-complementary AAV vectors with transgenes as large as ~1670 bp when used with a 120 base polyA. This has allowed the construction of a scAAV vector incorporating the *HEXM* transgene, scAAV9.47-*HEXM.*
- Neonatal Sandhoff mice intravenously injected with scAAV9.47-*HEXM* show a significant reduction in GM2 ganglioside storage in the mid-brain and a significant increase in survival.
- While this *in vivo* study has limitations, it shows the potential of using a relatively non-invasive method for treating GM2 gangliosidosis. Additional model development and research are needed to enhance the delivery of vectors to broad regions of the CNS of large animals with an intact blood brain barrier.

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3. S. J. Gray, V. Matagne, L. Bachaboina, S. Yadav, S. R. Ojeda, R. J. Samulski, Preclinical differences of intravascular AAV9 delivery to neurons and glia: a comparative study of adult mice and nonhuman primates. *Mol. Ther.* **19**, 1058-1069 (2011)