Sandhoff Disease is Corrected in a Murine Model by scAAV9-*HEXM* Gene Transfer

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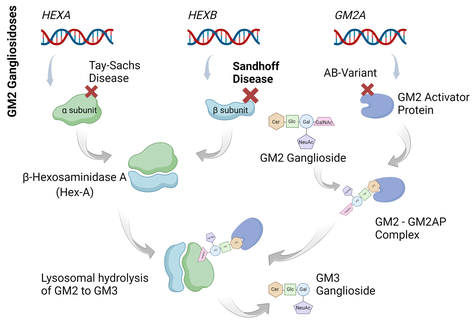


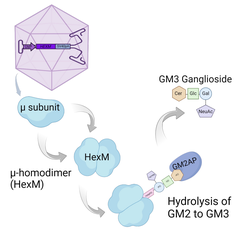
Infantile
Onset: ~ 6 months of age; death: ~ 4 years.
<2% residual HexA activity.
Most prevalent form of SD

Juvenile
Onset: 3-10 years, may survive to adulthood.
2-10% residual HexA activity.

Adult
Onset: Adolescence/ early adulthood.
Slowest progression of the three.
7-10% residual HexA activity.

Sandhoff disease (SD) is caused by the excessive accumulation of GM2 gangliosides in the lysosomes of neuronal cells. Typically, these lipids are hydrolyzed by β-hexosaminidase A (Hex-A), a heterodimer comprised of an α- and a β-subunit. Mutations in the gene encoding either subunit can lead to improper functioning of the enzyme. SD is caused by a mutation in the *HEXB* gene resulting in a deficient or absent β-subunit and subsequent accumulation of GM2 gangliosides. This causes widespread cell death, and consequently progressive symptoms and rapid neurological decline culminating in death.

A homodimer formed by a novel hybrid μ-subunit called HexM, an isoenzyme of human Hex-A, has been recently developed and shown to hydrolyze GM2 gangliosides *in vivo*[1]. Previous studies have determined the effectiveness of gene transfer with the gene, *HEXM,* packaged in a self-complementary adeno-associated viral vector, serotype 9 (scAAV9), through increased life span in a SD mouse model (*Hexb*$^{(-/-)}$) [2].



This study aims to determine the dose response of the scAAV9-*HEXM* treatment in the SD mouse model through dual delivery of treatment via intra-cisterna magna (ICM) and intravenous (IV) routes, along with the ancillary administration of immunosuppressant drugs.

Genotype	N	Treatment Group		Dose via ICM infusion	Dose via IV infusion	Total Dose
Heterozygous	8	Vehicle		0	0	0
Hexb$^{(-/-)}$	9	Vehicle		0	0	0
Hexb$^{(-/-)}$	9	ICM	Low	1.0e11 vg	0	1.0e11 vg
Hexb$^{(-/-)}$	10	ICM	High	2.5e11 vg	0	2.5e11 vg
Hexb$^{(-/-)}$	9	Dual: ICM high + IV	Low	2.5e11 vg	2.5e11 vg	5.0e11 vg
Hexb$^{(-/-)}$	9	Dual: ICM high + IV	Medium	2.5e11 vg	7.5e11 vg	1.0e12 vg
Hexb$^{(-/-)}$	7	Dual: ICM high + IV	High	2.5e11 vg	2.5e12 vg	2.75e12 vg
Hexb$^{(-/-)}$	9	IV only	Low	0	5.0e11 vg	5.0e11 vg
Hexb$^{(-/-)}$	9	IV only	Medium	0	1.0e12 vg	1.0e12 vg
Hexb$^{(-/-)}$	9	IV only	High	0	2.75e12 vg	2.75e12 vg

Table 1. 10 cohorts of mice received concurrent infusions through both ICM and IV routes. There were 3 possible infusates for the ICM route (vehicle, low or high vector dose) and 6 possible infusates (vehicle, 6 different vector doses) for the IV route.

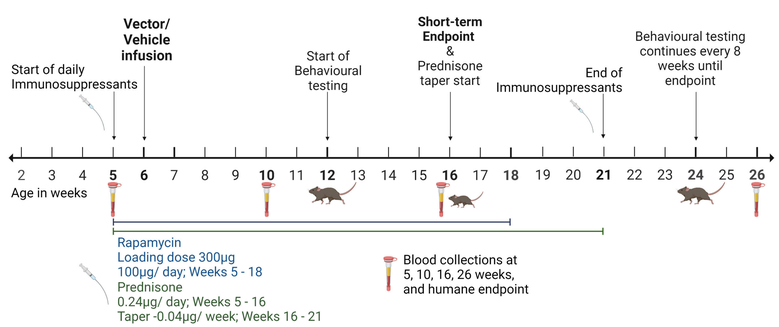


Figure 1. Timeline of Experimental Mice. Baseline blood collection and start of daily immunosuppressant regimen at 5-weeks. Administration of scAAV9-*HexM* or Vehicle infusions at 6-weeks. Immunosuppressant regimen maintained until 18-weeks (Rapamycin) and 21-weeks (Prednisone, tapered). Bimonthly behavioural testing, and blood collections at specific time points until mice reached their humane endpoint. At termination, blood, gross organs, brain, and spinal cord were collected for analysis of GM2 ganglioside accumulation, vector copy number, Hex enzyme activity, cellular and humoral immune response, and histology.

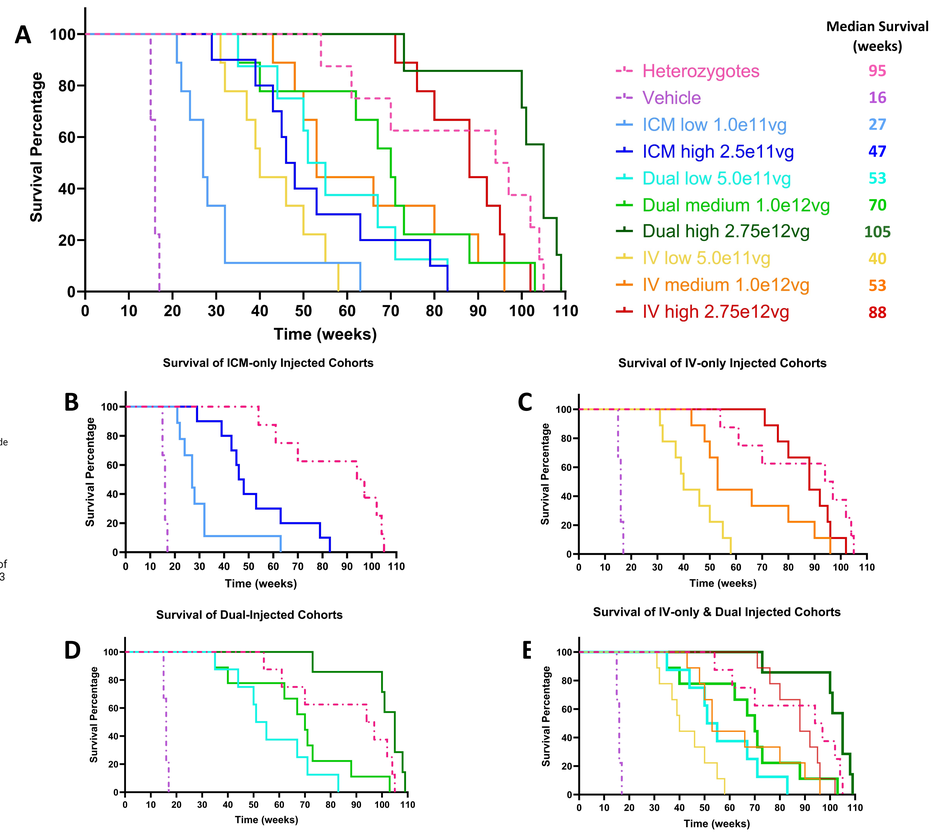


Figure 2. Kaplan-Meier Survival Curves. (A) All cohorts, showing increased survival in all treatment groups compared to the **vehicle-only control group** ($p < 0.001$ in all cases). **(B)** The **ICM low (1.0e11vg)** cohort shows a ~2-fold increase in median survival, and the **ICM high (2.5e11vg)** cohort has a ~3-fold increase. **(C)** Median survival increases 2.5-fold in the **IV low group (5.0e11vg)**, 3.3-fold in the **IV medium group (1.0e12vg)**, and 5.4-fold in the **IV high group (2.75e12vg)**. **(D)** Median survival increases >3-fold in the **dual low (5.0e11vg)** cohort, >4-fold in the **dual medium cohort (1.0e12vg)**, and 6.5-fold in the **dual high (2.75e12vg)** cohort. **(E)** Dose-matched cohorts with log-rank analyses. At the low dose, the dual cohort has higher survival than the IV-only (5.0e11vg; $z = 1.94$, marginally significant at $p = 0.052$). The medium dose has no significant difference (1.0e12vg; $z = 0.41$, $p = 0.68$, n.s.). At the high dose, the dual-injected cohort has a significant increase in survival over the IV-only cohort ($z = 2.71$, $p = 0.00667$). Among the high-dose groups, the **IV high group's** survival was comparable to the **Heterozygous** controls, ($z = 1.16$, $p = 0.25$) while the survival of the **dual high (2.75e12vg)** cohort actually exceeded that of the **Heterozygous** controls ($z = 2.23$, $p = 0.026$).

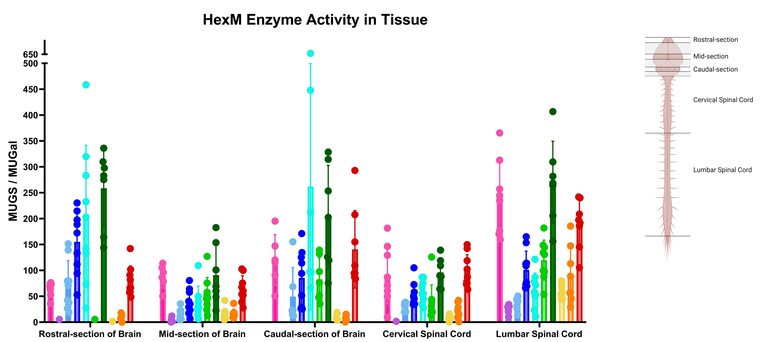


Figure 3. HexM Enzyme Activity in CNS Tissues. In tissue collected at humane endpoints there is evidence of the sustained long-term expression of the *HEXM* gene. Enzyme expression shows a dose response and echoes the survival of cohorts, with the longest-lived cohorts (**IV** and **Dual** high) showing high levels of HexM enzyme activity 2 years post-injection.

Key Findings and Conclusions:

- **The survival proportions show a strong dose-response and a clear benefit to splitting the dose between the ICM and IV routes at the highest total dose (Figure 2).**
- **This study has shown up to a >6-fold increase in median survival following scAAV9-*HEXM* vector infusion in six-week-old Sandhoff mice with survival of the longest-lived cohort (Dual high) exceeding that of the heterozygote control group (Figure 2).**
- **The longest-lived treated cohorts (Dual high and IV high) showed consistent behavioural performance over a two-year lifespan with no observable differences to that of the heterozygote control group (Figure 4).**
- Results of the GM2 ganglioside accumulation and Hex activity assays echo the survival of the respective cohorts, with indication of dose-responses and significant differences seen in multiple treated cohorts (Figures 4 & 5).
- The equivalent survival and behavioural outcomes to the heterozygote group, coupled with the biochemical results, indicate that **in the two high-dose cohorts (Dual high and IV high) Sandhoff disease has been corrected.**
- This novel gene therapy for Sandhoff and Tay-Sachs Disease has tremendous implications for improved survival and quality of life in a clinical setting.

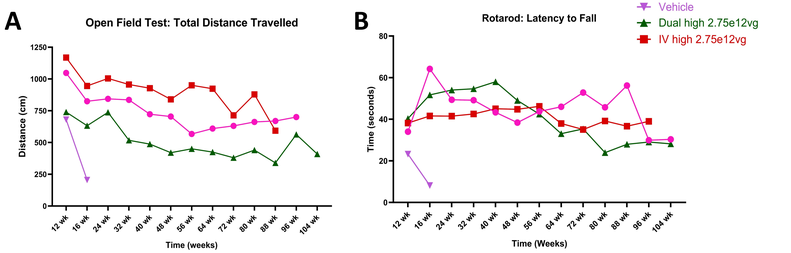


Figure 4. Behavioural Testing. Showed that the capabilities of **IV high** and **Dual high** treated mice were comparable to the **heterozygous** mice. **(A) Open Field Testing** showed no significant differences between cohorts for their Total Distance Travelled. Nor for the other parameters, not shown: Resting time, Mean speed, and Maximum speed. **(B) Rotarod testing** likewise showed no significant difference regarding their Latency to Fall, nor in the parameters not shown: Distance and End RPM.

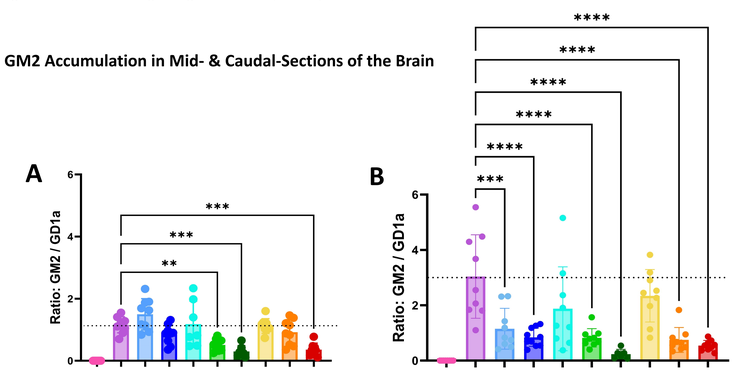


Figure 5. GM2 Ganglioside Accumulation. (A) In the mid-section of the brain, multiple treated cohorts show a significant decrease in accumulated GM2 gangliosides compared to **vehicle-only controls**, the greatest being **Dual high** with a >3.8-fold decrease (*** $p < 0.001$). **(B) In the caudal-section of the brain**, almost all treated cohorts have a significant decrease in GM2 accumulation compared to **vehicle-only controls**, the greatest being a >12.9-fold decrease in the **Dual high** cohort (**** $p < 0.0001$). Samples were collected at humane endpoint, at which stage GM2 ganglioside accumulation is expected to be at its highest.

Acknowledgements

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References

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[2] K. J. L. Osmon *et al.*, "Systemic Gene Transfer of a Hexosaminidase Variant Using an scAAV9.47 Vector Corrects GM2 Gangliosidosis in Sandhoff Mice," *Human Gene Therapy*, vol. 27, no. 7, pp. 497–508, Jul. 2016, doi: 10.1089/hum.2016.015.

GM2 Gangliosidoses, HexM, and Timeline Figures created with **BioRender.com**