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

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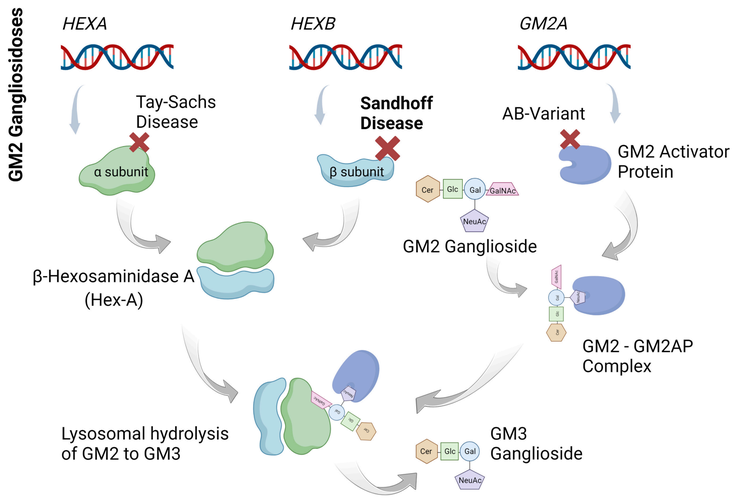


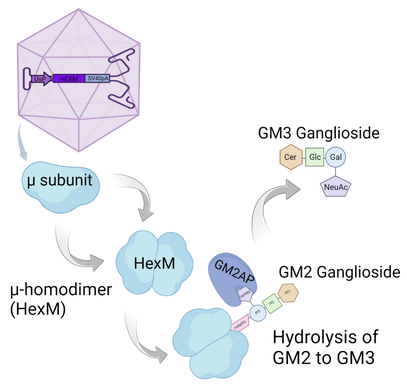
Infantile
Onset: ~ 6 months of age; death: ~ 4 years.
<2% residual HexA activity.
Most prevalent form of SD

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.

Cohort & 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 total dose	0	5.0e11 vg	5.0e11 vg
Hexb$^{(-/-)}$	9	IV only, medium total dose	0	1.0e12 vg	1.0e12 vg
Hexb$^{(-/-)}$	9	IV only, high total dose	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, 5 different vector doses) for the IV route.

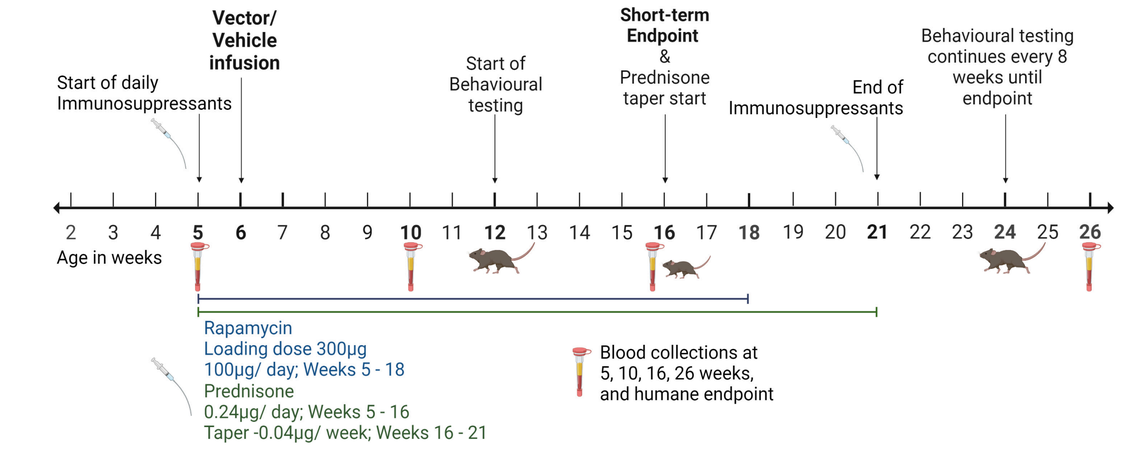


Figure 1. Timeline of Experimental Mice. Baseline blood collection and start of daily immunosuppressant regimen at 5-weeks. Administration of *scAAV9-HexM* or Vehicle infusions at 6-weeks. Immunosuppressant regimen maintained until 18-weeks (Rapamycin) and 21-weeks (Prednisone, tapered). Bimonthly behavioural 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.

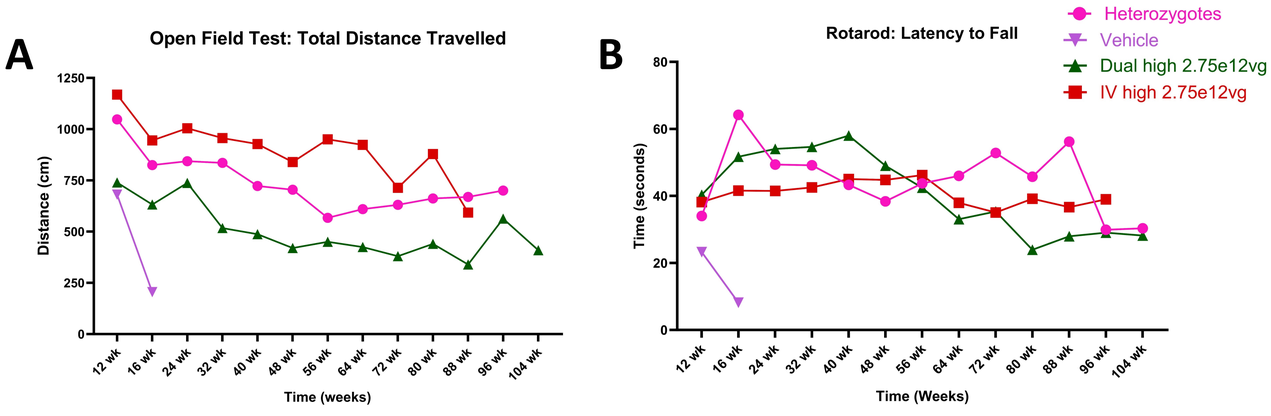


Figure 2. Behavioural Testing. Showed that the capabilities of **IV high** and **Dual high** treated mice were comparable to the **heterozygous** mice. **(A) Open Field Testing** showed no significant differences between cohorts for their Total Distance Travelled. Nor for the other parameters, not shown: 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.

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 when compared to the equivalent total dose in the IV-only cohorts (Figure 5).**
- **This study has shown up to a >6-fold increase in mean survival following *scAAV9-HEXM* vector infusion in six-week-old Sandhoff mice with survival of the longest-lived cohorts similar to the heterozygous control group (Figure 5).**
- **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 2).**
- Results of the GM2 ganglioside accumulation assays echo the survival of the respective cohorts, with significant differences seen in multiple treated cohorts (Figure 6). The greatest reduction in GM2 gangliosides is seen in the **Dual high** cohort, which likewise was the longest surviving cohort, surpassing even the **heterozygous** control group.
- The equivalent survival and behavioural outcomes to the **heterozygous** 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.

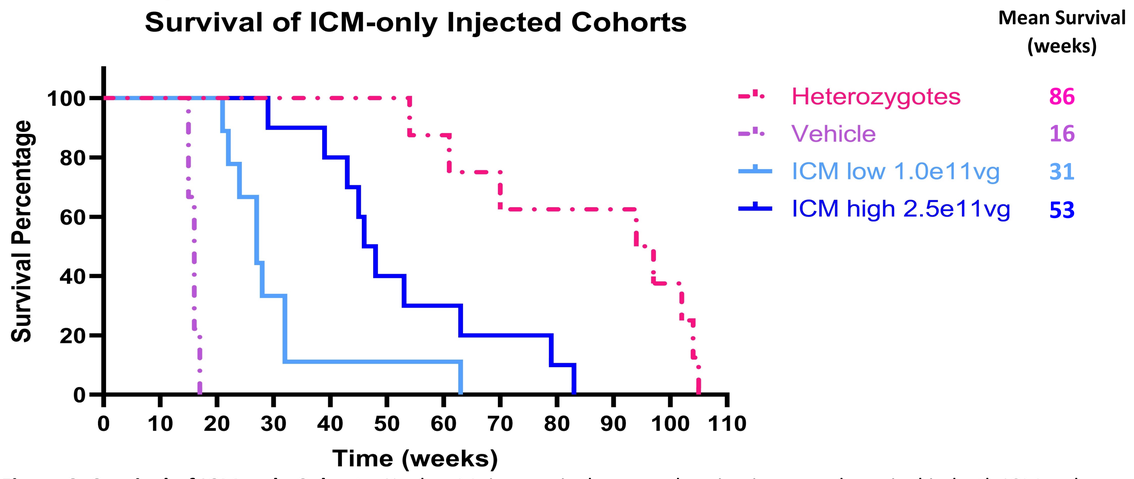


Figure 3. Survival of ICM-only Cohorts. Kaplan-Meier survival curves showing increased survival in both ICM-only cohorts. The **ICM low (1.0e11vg)** cohort shows a ~2-fold increase in mean survival, and the **ICM high (2.5e11vg)** cohort has a >3-fold increase compared to the **vehicle-only control** group.

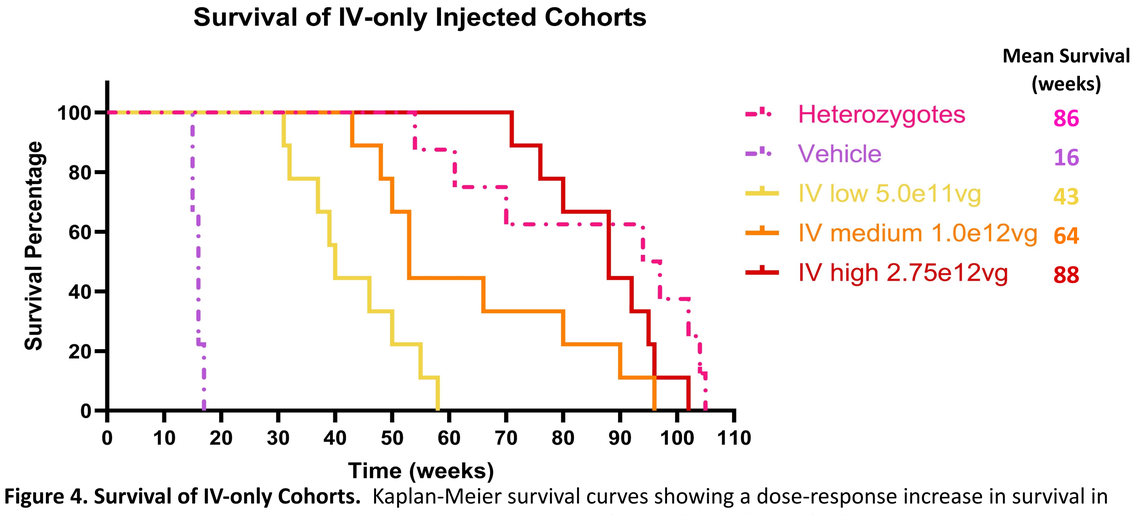


Figure 4. Survival of IV-only Cohorts. Kaplan-Meier survival curves showing a dose-response increase in survival in treatment groups compared to the **vehicle-only control** group ($p < 0.001$ in all cases). Mean survival increases almost 3-fold in the **IV low group (5.0e11vg)**, 4-fold in the **IV medium group (1.0e12vg)**, and almost 6-fold in the **IV high group (2.75e12vg)**. There is no significant difference between the **IV high (2.75e12vg)** cohort and the **heterozygous** cohort.

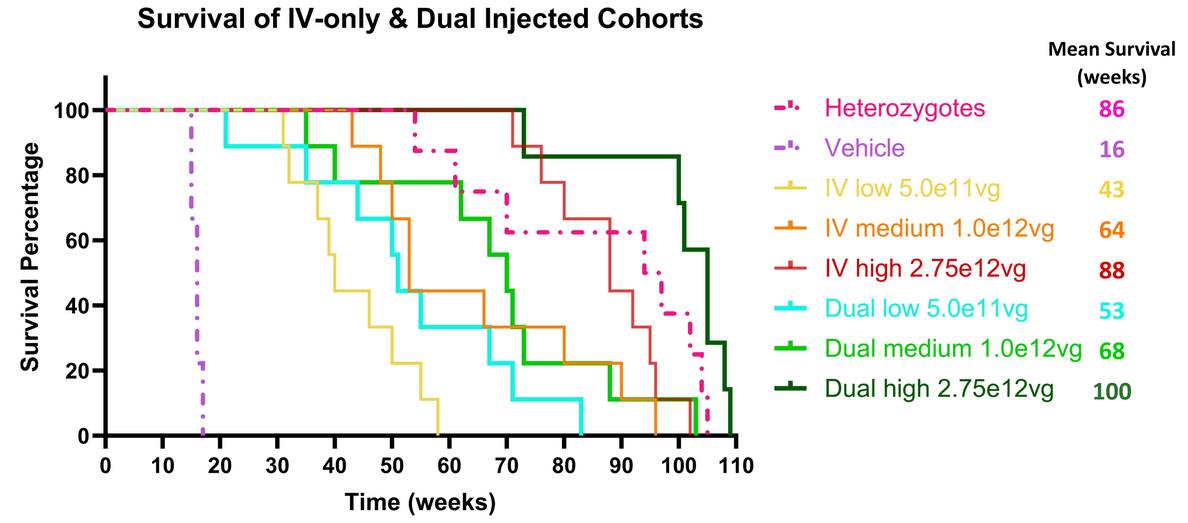


Figure 5. Survival of Dual-injected Cohorts Compared to IV-only. Kaplan-Meier survival curves showing a dose-response increase in survival in dual-treated groups compared to the **vehicle-only control** group ($p < 0.001$ in all cases). Mean survival increases >3-fold in the **dual low (5.0e11vg)** cohort, >4-fold in the **dual medium cohort (1.0e12vg)**, and >6-fold in the **dual high (2.75e12vg)** cohort. Treatments split between the IV and ICM routes show a greater increase in mean survival compared to the equivalent dose delivered entirely IV.

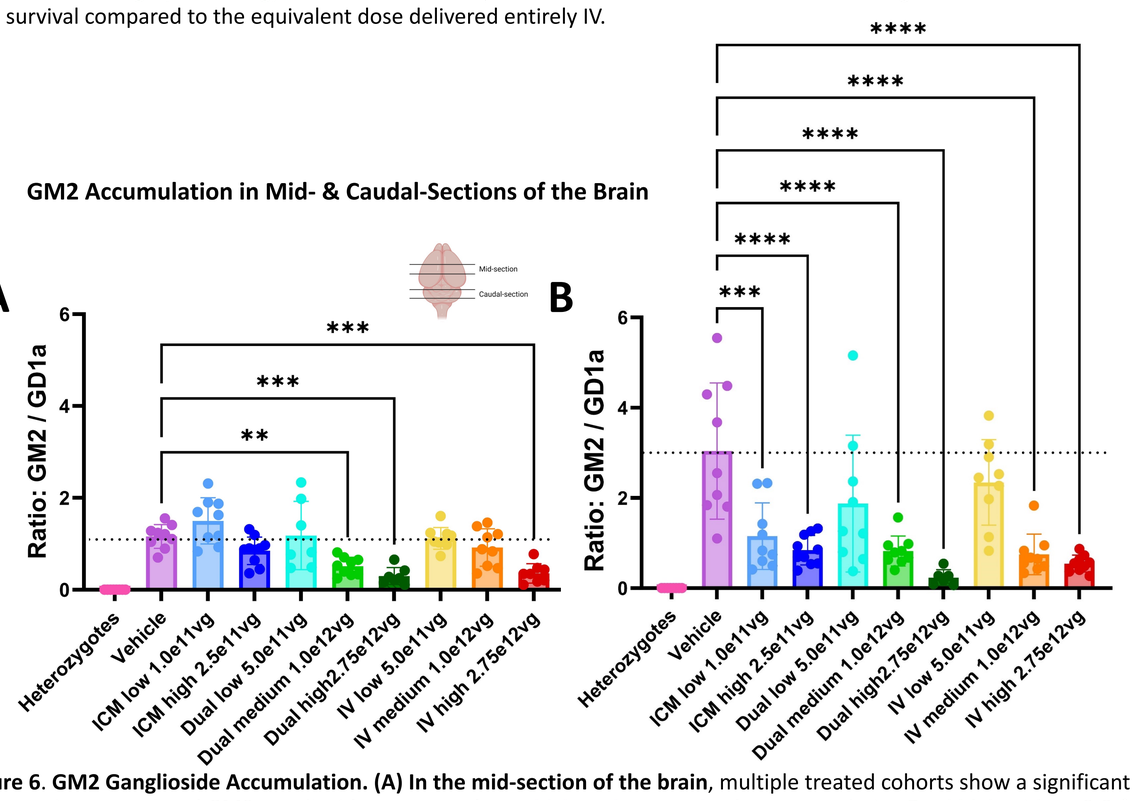


Figure 6. **GM2 Ganglioside Accumulation. (A) In the mid-section of the brain**, multiple treated cohorts show a significant decrease in accumulated GM2 gangliosides compared to **vehicle-only controls**, the greatest being **Dual high** with a >3.8-fold decrease (*** $p < 0.001$). **(B) In the caudal-section 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

[1] M. B. Tropak *et al.*, "Construction of a hybrid β-hexosaminidase subunit capable of forming stable homodimers that hydrolyze GM2 ganglioside in vivo," *Molecular Therapy - Methods and Clinical Development*, vol. 3, p. 15057, Mar. 2016, doi: 10.1038/mtm.2015.57.

[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, CNS sections, and Timeline Figures created with **BioRender.com**