Assessment of Immune Responses to scAAV9-HEXM in Tay-Sachs Mice

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INTRODUCTION

Tay-Sachs and Sandhoff diseases are neurodegenerative diseases resulting from deficiency of the heterodimeric isoenzyme β-Hexosaminidase A and the subsequent accumulation of GM2 gangliosides.

Previous AAV gene therapy approaches providing one of the two subunits failed to generate sufficient heterodimer to treat the disease. Providing both subunits is challenging due to size.

A novel enzyme was designed that instead consisted of a stable homodimer (Figure 1). This novel enzyme (HexM) was shown to hydrolyze GM2 independent of the endogenous subunit (Tropak et al.,2016). Treatment with scAAV9-*HEXM* provided therapeutic benefit in mouse models of both Tay-Sachs and Sandhoff diseases (Karumuthil-Melethil et al., 2016; Osmon et al., 2016)

The goal of this study was to determine whether the introduction of the novel HexM protein induces an immune response that may interfere with long-term therapeutic efficacy.

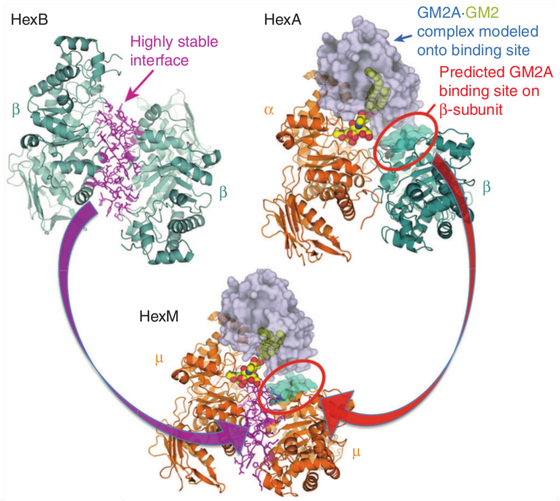

Figure 1. **Model of HexM.**
Figure taken from Tropak MB, et al. (2016) Construction of a hybrid β-hexosaminidase subunit capable of forming stable homodimers that hydrolyze GM2 ganglioside *in vivo*. *Mol. Ther. Meth. Clin. Devel.* **3**: 15057.

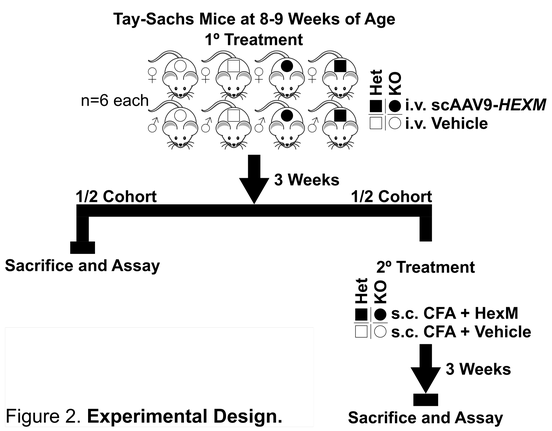

Figure 2. **Experimental Design.**

RESULTS

Table 1. **Composition of HexM Peptide Pools.**

	# Peptides in the Pool	100% Homology to Human HexA	100% Homology to Mouse HexA
Mouse-Human Homologous HexA Peptides	5	Yes	Yes
Novel HexM Peptides	15	No	No
Human-Specific HexA Peptides	32	Yes	No

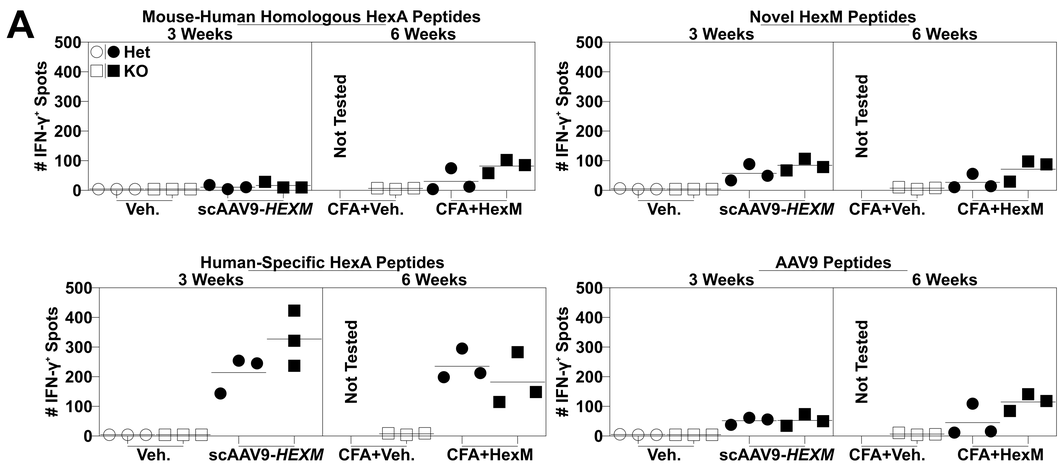

Figure 3. **scAAV9-*HEXM* therapy elicits T cell responses.**
A, ELISPOT for IFN-γ⁺ splenocytes following 48 hr stimulation with the indicated peptide libraries as described in Table 1 (each peptide at 5 μg/mL).
B, Proliferation assay for CD4⁺ and CD8β⁺ splenocytes after 48 hr stimulation with the total HexM peptide library. The percentage of cells having gone through at least one generation (CFSE dilution) is shown.

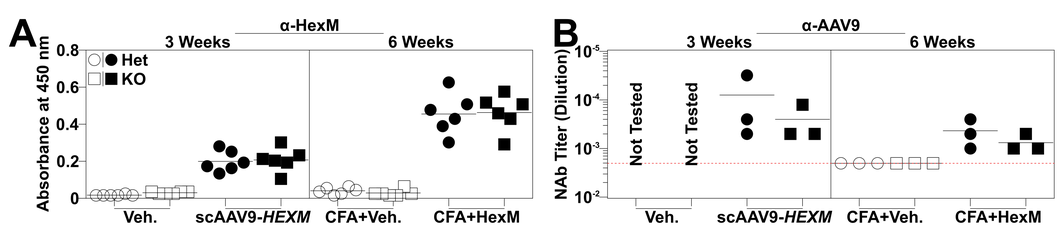

Figure 4. **scAAV9-*HEXM* therapy elicits antibody responses against HEXM and AAV9.**
A, ELISA for anti-HexM antibodies. Sheep anti-HexA antibody binding to HexM was used to generate the standard curve.
B, Neutralizing antibody assay for anti-AAV9 antibodies. AAV9-GFP was incubated with serial dilutions of serum and then used to infect Lec2 cells. The dilution at which GFP⁺ Lec2 cells fell to 50% of the positive control was considered the neutralizing antibody titer. Dashed red-line indicates lowest dilution tested.

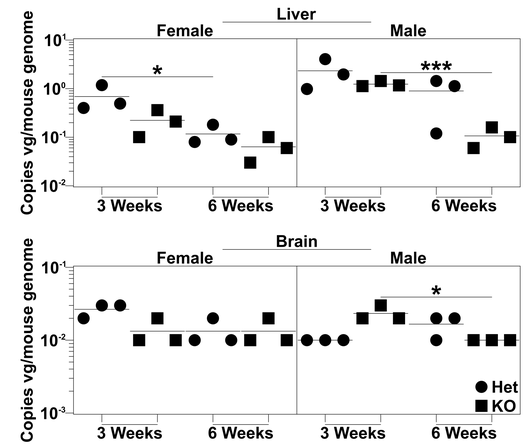

Figure 5. **Loss of *HEXM* transgene in liver and brain over time.**
Liver (top panel) and brain (bottom panel) samples were collected at necropsy and DNA was harvested. qPCR was used to determine the number of vector genomes per mouse genome present in the sample. Data from vehicle-treated mice are not shown as no vector genomes were detected in those samples.
Differences in group means were determined by unpaired Student's t test: *, p<0.05; ***, p<0.001.

CONCLUSIONS

scAAV9-*HEXM* therapy induces IFN-γ⁺ T cell as well as antibody responses against both HexM and AAV9.

The dominant IFN-γ⁺ T cell response was towards human-specific HexA peptides, but a clear response to the novel HexM peptides was observed.

The immune response may negatively impact the therapeutic efficacy of HexM.

The clear presence of an immune response against the human HexA peptides suggests a real danger for the treatment of null patients; even if *HEXA* or *HEXB* were delivered instead of *HEXM*.

FUTURE DIRECTIONS

Track the magnitude of T and B cell responses over-time in response to scAAV9-*HEXM* therapy.

Dissect the specific responses of CD4⁺ and CD8⁺ T cells.

Provide immunosuppressive drugs to prevent the formation of anti-HexM T and B cell responses.

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