Antisense Suppression of GFAP as a Therapeutic Strategy for Alexander Disease

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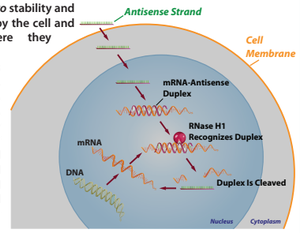
ABSTRACT - POSTER #S275

Alexander disease is a fatal leukodystrophy caused by autosomal dominant gain-of-function mutations in the gene for glial fibrillary acidic protein (GFAP), an intermediate filament protein primarily expressed in astrocytes of the central nervous system. A key feature of pathogenesis is over-expression and accumulation of GFAP, with formation of characteristic cytoplasmic aggregates known as Rosenthal fibers. We have used mouse models with knock-in mutations corresponding to known human GFAP mutations to explore the utility of antisense suppression of GFAP expression as a therapeutic strategy for this disorder. Antisense oligonucleotides were designed to target various regions of the murine Gfap transcript, and screened using primary mouse cortical cultures. Lead oligonucleotides were then tested for ability to reduce GFAP transcripts and protein, first in wild type mice with normal levels of GFAP, and then in adult mutant mice with established pathology and elevated levels of GFAP. Nearly complete and long-lasting elimination of GFAP occurred following single bolus intracerebroventricular injections, with reversal of Rosenthal fibers and downstream markers of microglial and other stress responses. Antisense suppression therefore shows great promise as a therapeutic approach for Alexander disease.

BACKGROUND

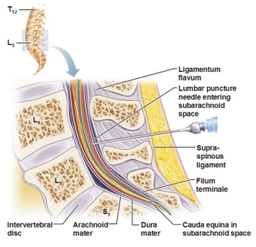
Antisense Mechanism of Action
RNase H1-mediated Degradation

ASOs are DNA oligomers that are chemically modified for *in vivo* stability and efficacy. ASOs are taken up by the cell and reach the nucleus, where they hybridize to the targeted RNA. The RNA-DNA hybrid is recognized by RNase H1, which cleaves the RNA strand. The resulting RNA fragments are rapidly degraded, leading to a loss of the mRNA and reduced expression of the encoded protein. The ASO released from the hybrid is stable and free to hybridize another target RNA molecule.



Mode of Delivery - Lumbar Puncture

Ionis' Neurology clinical programs deliver ASOs directly to the cerebrospinal fluid via lumbar puncture every 3 to 6 months. SPINRAZA® is an Ionis-developed ASO-based therapy approved by the FDA for the treatment of Spinal Muscular Atrophy. Ionis has on-going clinical trials in Huntington's Disease, Alzheimer's Disease and ALS.

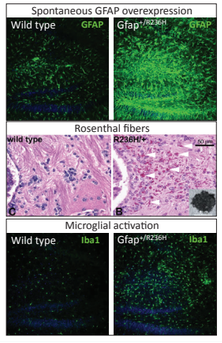


Features of Alexander Disease (AxD) Knockin Mouse Model

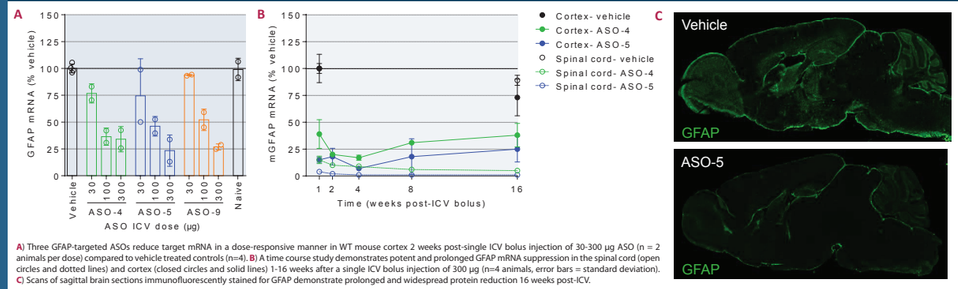
GFAP$^{+/R236H}$ mice carry a point mutation in Gfap that is orthologous to a common human Alexander disease-causing mutation.

The mice exhibit:

- ▲ Spontaneous GFAP overexpression
- ▲ Rosenthal fibers
- ▲ Activation of stress response
- ▲ Microglial activation/recruitment

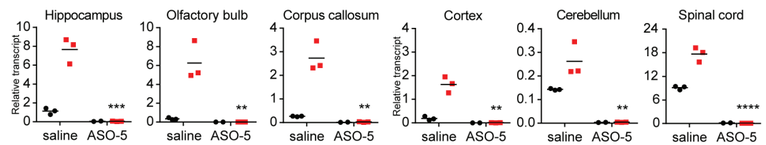


GFAP targeted ASOs cause significant and prolonged suppression of GFAP in WT mice



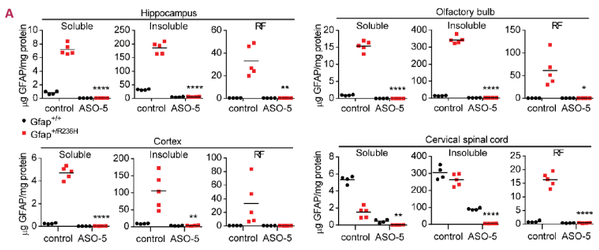
A) Three GFAP-targeted ASOs reduce target mRNA in a dose-responsive manner in WT mouse cortex 2 weeks post-single ICV bolus injection of 30-300 µg ASO (n = 2 animals per dose) compared to vehicle treated controls (n=4). **B)** A time course study demonstrates potent and prolonged GFAP mRNA suppression in the spinal cord (open circles and dotted lines) and cortex (closed circles and solid lines) 1-16 weeks after a single ICV bolus injection of 300 µg (n=4 animals, error bars = standard deviation). **C)** Scans of sagittal brain sections immunofluorescently stained for GFAP demonstrate prolonged and widespread protein reduction 16 weeks post-ICV.

GFAP targeted ASOs reduce GFAP mRNA in AxD model mice

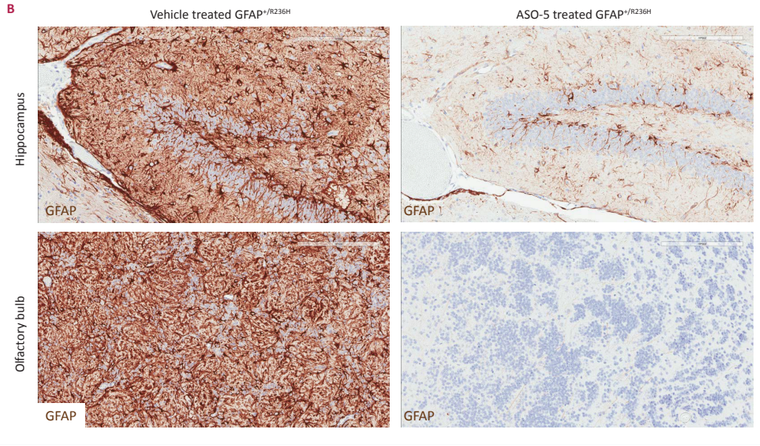


Gfap$^{+/R236H}$ (red squares) and *Gfap*$^{+/+}$ (black circles) littermates were treated with 500 µg ASO-5 or saline at 12 weeks of age, and transcript expression analyzed by qRT-PCR 2 weeks post-injection (n = 2-3). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 by 2-tailed unpaired t-test of control vs ASO treated *Gfap*$^{+/R236H}$ mice

ASOs significantly reduce GFAP protein in knock-in AxD mice

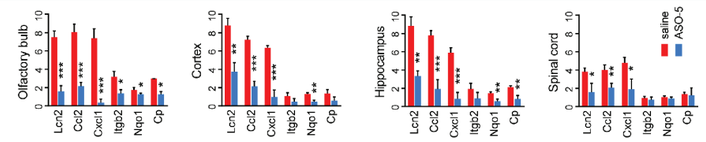


A) Mice were treated with either 500 µg ASO-5, a non-targeted control ASO, or saline at 8 weeks of age, and CNS regions collected 8 weeks post-treatment. Tissue lysates were fractionated into Triton-X-100 soluble, Triton insoluble (urea soluble), and RF-enriched (SDS soluble) fractions, and GFAP protein measured by ELISA (values from saline and control samples were combined for analysis, n = 4-5 males). Asterisks represent 2-tailed unpaired t-test comparing control versus ASO treated *Gfap*$^{+/R236H}$ mice *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.



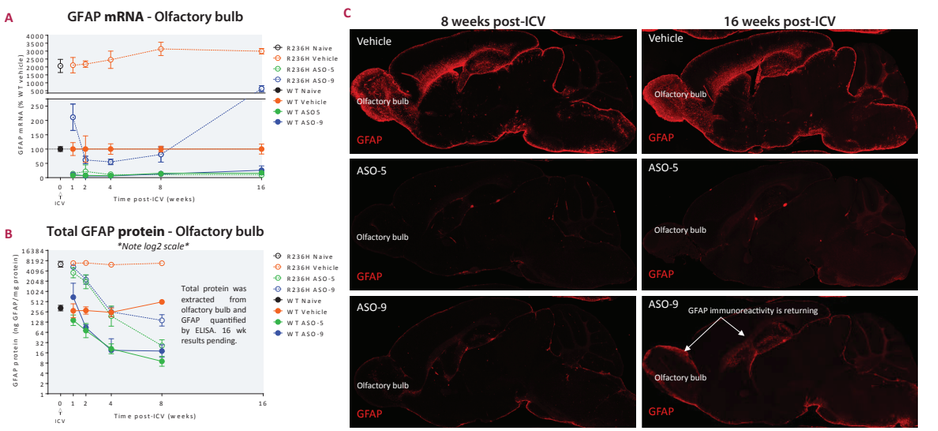
B) *Gfap*$^{+/R236H}$ littermates were given a single ICV bolus of vehicle (left) or 500 µg ASO-5 (right) and sacrificed 8 weeks later. Immunohistochemistry for GFAP demonstrates marked reduction of GFAP protein in knockin mice in brain regions where it is dramatically overexpressed, such as the hippocampus (upper panels) and olfactory bulb (lower panels).

Suppression of Gfap transcript leads to reduction in stress response



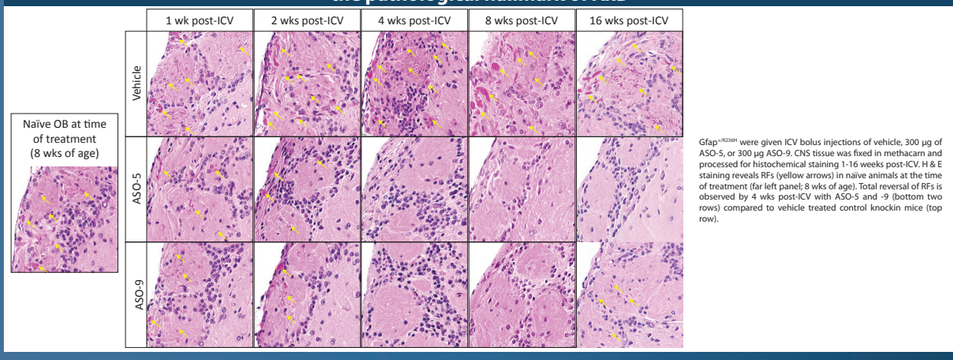
Fold-change for stress and immune-related transcripts in brain and spinal cord from ASO and saline-treated *Gfap*$^{+/R236H}$ mice compared to saline-treated *Gfap*$^{+/+}$ mice (log 2 scale, n = 3 mice treated at 12 weeks, 500 µg ASO-5, 2 weeks post-treatment). Asterisks 2-tailed unpaired t-test comparing saline versus ASO-treated *Gfap*$^{+/R236H}$ mice, *p<0.05, **p<0.01, and ***p<0.001. Error bars = standard deviation.

GFAP ASOs induce marked and prolonged GFAP suppression in knockin AxD mice



Gfap$^{+/R236H}$ and Gfap$^{+/+}$ littermates were given ICV bolus injections of vehicle, 300 µg of ASO-5, or 300 µg ASO-9. CNS regions were collected 1-16 weeks post-ICV. **A)** GFAP mRNA level was measured using qRT-PCR in various CNS regions (the worst affected area- olfactory bulb- is shown; other regions showed similar expression patterns). ASO-5 (green) reduces GFAP mRNA in Gfap$^{+/R236H}$ (open circles, dotted lines) to below WT levels (orange closed circles, solid line) from 1 to 16 wks, whereas ASO-9 (blue) did so only between 2-8 wks post-ICV. **B)** Total GFAP protein was measured by ELISA. It is reduced in Gfap$^{+/R236H}$ to WT levels by 4 wks post-ICV with both ASOs and levels continue to decrease out to 8 wks. 16 wk data is pending. **C)** Immunofluorescent staining in Gfap$^{+/R236H}$ of GFAP demonstrates excellent GFAP suppression with both ASOs at 8 wks post-ICV (left panels), and the return of GFAP immunoreactivity by 16 wks post-ICV (right panels) only with ASO-9.

GFAP ASOs induce rapid and sustained clearance of Rosenthal fibers (RFs), the pathological hallmark of AxD



Gfap$^{+/R236H}$ were given ICV bolus injections of vehicle, 300 µg of ASO-5, or 300 µg ASO-9. CNS tissue was fixed in methacarn and processed for histochemical staining 1-16 weeks post-ICV. H & E staining reveals RFs (yellow arrows) in naïve animals at the time of treatment (far left panel; 8 wks of age). Total reversal of RFs is observed by 4 wks post-ICV with ASO-5 and -9 (bottom two rows) compared to vehicle treated control knockin mice (top row).

SUMMARY OF FINDINGS

GFAP targeted ASOs:

- ▲ Induce marked and sustained reduction of GFAP mRNA and protein, even within the context of spontaneous overexpression in AxD knockin mice
- ▲ Reduce expression of stress response genes in AxD knockin mice after only 2 wks of treatment
- ▲ Clear Rosenthal fibers, the pathological hallmark of AxD, 4 wks post-treatment

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