Intrathecal delivery of an AAV encoding human ABCD1 shows dose-responsive expression and activity in a mouse model of Adrenomyeloneuropathy

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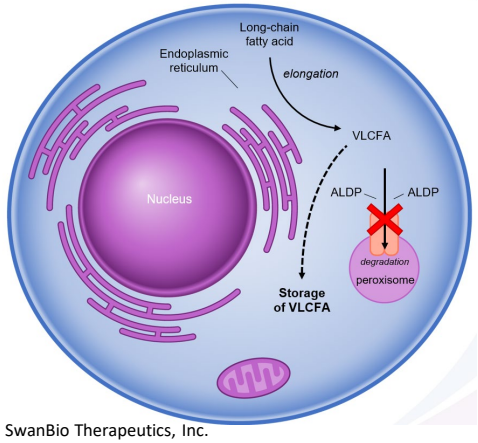
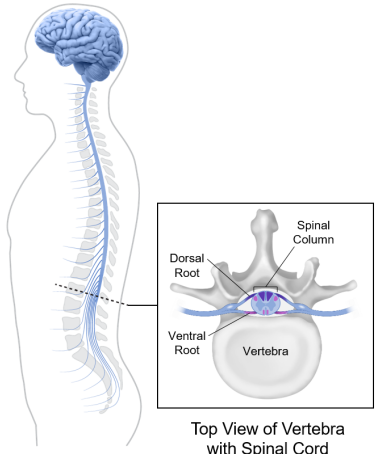
Introduction and Background on Adrenomyeloneuropathy

- X-linked adrenoleukodystrophy (ALD) is an inherited neurodegenerative disease caused by mutations in the *ABCD1* gene encoding a peroxisomal membrane transporter for very long-chain fatty acids (VLCFA).
- Adrenomyeloneuropathy (AMN) is the most frequent clinical manifestation of ALD affecting virtually all adult males and >80% of females.
- Adrenomyeloneuropathy is characterized by adult onset slowly progressive spinal cord disease leading to loss of mobility, incontinence, and debilitating pain.
- There are currently no approved treatments for AMN.
- We are developing SBT101 gene therapy for AMN and herein present preclinical data supporting a potential therapy.

Introduction and Background on AMN/SBT101

Molecular mechanisms associated with pathogenesis of X-ALD





Top View of Vertebra with Spinal Cord

- Mutations in the *ABCD1* gene cause dysfunction of the transporter protein resulting in impaired degradation and subsequent build-up of very long-chain fatty acids (VLCFA).
- In the absence of functional ABCD1:
  - Increased VLCFA levels
  - Changes in mitochondrial DNA levels, a marker of oxidative stress, in mice
  - Dying-back axonopathy in adults

The SBT101 Gene Therapy Vector:

- AAV9 vector encoding hABCD1

We Present an Examination of SBT101's Activity in:

- Mixed glial cultures derived from *Abcd1*-knockout mice
  - hABCD1 expression levels
  - VLCFA levels
- *Abcd1*-knockout mice following intrathecal bolus delivery
  - hABCD1 expression levels
  - Mitochondrial DNA levels
  - VLCFA levels

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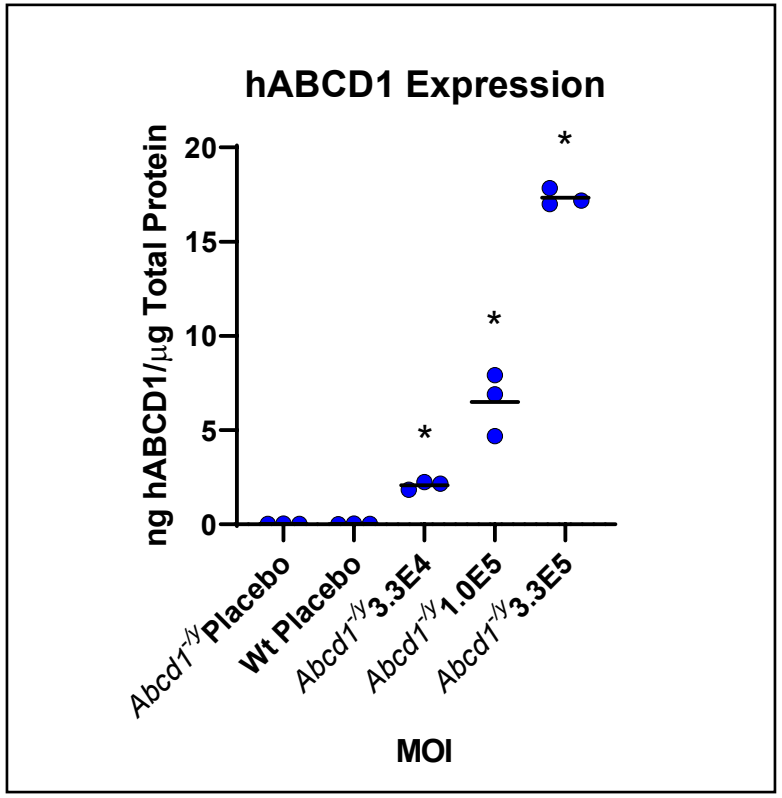
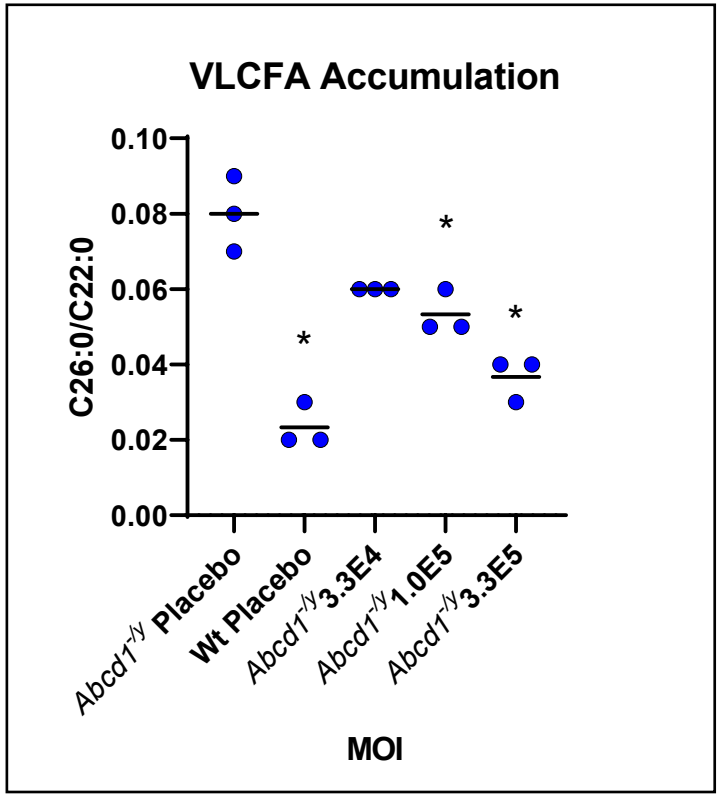
The Impact of a AAV9-*ABCD1* Therapy in a Disease Model *Ex Vivo* Glial Cell Culture

Model: Mixed glial cultures from *Abcd1*-knockout mice accumulate VLCFA levels like human AMN disease

Experiment: SBT101 transduction of glial cell cultures to examine VLCFA level correction





Results:

- Transduction of SBT101 results in dose-dependent expression of hABCD1 and a concomitant reduction of VLCFA levels

* $p < 0.05$ vs *Abcd1*$^{-/y}$ Placebo

MOI = multiplicity of infection

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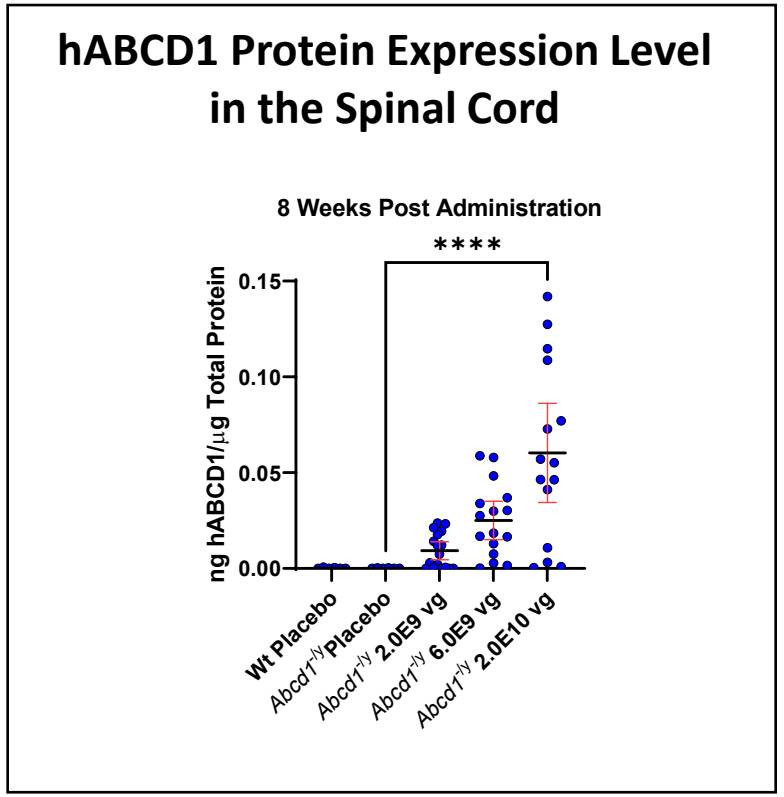
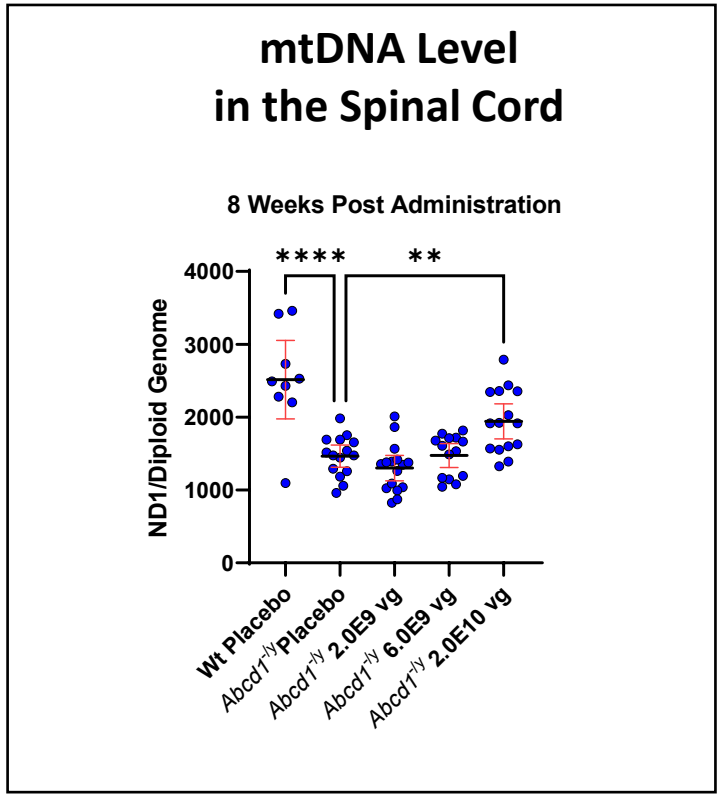
In Vivo Translation – Short Term Expression in Aged Mice

Experiment: Intrathecal bolus dosing of SBT101* in control and knockout mice at 18 - 24 months of age





Results:

- hABCD1 protein was detected in a dose dependent manner in whole spinal cord at 8 weeks
- Altered mtDNA levels were detected in KO-placebo at 8 weeks post-infusion
- A dose dependent increase in mtDNA levels towards normal was detected in whole spinal cord at 8 weeks

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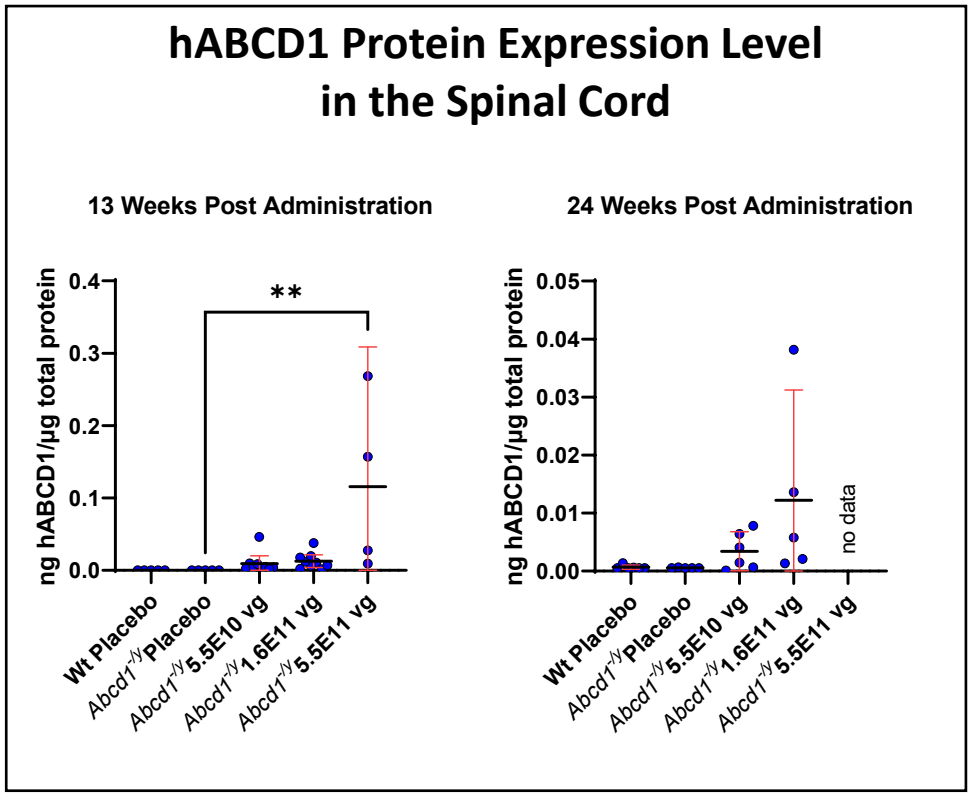
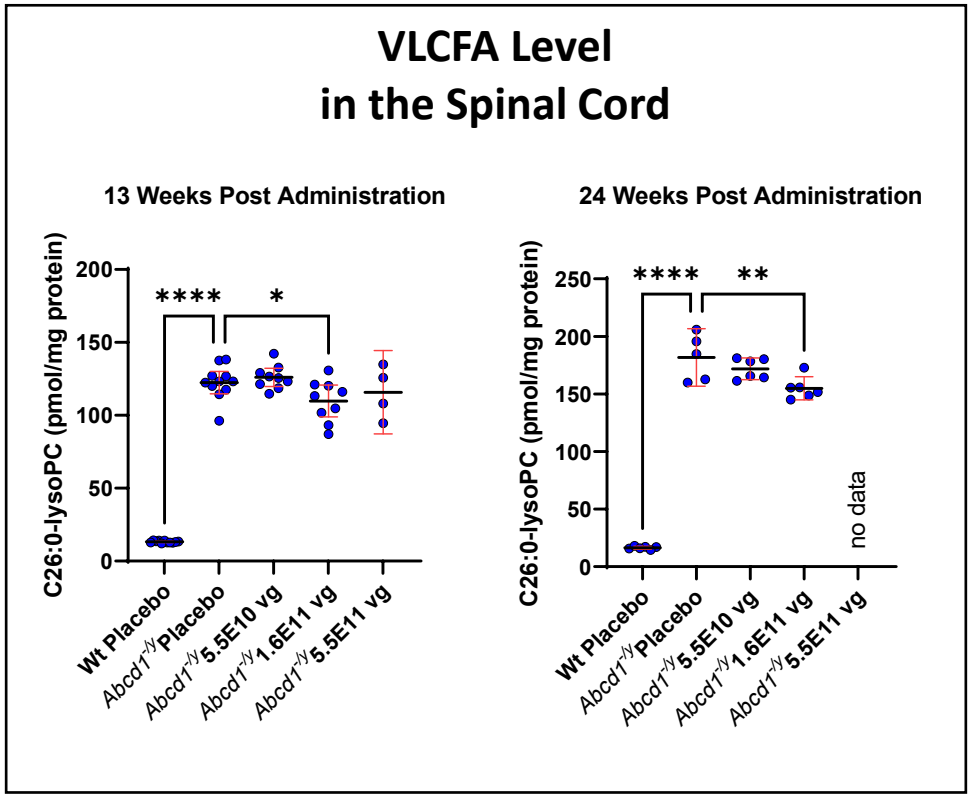
* Preliminary construct with identical expression cassette

In Vivo Translation – Long Term Expression Effects on VLCFA in Young Mice

Experiment: Intrathecal bolus dosing of SBT101 in control and knockout mice at 9 - 11 months of age





Results:

- hABCD1 protein was detected in a dose dependent manner in whole spinal cord at 13 and 24 weeks
- VLCFA levels were significantly elevated in knockout placebo at 13 and 24 weeks as compared to WT mice
- SBT101 treatment significantly reduced VLCFA levels at both timepoints

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Summary and Conclusions

- The transduction of knockout glial cell cultures with SBT101 reduced accumulated VLCFA to near normal levels.
- SBT101 treatment can deliver sustained hABCD1 transgene expression through 6 months duration in a knockout mouse model.
- Intrathecal delivery of SBT101 in *ABCD1* knockout mice can reduce the elevated VLCFA levels and increase mtDNA content towards normal levels.
- Taken together, successful targeting of the spinal cord with SBT101 was demonstrated with dose-dependent improvement of disease markers in a mouse model of AMN.
- These data support further preclinical investigation of SBT101 as a potential therapeutic for the treatment of AMN.