An AAV encoding human ABCD1 shows dose-responsive expression and function following spinal cord delivery in a rodent model of adrenomyeloneuropathy

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Disclosures

- **DW Anderson** – employee, stock/shareholder: SwanBio Therapeutics
- **H Park** – employee, stock/shareholder: SwanBio Therapeutics[a]
- **IME Dijkstra** – nothing to disclose
- **C Ng** – nothing to disclose
- **Y Gong** – nothing to disclose
- **T del Rio** – employee, stock/shareholder: SwanBio Therapeutics[a]
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- **K Kozarsky** – employee, stock/shareholder: SwanBio Therapeutics

[a]At the time the study was performed

Introduction

- AMN is an inherited neurodegenerative disease caused by pathogenic variants in the *ABCD1* gene
- Symptoms of AMN typically occur in adulthood and affect virtually all males and > 80% of females
- Patients typically experience slowly progressive spinal cord disease, characterized by a dying-back axonopathy leading to sensory ataxia, incontinence, debilitating pain and loss of mobility over time
- Given the lack of current approved treatments for AMN, AAV-mediated gene replacement via delivery of a functional copy of *ABCD1* to the spinal cord is a promising therapeutic strategy

Objective: to present preclinical data supporting the development of SBT101, an AAV9-based gene therapy candidate for the treatment of AMN

AAV, adeno-associated virus; *ABCD1,* ATP-Binding Cassette sub-family D Member 1; AMN, adrenomyeloneuropathy; ATP, adenosine triphosphate

Molecular Mechanisms Associated with AMN Pathogenesis

- *ABCD1* encodes a peroxisomal membrane protein called ALDP that transports VLCFA into the peroxisome
- Absence of functional *ABCD1* leads to:
  - increased VLCFA levels
  - cell stress and dysfunction
  - changes in mitochondrial DNA levels as a result of oxidative stress
  - dying-back axonopathy
  - adrenal dysfunction

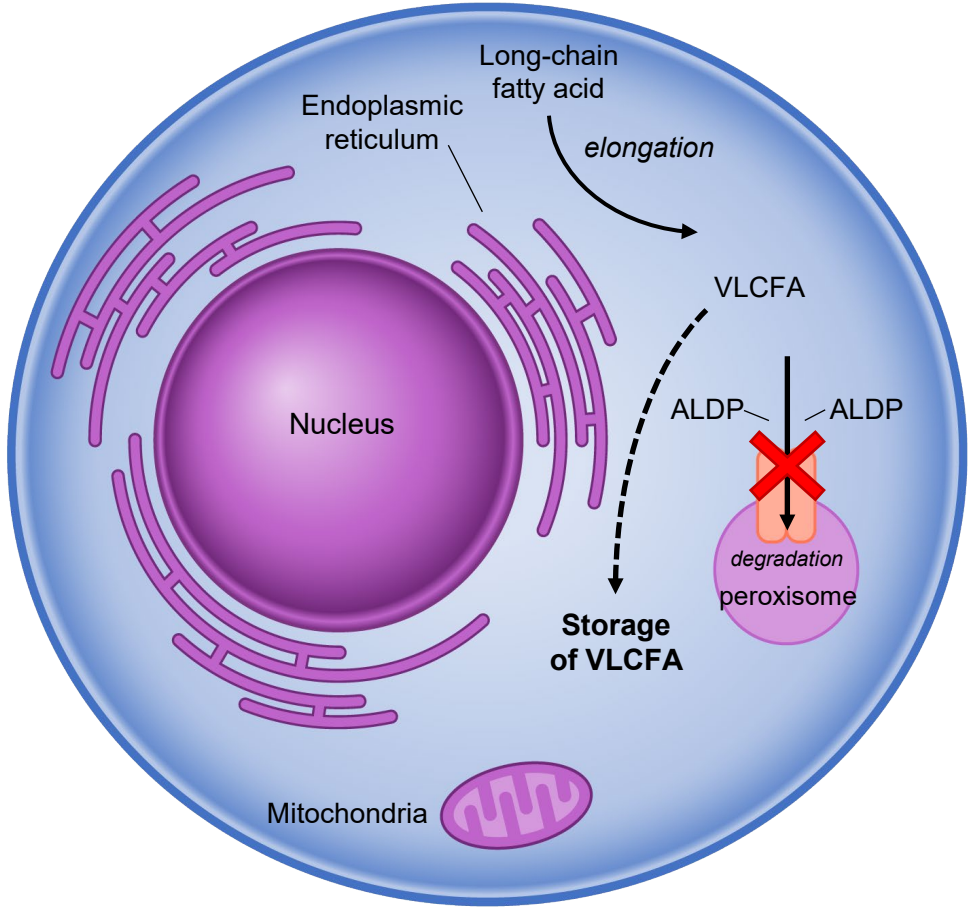


Figure adapted from: https://adrenoleukodystrophy.info/mutations-biochemistry/vlcfa

ABCD1, ATP-Binding Cassette sub-family D Member 1; ALDP, adrenoleukodystrophy protein; AMN, adrenomyeloneuropathy; ATP, adenosine triphosphate; VLCFA, very long-chain fatty acid

SBT101: A Novel AAV9-*hABCD1* Vector

- Here we present an examination of SBT101 activity in:

mixed glial cultures derived from *Abcd1*-null mice, that accumulate VLCFA similar to human AMN disease

- hABCD1 expression levels
- VLCFA levels

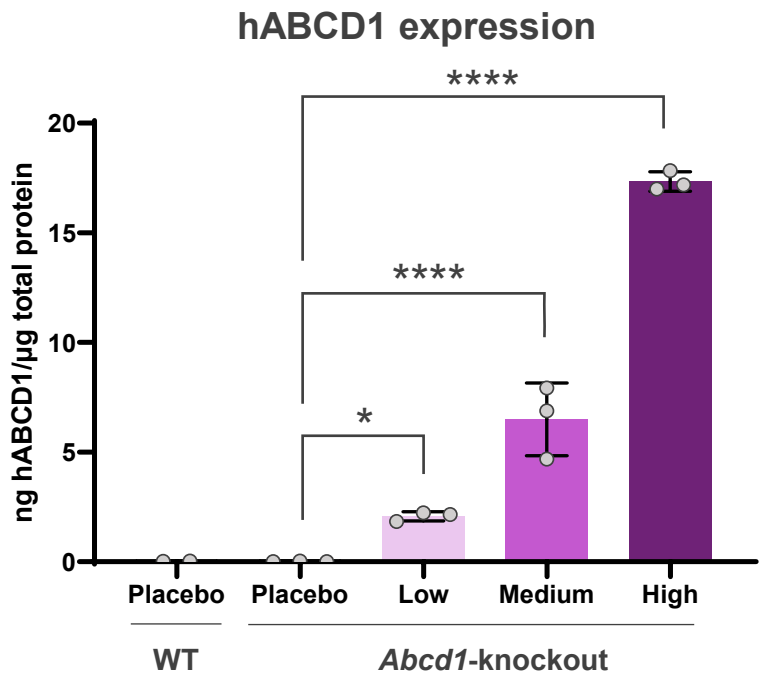
Abcd1-knockout mice following intrathecal bolus delivery

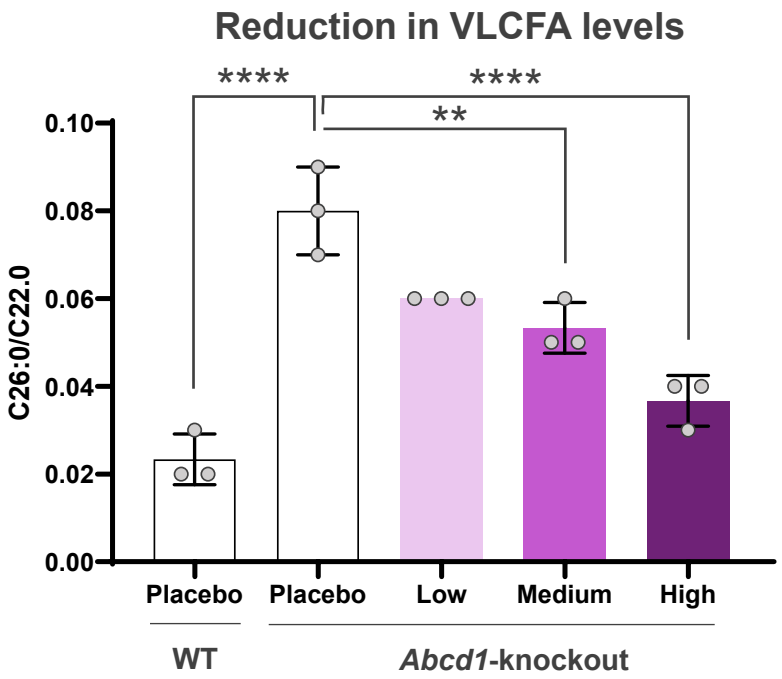
- hABCD1 expression levels
- VLCFA levels
- Mitochondrial DNA levels

AAV, adeno-associated virus; AMN, adrenomyeloneuropathy; ATP, adenosine triphosphate; hABCD1, human ATP-Binding Cassette sub-family D Member 1; VLCFA, very long-chain fatty acid

SBT101 Transduction of Glial Cell Cultures Derived from *Abcd1*-knockout Mice

- SBT101 transduction led to dose-dependent expression of hABCD1 and reduction of VLCFA levels





One-time SBT101 dose

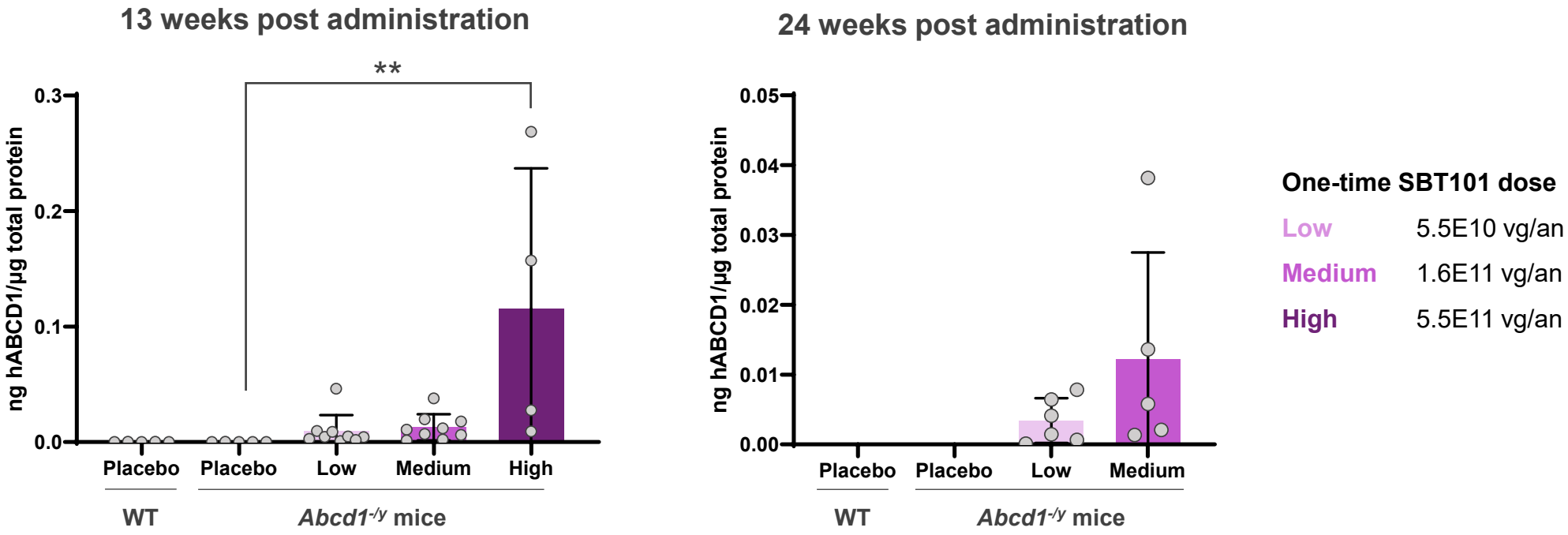
Low	3.3E4 vg/cell (MOI)
Medium	1.0E5 vg/cell (MOI)
High	3.3E5 vg/cell (MOI)

* $p < 0.05$; ** $p < 0.01$; **** $p < 0.0001$. Data represent mean ± standard deviation

ATP, adenosine triphosphate; hABCD1, human ATP-Binding Cassette sub-family D Member 1; MOI, multiplicity of infection; vg, vector genome; VLCFA, very long-cain fatty acid; WT, wild type

SBT101 Transduction of Spinal Cord in *Abcd1*-knockout Mice

- hABCD1 protein was detected in a dose-dependent manner in whole spinal cord at 13 and 24 weeks post administration of SBT101 in *Abcd1*-knockout mice (9–11 weeks of age at treatment)



** $p < 0.01$. Data represent mean ± standard deviation

an, animal; ATP, adenosine triphosphate; hABCD1, human ATP-Binding Cassette sub-family D Member 1; vg, vector genome; VLCFA, very long-cain fatty acid; WT, wild type

In Vivo Translation – Expression at 24 weeks

Note to SwanBio: This slide presents new data since the original ESGCT 2021 presentation.

- SBT101 was administered to *Abcd1*-knockout mice (9–11 weeks of age at treatment)
- All samples exhibited diffuse vacuolation (black arrows) owing to storage in ethanol (not pathological)
- In both WT and knockout mice receiving placebo, tissue showed normal histology
- SBT101-dosed knockout mice exhibited minimal white matter axonal degeneration

Group		Histological Score	Mice, n
WT	Placebo	0.3	6
Abcd1$^{-/y}$	Placebo	0.0	5
	Low-dose SBT101, 5.5E10 vg/an	0.0	3
	Medium-dose SBT101, 1.6E11 vg/an	0.3	3

Pathological analysis of spinal cord tissue

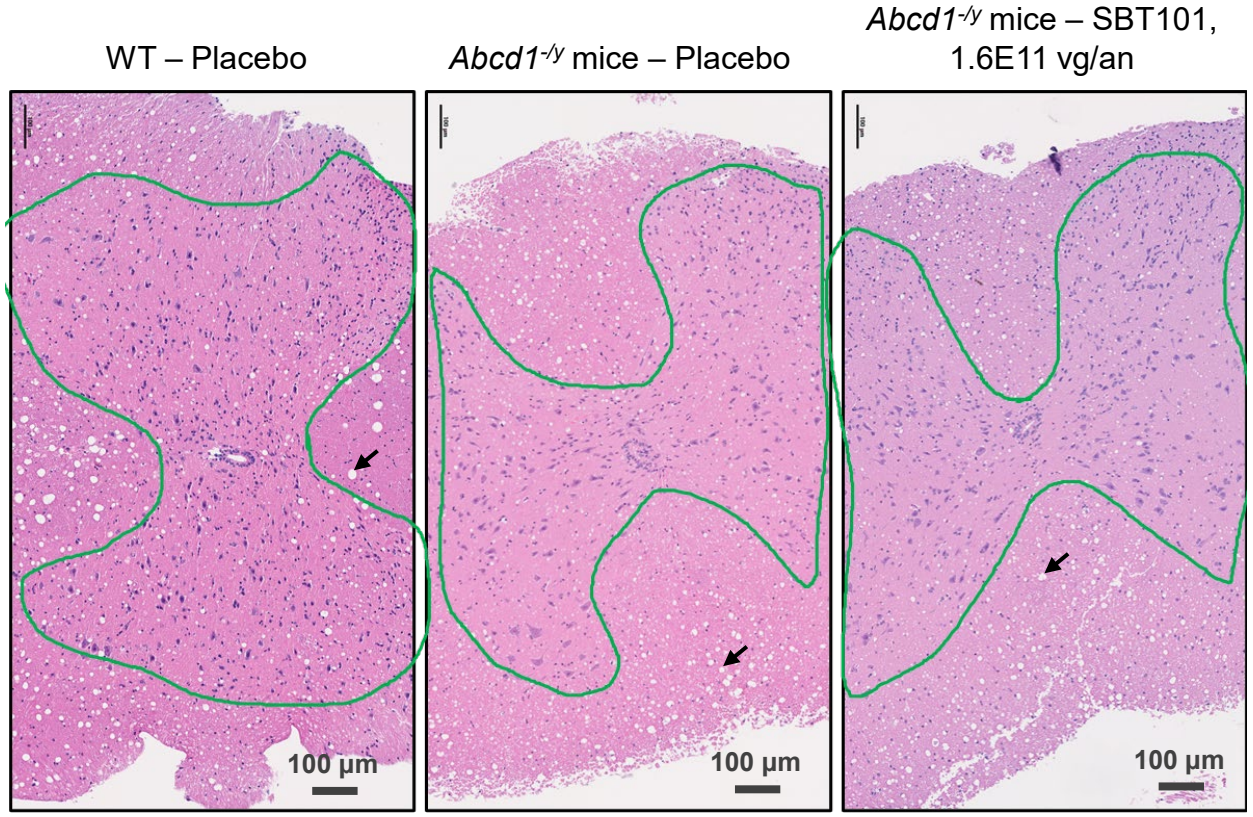
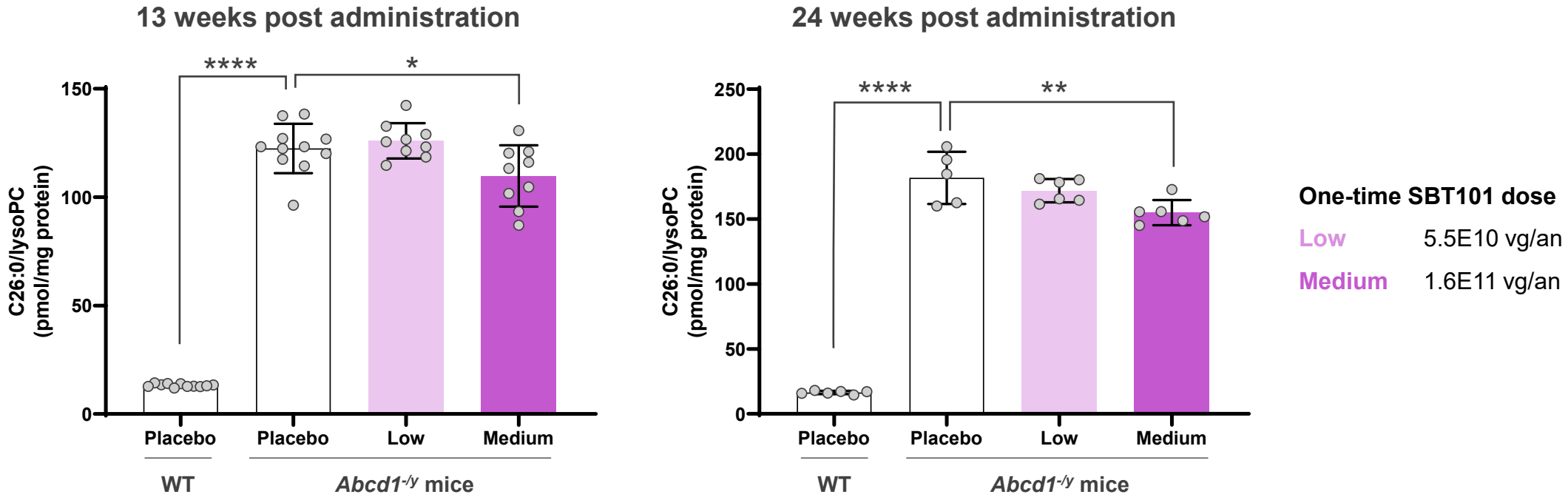


Figure shows representative images from each group; haematoxylin and eosin-stained tissue. Green outlines depict gray matter. Histological scoring ranged 0–4: 0, unremarkable; 1, minimal; 2, mild; 3, moderate; 4, severe

Abcd1, ATP-Binding Cassette sub-family D Member 1; an, animal; ATP, adenosine triphosphate; vg, vector genome; WT, wild type

VLCFA Levels in *Abcd1*-knockout Mice

- Compared with placebo, administration of medium-dose SBT101 led to significant reductions in VLCFA levels of 12% (13 weeks) and 15% (26 weeks) in *Abcd1*-knockout mice (9–11 weeks of age at treatment)

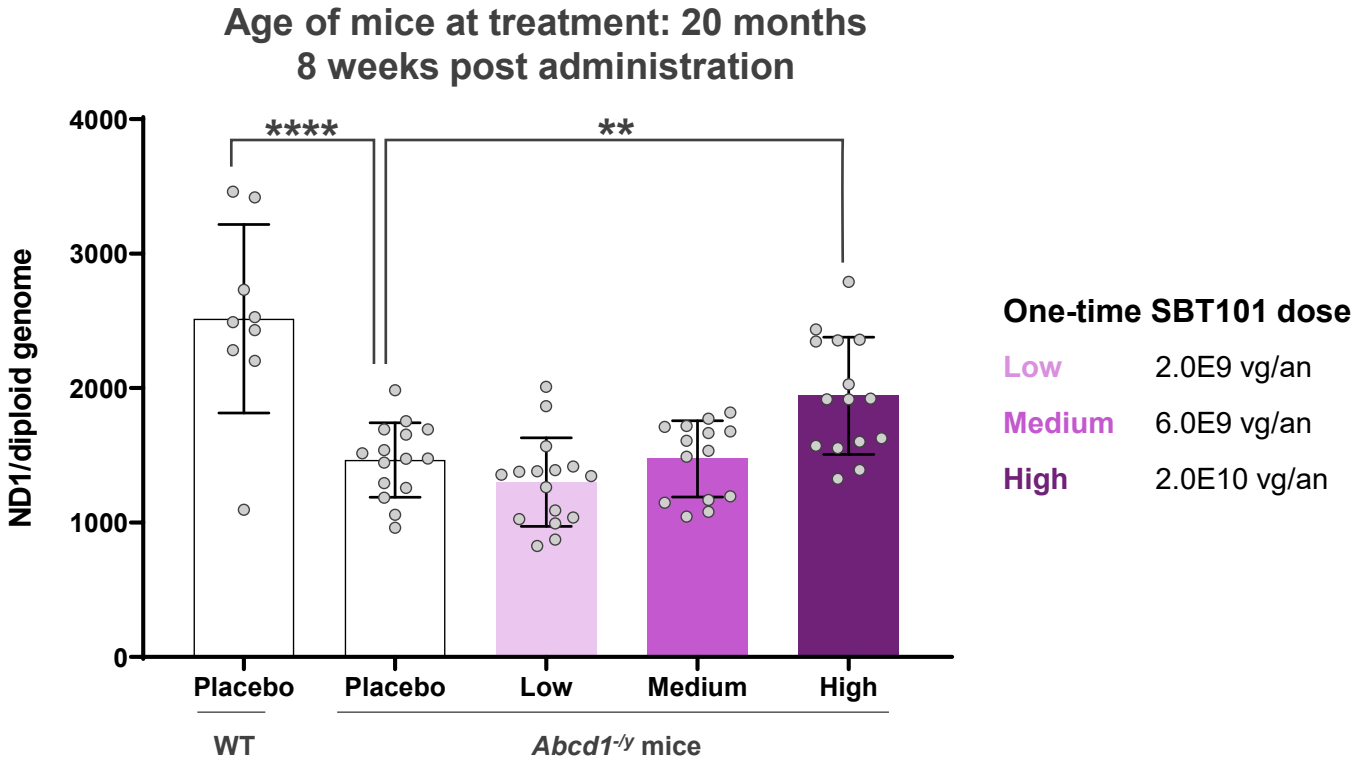


* $p < 0.05$; ** $p < 0.01$; **** $p < 0.0001$. Data represent mean ± standard deviation

Abcd1, ATP-Binding Cassette sub-family D Member 1; an, animal; ATP, adenosine triphosphate; lysoPC, lysophosphatidylcholine; vg, vector genome; VLCFA, very long-cain fatty acid; WT, wild type

Mitochondrial DNA Levels in Older *Abcd1*-knockout Mice

- High-dose SBT101 significantly attenuated the reduction of mitochondrial DNA in older *Abcd1*-knockout mice



** $p < 0.01$; **** $p < 0.0001$. Data represent mean ± standard deviation
Abcd1, ATP-Binding Cassette sub-family D Member 1; an, animal; ATP, adenosine triphosphate; ND1, NADH dehydrogenase 1; vg, vector genome; WT, wild type

Summary and Conclusions

- SBT101 transduction of *Abcd1*-knockout glial cell cultures reduced VLCFA levels to near normal levels
- *Abcd1*-knockout mice exhibited late-onset mtDNA abnormalities in an age-dependent manner
- Intrathecal delivery of SBT101 in *Abcd1*-knockout mice can both reduce elevated VLCFA levels and increase mtDNA content towards normal levels
- SBT101 treatment can deliver sustained hABCD1 transgene expression through 6 months duration in a knockout mouse model with minor to undetectable white matter pathological findings in the spinal cord
- Taken together, these findings demonstrate successful targeting of SBT101 to the spinal cord with dose-dependent improvement of disease markers in a mouse model of AMN
- These data support further preclinical investigation of SBT101 as a potential treatment for AMN

AMN, adrenomyeloneuropathy; ATP, adenosine triphosphate; hABCD1, human ATP-Binding Cassette sub-family D Member 1; mtDNA, mitochondrial DNA; VLCFA, very long-chain fatty acid

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