



A Phase 1/2 Randomized, Blinded, Dose-escalation Study to
Evaluate the Safety and Efficacy of Intrathecal Administration
of AAV9-ABCD1 Gene Therapy (SBT101) in Adult Patients
with Adrenomyeloneuropathy

Study Number:	SBT101-CT101
Study Phase:	1/2
Sponsor:	SwanBio Therapeutics, Ltd
Original Protocol:	Version 9.0, 13 December 2023
IND Number:	28043
EudraCT:	2021-004410-19

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Phase 1/2 Clinical Research Protocol
SBT101-CT101

SwanBio Therapeutics, Ltd.
Version 9.0; 13 December 2023

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12/13/2023

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ABBREVIATIONS AND DEFINITIONS

Abbreviation	Definition
2MWT	2-minute Walk Test
5XSST	5 Times Sit-to-Stand Test
6MWT	6-minute Walk Test
AASLD	American Association for the Study of Liver Diseases
AAV	Adeno-associated virus
AAV9	Adeno-associated virus serotype 9
AAV9-h <i>ABCD1</i>	Adeno-associated virus serotype 9 containing the human <i>ABCD1</i> gene
<i>ABCD1</i>	Adenosine triphosphate-binding cassette transporter subfamily D member 1
<i>Abcd1</i> ^{-/-}	Male <i>Abcd1</i> knockout
ACTH	Adrenocorticotrophic hormone
AE	Adverse event
ALD	Adrenoleukodystrophy
ALT	Alanine aminotransferase
AMN	Adrenomyeloneuropathy
AST	Aspartate aminotransferase
ATC	Anatomical Therapeutic Chemical
ATP	Adenosine triphosphate
bGH	Bovine growth hormone
cDNA	Complementary deoxyribonucleic acid
CGI	Clinical Global Impression
CGI-C	Clinical Global Impression –Change
CMV	Cytomegalovirus
CNS	Central nervous system
COVID-19	Coronavirus disease 2019
CSF	Cerebrospinal fluid
CTCAE	Common Terminology Criteria for Adverse Events
DLT	Dose-limiting toxicity(ies)
DNA	Deoxyribonucleic acid
DRG	Dorsal root ganglia
DSMB	Data Safety Monitoring Board
DTI	Diffusion tensor imaging
ECG	Electrocardiogram
eCRF	Electronic case report form
EC	Ethics Committee

Abbreviation	Definition
EDC	Electronic data capture
EDSS	Expanded Disability Status Score
ELISpot	Enzyme-linked immunosorbent spot
EOS	End of Study
EP	Electrophysiological
EU	European Union
FAS	Full Analysis Set
FDA	Food and Drug Administration
FIH	First-in-human
GCP	Good Clinical Practice
GFAP	Glial fibrillary acidic protein
GLP	Good Laboratory Practice
<i>hABCD1</i>	Human adenosine triphosphate-binding cassette transporter subfamily D member 1
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HEK	Human embryonic kidney
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human immunodeficiency virus
HIV-1/-2	Human immunodeficiency virus type 1/2
ICF	Informed consent form
ICH	International Council for Harmonisation
ID	Identification number
IMP	Investigational medicinal product
IMP Procedure	SBT101 infusion or imitation (sham) procedure
IRB	Institutional Review Board
IT	Intrathecal(ly)
ITR	Inverted terminal repeat
IV	Intravenous(ly)
IXRS	Interactive Voice/Web Response System
LFT	Liver function test
LPC	Lysophosphatidylcholine
LPC-VLCFA	Lysophosphatidylcholine very long chain fatty acid
M	Month
MED	Minimum effective dose
MedDRA	Medical Dictionary for Regulatory Activities
miniBESTest	Balance Evaluation System Test

Abbreviation	Definition
mITT	Modified Intent to Treat
MRI	Magnetic resonance imaging
MSQoL-54	Multiple Sclerosis Quality of Life-54
MSWS-12	Multiple Sclerosis Walking Scale
MT	Magnetization transfer
MTD	Maximum tolerated dose
mtDNA	Mitochondrial deoxyribonucleic acid
NAb	Neutralizing antibody
NCS	Nerve conduction study(ies)
NfL	Neurofilament light chain
NHP	Non-human primate
pA	PolyA
PBMC	Peripheral blood mononuclear cell
PGI	Patient Global Impression
PGI-C	Patient Global Impression – Change
PGI-S	Patient Global Impression – Severity
PRO	Patient-reported outcome
qPCR	Quantitative polymerase chain reaction
QTcF	Corrected QC interval by Fredericia
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SSEP	Somatosensory evoked potentials
SFU	Spot forming unit
SSPROM	Severity Score System for Progressive Myelopathy
SUSAR	Suspected unexpected serious adverse reaction
SV40	Simian virus 40
SwanBio	SwanBio Therapeutics, Ltd
TEAE	Treatment-emergent adverse event
TUG	Timed Up and Go
TUG-DT	Dual-task Timed Up and Go
UCHL-1	Ubiquitin carboxy-terminal hydrolase L1
ULN	Upper limit of normal
US	United States
vg	Vector genome
VLCFA	Very long chain fatty acid
WHO	World Health Organization

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SYNOPSIS

Name of Sponsor/Company: SwanBio Therapeutics, Ltd	
Title of Study: A Phase 1/2 Randomized, Blinded, Dose-escalation Study to Evaluate the Safety and Efficacy of Intrathecal Administration of AAV9-ABCD1 Gene Therapy (SBT101) in Adult Patients with Adrenomyeloneuropathy (AMN)	
Study Number: SBT101-CT101	Phase of Development: Phase 1/2
Study Design: This is a Phase 1/2 randomized, blinded, dose-escalation study to evaluate the safety and efficacy of intrathecal (IT) administration of SBT101, a recombinant adeno-associated virus serotype 9 (AAV9) containing a functional copy of the human adenosine triphosphate (ATP)-binding cassette transporter subfamily D member 1 (<i>ABCD1</i>; <i>hABCD1</i>) gene, in adult patients with adrenomyeloneuropathy (AMN) aged 18-65 years. The core study consists of a parallel design with a control crossover in Part 2. During dose escalation, Dose Level 1 and Dose Level 2 patients will receive open-label SBT101 to establish safety and the maximum tolerated dose (MTD). After confirmation of safety, the MTD will be selected for the randomized, blinded Cohort 3 dose expansion phase. Patients will receive a single dose of SBT101 via IT route and will be followed for safety and efficacy for 5 years. The study consists of two parts after infusion of SBT101: - Part 1: A 24-month core study period to evaluate the safety and potential impact of SBT101 on disease progression. Part 1 will consist of 2 phases: - Dose Escalation Phase (open-label): Two (2) doses of SBT101 will be evaluated to establish the maximum tolerated dose (MTD). - Dose Level 1 Cohort – 1.0E14 vector genomes (vg) - Dose Level 2 Cohort – 3.0E14 vg - Dose Expansion Phase (randomized, blinded): Additional patients will be enrolled to receive SBT101 at the MTD - Part 2: An unblinded 3-year long-term safety follow-up period with annual follow-up visits to evaluate the safety of SBT101 and disease progression. Informed consent will be obtained from all patients at the Screening Visit (Visit 1) prior to conducting any screening procedures to assess study eligibility. The duration of the study for patients receiving SBT101 will be up to 66 months, including up to 4 months for Screening, the 24-month core study period (i.e., Part 1), and 36 (±2) months for long-term safety follow-up (i.e., Part 2). The duration of the study for patients randomized to the control arm who choose to receive SBT101 after the cohort primary analysis will be a maximum of 90 months, including up to 4 months for Screening, the 24-month core study period, another 24 months for follow-up post-administration of open-label SBT101, and 36 (±2) months for long-term safety follow-up. In Part 1, a minimum of 16 patients will be enrolled in 2 phases: Dose Escalation Phase and Dose Expansion Phase. The Dose Escalation Phase aims to establish the MTD. In the Dose Escalation Phase, a minimum of 8 patients will be enrolled, with a minimum of 4 patients enrolled to Dose Level (1.0E14 vg) and a minimum of 4 patients enrolled to Dose Level 2 (3.0E14 vg). In the Dose Expansion Phase, an additional 8 patients will be randomized to treatment on a 3:1 basis to receive SBT101 at MTD. The decision on dose escalation and treatment of additional patients at each dose level during the Dose Escalation Phase will be made after review of safety by the independent Data Safety Monitoring Board (DSMB) and the Sponsor. - An independent DSMB will be established; members will include at least 3 independent physicians with expertise in neurological diseases or gene therapy, and an independent statistician. - The Sponsor's Medical Monitor will review the safety data after each patient completes the Day 30 Visit before treatment of subsequent patients within that Dose Level Cohort.	

- The DSMB will review the available safety data after at least 2 patients complete the Day 30 visit at each Dose Level prior to recommending treatment of additional patients or initiation of dose escalation or expansion.

The Sponsor, in consultation with the DSMB, may:

- Decide to investigate an additional dose of SBT101 in the Dose Expansion Cohort different than Dose Level 1 and Dose Level 2;
- Elect to increase or decrease the number of patients in a Dose Level Cohort during the study.

All patients in the control arm will have the option to be treated with open-label SBT101 after the last patient in the cohort has completed the Month 24 Visit. Patients who opt to be treated with open-label SBT101 must continue to meet all exclusion and inclusion criteria (except for inclusion criterion (#1 and #3) and exclusion criterion (#3)). If they choose to be treated with open-label SBT101, they will restart all procedures at Day -1 and will be followed for another 5 years.

Selection of Dosage

Selection of dosages in this study was made based on safety and efficacy preclinical data from rodent and non-human primate (NHP) models, which suggest potentially efficacious exposures; these data were analyzed to determine exposure-based safety margins. An 8-week dose-finding proof-of-concept study (NCP-MOU-101-01) was conducted using male *Abcd1* knockout (*Abcd1^{-y}*) mice. There was a dose-dependent increase in ABCD1 protein levels in the spinal cords of all treated mice. As compared to untreated littermate controls, mice receiving 2.0E10 vg/animal (vg/an) had a statistically significant improvement in mitochondrial deoxyribonucleic acid (mtDNA) at 8 weeks post-administration. The minimum effective dose (MED) was, thus, established to be 2.0E10 vg/an (see conversion of the dose for humans in [Table 1](#)). In NHPs, SBT101 administered via IT infusion at $\leq 7.6E13$ vg/an was well tolerated (NCP-NHP-101-05).

The MED is also supported by the functional improvement observed in mouse study NCP-MOU-101-05, a 20-month dose-finding proof-of-concept study. Compared to control mice, administered AAV9-Null, there was an increased grip strength at 7-8 months post administration of SBT101 to *Abcd1/Abcd2* double knock out (*Abcd1^{-y}/Abcd2^{-/-}*) mice at 3.3E10 vg/an and 3.3E11 vg/an, equal to the grip strength of wild-type mice. The grip strength in *Abcd1^{-y}/Abcd2^{-/-}* mice was observed to be maintained up through 10 months post administration, compared to *Abcd1^{-y}/Abcd2^{-/-}* mice administered AAV9-Null vector. The increase in grip strength occurred together with a dose dependent decrease of VLCFA compared to *Abcd1^{-y}/Abcd2^{-/-}* animals administered AAV9-Null vector. The lowest dose (3.3E10 vg/an) in mice at which functional improvement and reduction in VLCFA were observed translates to a human dose of 1.24E14 vg/person.

Safety was demonstrated at similar and higher doses in NHPs; in study NCP-NHP-101-05, single IT administrations up to doses of 7.62E13 vg/an (corresponding to human doses up to 8.77E14 vg/person) were well tolerated by NHPs. Based on efficacy, biodistribution, and safety data, and taking into consideration differences in CSF volumes across species, a dose of SBT101 was calculated (see dose conversion for humans in [Table 1](#)). Because SBT101 is intended for IT administration, the scaling of doses between species was based on CSF volume. The proposed doses in patients, 1.0E14 and 3.0E14 vg/person, fall within dose ranges that have been shown to be associated with activity and efficacy in mice. These dose levels also translate into doses that efficiently transduce the spinal cord and DRG neurons in NHPs at >25% and have shown safety in both species with a safety margin for the high dose of approximately 2.75-fold.

(see [Table 1](#)).

Table 1 CSF Volumes in Different Species and Comparison of Doses Between Species

Species	CSF Volume (mL)	Relative to Humans	Dose (vg)	Equivalent Dose in Humans (vg)
Mouse	0.04 ^{a,b}	0.000267	mtDNA effect (MED): 2.0E10 ^c	MED: 7.50E13
			Grip effect: 3.3E10 ^f	1.24E14
			VLCFA effect: 5.5E10 ^g	2.06E14
NHP	13 ^c	0.086	1.26E13 ^h	1.45E14
			1.50E13 (low) ⁱ	1.73E14 (low)
			3.38E13 ^h	3.90E14
			7.62E13 (high) ⁱ	8.79E14 (high)
Human	150 ^{a,d}	1		

Calculation of equivalent human dose used non-rounded conversion factors

Abbreviations: CSF = cerebrospinal fluid; MED = minimum effective dose; mtDNA = mitochondrial deoxyribonucleic acid; NHP = non-human primate; vg = vector genomes; VLCFA = very long chain fatty acid

^a Johanson et al. (2008). *Cerebrospinal Fluid Res* 5:10.

^b Simon and Iloff (2016). *Biochim Biophys Acta* 1862: 442-451.

^c Bourke et al. (1973). *Cancer Res* 33: 1735-1746.

^d Telano and Baker (2020). In *StatPearls*. StatPearls Publishing.

Biodistribution

Biodistribution analyses conducted on data from 2 NHP studies (NCP-NHP-101-01; NCP-NHP-101-02) have demonstrated consistent and higher uptake of vector genomes into the cervical, thoracic, and lumbar spine following administration over 6 to 24 hours compared to bolus administration. In line with this, SBT101 will be infused over a period of approximately 5 hours.

SBT101 Administration

In this protocol, “Investigational Medicinal Product (IMP) procedure” refers to SBT101 infusion in patients randomized to the SBT101 arm and the imitation (sham) procedure (see below) in patients randomized to the control arm.

All patients will be admitted to the study site for the IMP procedure for a duration of 3 days (Day -1 [Visit 2], Day 1 [Visit 3], and Day 2 [Visit 4]). In patients receiving SBT101, SBT101 will be instilled into the CSF via IT infusion using a catheter that will be inserted at the lower lumbar area (eg. L3-L4 interspace) and forwarded up at the T8-T10 level, where SBT101 will be infused. It is estimated that the infusion will involve up to 40 cc. Prior to SBT101 infusion, 12-16 cc of CSF will be collected via gravity for clinical laboratory assessments and to measure biomarkers and total and neutralizing antibody (Nab) titers. The infusions will be performed by trained site staff (e.g., neuroradiologists, neurosurgeons, or anesthesiologists) using fluoroscopic guidance. Each infusion will be administered by slow and continuous delivery over approximately 5 hours at a rate of up to 8 mL/hour. Patients will be allowed and encouraged to walk once every hour during the infusion.

Patients randomized to the control arm will undergo an imitation (sham) procedure consisting of a lumbar puncture for collection of CSF samples. The procedure for control patients will be comparable to that of SBT101; catheter tubing will be attached to a Collection EVA bag (a minibag) placed below the injection site. The infusion bag will contain normal saline 0.9% (Placebo_{IMP}), which will not be instilled into the CSF but collected in the Collection EVA bag through the tubing. The duration of infusions will be the same for control and SBT101 patients; approximately 5 hours.

Immunosuppression Regimen

Patients will receive an immunosuppressive regimen to prevent the potential development of an immune response to the AAV9 capsid and hABCD1 transgene. Induction will be achieved with methylprednisolone sodium succinate for injection (from here on referred to as methylprednisolone) and maintenance will be achieved with prednisone/prednisolone. Maintenance therapy will be continued until Day 90, at a minimum. If clinically indicated, prednisone/prednisolone dose can be increased. Initiation of additional immunosuppression, changes to dose, and/or additional alterations to the immunosuppression regimen can be made after discussions between the Investigator and Medical Monitor.

All study patients (control arm included) will be administered 1 g methylprednisolone (Solu-Medrol™) via intravenous (IV) infusion over the span of 2 ($\pm$ 1) hours on Day -1 (Visit 2), and after the IMP procedure on Day 1 (Visit 3) and Day 2 (Visit 4). Thereafter, patients will receive oral prednisone/prednisolone at a dose of 0.5 mg/kg/day, starting on Day 3 until Day 90 at a minimum, this can be extended as needed per clinical judgement.

A standard clinical tapering regimen will be initiated per [Appendix 1](#).

In the event of a developing cellular immune response, as indicated by increased T-cell reactivity measured by enzyme-linked immunosorbent spot (ELISpot) and/or clinical signs and symptoms, the Medical Monitor should be consulted. Patients may be treated with sirolimus, or another immune suppressor based on clinical indication as adjuvant to the ongoing prednisone/prednisolone regimen.

All patients will be evaluated at the Day 90 Clinic visit to determine appropriateness for initiation of the tapering protocol by the Investigator in consultation with the Medical Monitor.

See [Appendix 2](#) for details on the approach to the use of sirolimus.

Blinding

The patients and study team members conducting efficacy assessments will be blinded to treatment assignment. The Investigators who perform the IMP procedure will be unblinded. The Sponsor will be blinded during the Dose Expansion Phase.

To maintain blinding of patients and blinded study team members during Dose Expansion, patients in the control arm will undergo the full imitation (sham) procedure, as described above; a Collection EVA bag will be placed below the infusion site of patients in both the SBT101 and control arms.

All patients will receive the same doses of methylprednisolone and prednisone/prednisolone for immunosuppression. Safety laboratory assessments will be conducted on all patients.

Safety Monitoring

Prior to the IMP procedure, troponin, thrombocyte count, vitamin B12 level, and liver function will be assessed by laboratory testing (e.g., aspartate aminotransferase [AST], alanine aminotransferase [ALT], total bilirubin, and prothrombin time). Troponin, thrombocyte count, and liver function tests (LFTs) will be monitored for at least 3 months after the IMP procedure and until results are unremarkable (i.e., values within normal ranges and no Grade III or Grade IV elevated transaminases that are related or possibly related to SBT101). These laboratory assessments will be performed weekly for the first month, then every other week for the second and third months; LFTs will also be monitored biweekly for until 4 weeks after the prednisone/prednisolone dose has been tapered down to a daily dose of 10 mg. Weekly follow-up may be reinstated if elevation of transaminases are detected at any point during the study.

Serum AAV9 antibody titers, ELISpot for AAV9 and hABCD1, and viral shedding in serum, saliva, and urine will be monitored per the Schedule of Assessments. Additional ELISpot testing may be performed at the discretion of the site Investigator; based on the test results, the immunosuppression regimen may be altered.

Monitoring of changes in spinal cord and dorsal root ganglia (DRG) function will be performed through neurological examination, nerve conduction studies (NCS), Somatosensory Evoked Potentials (SSEP), and magnetic resonance imaging (MRI) of the lumbar spine as indicated in the Schedule of Assessments. The neurological examination will include monitoring for acute worsening of spinal cord function and sensory neuropathy by motor and sensory examination, deep tendon reflexes and assessments of gait balance and proprioception. Sural nerve amplitudes and H-reflex will be measured in NCS. Lumbar spine MRIs, with and without contrast, will be performed to assess contrast enhancement of the DRG at the lumbosacral level.

Patients with AMN are at risk of developing inflammatory cerebral disease across their life span, as such, all patients will undergo MRI of the brain (with and without contrast) as indicated in the Schedule of Assessments. If a patient shows signs of inflammatory cerebral disease (contrast enhancement) at any time during the study, the brain MRI will be repeated in 1-3 months and; the DSMB will be informed. If inflammatory cerebral disease is detected within 6 months following the IMP procedure, dosing will be paused while the DSMB is consulted. If the inflammatory lesion(s) continues to progress, the patient will be referred for management of inflammatory cerebral disease. Safety data related to gene therapy will continue to be collected.

If a patient shows signs of inflammatory cerebral disease (contrast enhancement) within 6 months following the IMP procedure, further enrollment will be suspended and the DSMB will be informed and a MRI of the brain will be repeated in 1-3 months and further enrollment will be postponed till the DSMB agrees on the action plan and follow-up proposed by the Investigator in conversation with the Medical Monitor. An MRI of the brain will be repeated in 1-3 months; if signs of inflammatory disease resolve on repeat MRI, enrollment will be resumed in consultation with the DSMB. If unresolved, enrollment will be postponed until the DSMB agrees on the action plan.

While on immunomodulatory regimens and tapering, all patients will be monitored for AEs, including opportunistic infections. Antibiotics and/or other medications to minimize the risk of opportunistic infections, and vaccinations may be prescribed at the discretion of the Investigator. All events related to the use of immunomodulatory drugs (e.g., hyperglycemia, weight gain, infections) will be recorded as AEs connected to their use.

Tapering of prednisone/prednisolone will be initiated immediately, or the dose will be reduced, if there is severe gastric pain or any evidence of bleeding (e.g., vomiting blood, blood in the stools). If excessive jitteriness occurs and the patient is still on prednisone/prednisolone, the protocol for oral tapering will be applied immediately.

In the event of a change in the prednisone/prednisolone dose, sirolimus or another immunosuppressive regimen may be initiated in consultation with medical monitor and continued until Day 90, or longer based on T-cell reactivity and/or clinical indication, as described in [Appendix 2](#).

Dosing Suspension Criteria

If a patient experiences an adverse event (AE) of $\geq$ Grade III toxicity, the event will be investigated in real time and further enrollment and dosing will be suspended after consultation with the DSMB. Additionally, any of the following dose-limiting toxicities (DLTs) events will result in suspension of further enrollment and dosing while the events are under investigation after consultation with the DSMB:

- Any study drug-related death during the study
- Any patient develops study drug-related or possibly study drug-related Grade III or Grade IV toxicity, excluding elevated transaminases
- Any patient develops study drug-related or possibly study drug-related Grade III or Grade IV elevated transaminases that fail to improve or resolve within 4 weeks on the immunosuppressive regimens
- Any patient develops study drug-related or possibly study drug-related Grade IV (>105-fold) elevated transaminases
- Any patient develops contrast enhancement of their spinal cord on MRI in the first 6 months of the study; or contrast enhancement of the DRGs or/and a >50% drop in sensory amplitudes in the first 3 months after administration of SBT101
- Any patient shows signs of inflammatory cerebral disease (contrast enhancement) within 6 months following the IMP procedure. An MRI of the brain will be repeated in 1-3 months.
- If any patient develops signs of inflammatory cerebral disease within 4-6 weeks after SBT101 administration, further enrollment will be put on hold and an MRI will be repeated in 1-3 months; if signs of contrast enhancement resolve on repeat MRI, the enrollment will be resumed after consulting with the DSMB
- Any patient experiences a medically important event that warrants further evaluation
- Should AAV vector-mediated insertional mutagenesis occur, it is not likely to be observed in the first year after the IMP procedure; therefore, the Sponsor intends to conduct long-term safety monitoring of patients in the extension study.

All Study Stopping Rules are documented in the DSMB Charter.

Any occurrence of malignancy at any point after vector infusion that is possibly, probably, or definitely related to the study drug

If a patient experiences any study drug-related death during the study or a Severe Adverse Event (SAE) included or not in the Dosing Suspension Criteria with a possible prolonged or permanent disability not expected as the natural progression of AMN, and Stopping Rules

Should AAV vector-mediated insertional mutagenesis occur, it is not likely to be observed in the first year after the IMP procedure; therefore, the Sponsor intends to conduct long-term safety monitoring of patients in the extension study. An event of insertional mutagenesis will be handled as a serious adverse event (SAE) and reported in the time frame as outlined in Section 7.3.8 (Reporting of Serious Adverse Events). The DSMB will review data relevant to the event and will receive input from the Sponsor and/or Investigator before providing appropriate recommendations. The event and the DSMB's recommendation will be discussed with the United States (US) Food and Drug Administration (FDA) and other regulatory authorities. All patients who were administered SBT101 will continue to comply with the follow-up schedule according to the protocol. Any pregnancies that occur in study patients' partners will be reported on the Pregnancy Form after consent has been provided.	
Number of Patients: Approximately 16	Number of Sites: Estimated total: Approximately 2 sites globally
Study Drug: Patients in the SBT101 arm will receive SBT101 via IT infusion. Patients in the control arm will undergo an imitation (sham) procedure using infusion bags filled with normal saline 0.9% (Placebo _{IMP}) but Placebo _{IMP} will not be infused in the CSF.	Arms and Interventions: Dose escalation Phase: Dose Level 1 (1.0E14 vg): Single IT administration of SBT101 (minimum of 4 patients treated with SBT101) Dose Level 2 (3.0E14 vg): Single IT administration of SBT101 (minimum of 4 patients treated with SBT101) Dose expansion Phase Single IT administration of SBT101 at MTD (6 patients treated with SBT101 and 2 controls)
Duration of Treatment: One-time administration of SBT101.	Period of Evaluation: Part 1: A 24-month core study period to evaluate the safety and potential impact of SBT101 on disease progression. Part 2: An unblinded 3-year long-term safety follow-up period with annual follow-up visits to evaluate the safety of SBT101 and disease progression.
Objectives and Endpoints:	
To characterize the safety and tolerability of one-time IT administered SBT101 in adults diagnosed with AMN.	Primary Endpoint: Percentage of patients with at least 1 Grade III or Grade IV AE that is at least possibly related to SBT101 at Month 24, as reported by the patients or observed by the Investigator, after SBT101 treatment.
To characterize the efficacy of IT administered SBT101 in adults diagnosed with AMN.	Secondary Endpoints: - Changes in the 6-minute Walk Test (6MWT) at Month 12 and Month 24 compared to controls, and compared to the reference changes determined by the prospective SBTNHX-CT901 study. - Changes in the 2-minute Walk Test (2MWT) at Month 12 and Month 24 compared to controls, and compared to the reference changes determined by the prospective SBTNHX-CT901 study. - Changes in the 5 times Sit-to-Stand Test (5XSST) at Month 12 and Month 24 compared to controls, and compared to the reference changes determined by the prospective SBTNHX-CT901 study. - Changes in postural body sway amplitudes at Month 12 and Month 24 compared to controls, and compared to the

	reference changes determined by the prospective SBTNHX-CT901 study. • Proportions of patients with Expanded Disability Status Score (EDSS) of ≥ 5.5 at Month 12 and Month 24 compared to controls, and compared to the reference changes determined by the prospective SBTNHX-CT901 study. • Proportions of patients with increased anti-spasticity medication dose at Month 12 and Month 24 compared to controls, and compared to the reference changes determined by the prospective SBTNHX-CT901 study. • Changes in Timed Up and Go (TUG) at Month 12 and Month 24 compared to controls, and compared to the changes observed in the prospective SBTNHX-CT901 study. • Changes in Dual-task TUG (TUG-DT) at Month 12 and Month 24 compared to controls, and compared to the changes observed in the prospective SBTNHX-CT901 study. • Changes in the Balance Evaluation System Test (miniBESTest) at Month 12 and Month 24 compared to controls, and compared to the changes observed in the prospective SBTNHX-CT901 study. • Changes in the Clinical Global Impression (CGI; performed by a qualified professional) score at Month 12 and Month 24 compared to controls. • Changes in the Patient Global Impression of Severity (PGI-S), Patient Global Impression of Severity – Balance (PGI-S Balance), and Patient Global Impression of Change – Balance (PGI-C Balance) scores at Month 12 and Month 24 compared to controls, and compared to the changes observed in the prospective SBTNHX-CT901 study. • Percentage of patients with at least 1 Grade III or Grade IV AE that is at least possibly related to SBT101 at Month 60, as reported by the patients or observed by the Investigator, after SBT101 treatment.
To assess the change in quality of life, patient abilities, and daily living activities from Baseline to Month 12 and Month 24.	Exploratory Endpoints: • Changes in the Severity Score System for Progressive Myelopathy (SSPROM) score at Month 12 and Month 24 compared to controls, and compared to the changes observed in the prospective SBTNHX-CT901 study. • Changes in the Multiple Sclerosis Quality of Life-54 (MSQoL-54) score at Month 12 and Month 24 compared to controls, and compared to the changes observed in the prospective SBTNHX-CT901 study. • Changes in the Multiple Sclerosis Walking Scale (MSWS-12) score at Month 12 and Month 24 compared to controls, and compared to the changes observed in the prospective SBTNHX-CT901 study. • Changes in the Urinary and Bowel Questionnaire at Month 12 and Month 24 compared to controls, and compared to the changes observed in the prospective SBTNHX-CT901 study.

	• Changes in the conduction velocity, distal latency, and F-wave- of peroneal compound motor action potential at Month 12 and Month 24 compared to controls, and compared to the changes observed in the prospective SBTNHX-CT901 study. • Changes in somatosensory evoked potentials at Month 12 and Month 24 compared to controls. • Changes in lower kinematics and kinetics parameters at Month 12 and Month 24 compared to controls, and compared to the changes observed in the SBTNHX-CT901 study. • Changes in the daily life profile, activity bouts, and sleep parameters, as measured by the CentrePoint Watch, compared to controls, and compared to the changes observed in the prospective SBTNHX-CT901 study. • Clinical Global Impression-Change by visit • Changes in the following parameters at Month 12 and Month 24 compared to controls: • Plasma and/or CSF neurofilament light chain (NfL), lysophosphatidylcholine (LPC) very long chain fatty acids (VLCFAs), and VLCFAs. • Cervical spine MRIs (diffusion tensor imaging [DTI], magnetization transfer [MT], and spectroscopy parameters). • T-cell reactivity to the capsid by visit from pre-IMP procedure through to Day 90.
To characterize the safety and tolerability of one-time IT administered SBT101 in adults diagnosed with AMN. To evaluate the safety of the catheter used to infuse SBT101 intrathecally in Europe (European only objective)	Safety Endpoints: • Incidence of treatment emergent adverse events (TEAEs). • Changes in the amplitude of sural nerve sensory action potential at Month 1 and Month 6 compared to Baseline. • Changes in the latency of H-reflex of soleus muscle at Month 1 and Month 6 compared to Baseline. • Changes in somatosensory evoked potentials at Month 3 compared to Baseline. • Incidence of abnormal values in clinical laboratory assessments, vital signs, and electrocardiogram (ECG) parameters posttreatment. • Changes in clinical laboratory assessments, vital signs, physical and neurological examinations, and ECG parameters post-treatment compared to Baseline. • Frequency and severity of adverse events related to the catheter used for infusion of SBT101 intrathecally in Europe (European only endpoint)
Diagnosis and Main Criteria for Inclusion: <u>Patients with AMN will be eligible for this study if they meet all of the following criteria:</u> 1. Male adults aged ≥ 18 and ≤ 65 years 2. Diagnosed with X-linked adrenoleukodystrophy (ALD) including proven mutation in the <i>ABCD1</i> gene through confirmatory genetic testing, and supported by historically elevated VLCFA. 3. Clinical evidence of spinal cord involvement with EDSS score between 1 and 4.5 and pyramidal function score of ≥ 1 in the Functional System Score of the EDSS. Signs of pyramidal tract dysfunction don't include hyperreflexia.	

4. Must agree to use reliable double-barrier contraception methods (e.g., condom and 1 other form of contraception for female sexual partners that are of childbearing potential, such as diaphragm, intrauterine device, spermicidal jelly, and/or hormonal contraceptive) and to not donate sperm for at least 6 months following the IMP procedure.
5. For patients who are receiving any other treatment for ALD, including off-label medications and/or supplements (e.g., antioxidants, Lorenzo's oil, statins, etc.) or physical rehabilitation support, such treatments must have been at a stable dose and/or frequency for ≥ 4 weeks prior to Screening and patients must agree to continue at the same dose and/or frequency through Part 1 of the study.
6. The patient provided written informed consent prior to any study procedures being performed.

Patients will be excluded from this study if they meet any of the following criteria:

1. Presence of inflammatory cerebral disease, established by radiographic review of brain MRI demonstrating a Loes score ≥ 0.5 on the 34-point scale, except for the abnormalities that can be observed in patients with AMN without inflammatory cerebral demyelination with Loes score ≤ 4 .
2. Pathological changes identified on brain MRI including all lesions from previous diagnosis of inflammatory cerebral disease with Loes score ≥ 0.5 on the 34-point scale, except for the abnormalities that can be observed in patients with AMN without inflammatory cerebral demyelination with Loes score ≤ 4 .
3. 15 years or more have elapsed since the initial onset of myeloneuropathy manifestations, such as walking or running difficulties, bladder dysfunction, increased muscular tone, spasticity, weakness, balance problems, etc.
4. Contraindications for MRI procedure and/or contrast materials.
5. History of a brain or spinal cord disease that would interfere with lumbar puncture procedures, CSF circulation, and/or safety assessments.
6. Contraindication to steroids, sirolimus, tacrolimus, and/or anesthetic medications.
7. Contraindication to SBT101 and/or any of its ingredients.
8. Unstable adrenal function (e.g., untreated or inappropriately treated adrenal insufficiency).
 - a. Adrenal function will be evaluated through laboratory assessments of cortisol, plasma adrenocorticotrophic hormone (ACTH), plasma renin, aldosterone, sodium, and potassium levels at Screening. Monitoring of replacement therapy will be mainly clinical. Determination of inappropriately treated adrenal insufficiency will be made by the Investigator in consultation with the Medical Monitor.
 - b. Patients who meet the following criteria will be considered to have adrenal insufficiency: (i) basal cortisol concentration < 275 nmol (10 $\mu\text{g/dL}$) in the morning (i.e., 6 AM to 10 AM) and (ii) plasma ACTH $\geq 2 \times$ upper limit of normal (ULN).
9. Positive for human immunodeficiency virus (HIV) type 1 or 2 (HIV-1, HIV-2), hepatitis B virus (HBV), or hepatitis C virus (HCV).
 - a. Patients who have been vaccinated against HBV (e.g., HBV surface antibody-positive) who are negative for other markers of prior HBV infection (e.g., HBV core antibody-negative) are eligible.
 - b. Patients who are positive for anti-HCV antibodies are eligible, as long as they have a negative HCV load as measured by quantitative polymerase chain reaction (qPCR).
10. Presence of a clinically significant active bacterial, viral, fungal, parasitic, or prion-associated infection.
11. History of diabetes or abnormal fasting serum glucose (≥ 126 mg/dL) or hemoglobin A1C $\geq 6.5\%$.
12. Patients who have received a gene therapy.
13. Current use of medications that could potentially lead to changes in intracranial pressure (e.g., levothyroxine, vitamin A supplementation, oral contraceptives, tetracycline, acetazolamide [Diamox]).
14. Patients who are currently using strong CYP3A4 inducers or strong CYP3A4 inhibitors, and are unwilling or unable to stop prior to and during the immunosuppression regimen.
15. Patients who are currently receiving or have received an investigational drug or procedure within 3 months prior to Screening. The use of investigational drugs is prohibited throughout Part 1 of the study.
16. Patients with unstable, clinically significant neurologic (other than AMN), psychiatric, cardiovascular, ophthalmologic, pulmonary, hepatic, renal, metabolic, gastrointestinal, urologic, immunologic, hematopoietic, and/or endocrine disease (other than adrenal insufficiency) and/or other abnormality that may

impact the ability to participate in the study or that may potentially confound the study results. It is the responsibility of the Investigator to assess clinical significance; however, consultation with the Sponsor's Medical Monitor may be warranted.

17. Patients who, in the opinion of the Investigator, have any other medical or psychological condition or social circumstances that would impair their ability to participate reliably in the assessments, or who may increase the risk to themselves or others by participating.
18. Patients who are an immediate family member, study site employee, or are in a dependent relationship with a study team member involved in the conduct of this study (e.g., spouse, parent, child, sibling).

SCHEDULE OF ASSESSMENTS

Table 2 Schedule of Assessments for Screening to Day 90 (Month 3)

Activity/Assessment	Screening Visit 1 -120 days	Day -1 Admission	Day 1		Day 2 Discharge	Day 4 Visit 5 Phone Call	Day 8 Visit 6 Clinic	Day 15 Visit 7 Remote ¹	Day 22 Visit 8 Remote ¹	Day 30 (M1) Visit 9 Clinic	Day 38, 52, 68, and 82 ELISpot ¹³ Remote	Day 45 Visit 10 Remote ¹	Day 60 (M2) Visit 11 Clinic	Day 75 Visit 12 Remote ¹	Day 90 (M3) Visit 13 Clinic
			Pre - Dose	Post Dose											
Visit Window	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10	Visit 11	Visit 12	Visit 13	Visit 14	Visit 15
Visit Type	Clinic	Clinic	Clinic	Clinic	Clinic	Clinic	Clinic	Remote ¹	Remote ¹	Clinic	Remote	Remote ¹	Clinic	Remote ¹	Clinic
Informed Consent	X														
Inclusion/Exclusion Criteria	X	X													
Medical History and Demographics	X														
Physical Examination ²	X	X			X	X			X				X		X
Adrenal Function Laboratory Test	X														
HIV, HBV, and HCV Testing	X														
Genetic Testing for <i>ABCD1</i> Mutation	X														
Vital Signs	X		X		X	X			X	X			X		X
Height and Weight	X	X								X			X		X
Electrocardiogram	X				X	X				X			X		X
Neurological Examination	X									X			X		X
Electrophysiological Exam	X									X			X		X
Somatosensory Evoked Potentials	X														
MRI of the Full Spine ^{3,14}	X														X
MRI of the Brain ³	X									X					X
MRI of the Cervical Spine ¹³	X														
Expanded Disability Status Score	X														
SSPROM	X														
Balance Evaluation System Test (MiniBEST)	X														
6-minute Walk Test, Dual-task Timed Up and Go ⁴	X														
2-minute Walk Test, 5 Times Sit-to-Stand Test, Timed Up and Go ⁴	X														
Postural Body Sway ⁴	X														
Clinical Global Impression Scale	X														
MSWS-12, MSQoL-54, PGI Scales, and Urinary and Bowel Questionnaire	X														
Randomization ⁵	X														
IMP Procedure ⁶			X												
IV Methylprednisolone Infusion ⁷		X			X										

SCHEDULE OF ASSESSMENTS

Table 2 Schedule of Assessments for Screening to Day 90 (Month 3)

Activity/Assessment	Screening -120 days	Day -1 Admission	Day 1		Day 2 Discharge	Day 4 ±1 days Visit 5 Phone Call	Day 8 ±2 days Visit 6 Clinic	Day 15 ±2 days Visit 7 Remote ¹	Day 22 ±2 days Visit 8 Remote ¹	Day 30 (M1) ±2 days Visit 9 Clinic	Day 38, 52, 68, and 82 ±2 days ELISpot ¹³ Remote	Day 45 ±2 days Visit 10 Remote ¹	Day 60 (M2) ±2 days Visit 11 Clinic	Day 75 ±2 days Visit 12 Remote ¹	Day 90 (M3) ±7 days Visit 13 Clinic
			Pre - Dose	Post Dose											
Visit Window	Visit 1	Visit 2	Visit 3		Visit 4										
Visit Type	Clinic	Clinic	Clinic		Clinic										
Everyday starting on Day 3															
Oral Prednisone Treatment ⁸															
Blood Chemistry	X ⁹	X			X		X	X	X	X			X	X	X
Hematology	X	X			X		X	X	X	X			X	X	X
Urinalysis	X														
Neutralizing Antibody Titer Testing	X						X	X	X	X			X	X	X
Enzyme-linked Immunosorbent Spot ¹²		X					X	X	X	X			X	X	X
Plasma Biomarkers ⁹	X	X													
Cerebrospinal Fluid Biomarkers ¹⁰			X												
Viral Shedding ¹¹	X												X	X	X
CentrePoint Watch Data Collection															
Concomitant Medications	X	X	X	X	X	X	X	X	X	X			X	X	X
Adverse Events Monitoring		X	X	X	X	X	X	X	X	X			X	X	X
Continuous data collection (everyday)															

Abbreviations: HBV = hepatitis B virus; HBC = hepatitis C virus; HIV = human immunodeficiency virus; IMP = investigational medicinal product; IV = intravenous; MRI = magnetic resonance imaging; M = month; MSQoL-54 = Multiple Sclerosis Quality of Life-54; MSWS12 = Multiple Sclerosis Walking Scale; PGI = Patient Global Impression; SSPROM = Severity Score System for Progressive Myelopathy.

1. A qualified professional will conduct a home healthcare visit for sample collection in conjunction with the site staff, who will conduct assessments virtually. Patients will be contacted by the clinical site to review and record concomitant medications, AEs and functional progress since the last visit. The phone calls will be conducted until the patient has completed immunosuppression regimen tapering.

2. A full physical examination will be conducted at Screening. All other physical examinations will be brief physical examinations, as described in [Section 7.2.15](#).

3. With and without contrast.

4. Completed using the Mobility Lab (OPAL) sensors.

5. IMP Request and randomization (Dose Level 2 Cohort and Dose- Expansion Cohort) should occur after patient's eligibility has been confirmed, but before Day 1.

6. Patients receiving SBT101 will be administered SBT101 via IT infusion; patients randomized to the control arm will undergo an imitation (sham) procedure. All patients must remain at the study site for a 24-hour observational period prior to discharge.

7. The first dose of IV methylprednisolone will be administered on Day 1 and should be completed at least 6 hours before IMP infusion, the second IV methylprednisolone should be administered approximately 2 hours after completion of the IMP procedure, and the third infusion on Day 2 prior to discharge.

8. Maintenance immunosuppressant medication will be dispensed to patients prior to discharge. Patients will be on prednisone/prednisolone (0.5 mg/kg orally once a day) starting on Day 2 for a minimum of 3 months.

9. Patients should be fasted.

10. The CSF sample will be collected after the IMP catheter has been inserted and prior to the IMP infusion (i.e., SBT101 infusion or imitation (sham) procedure). Collection of the CSF sample will be performed via gravity. Patients should refrain from air travel for at least 2 days after cerebrospinal fluid collection.

11. Viral shedding (serum, saliva, urine) assessments are to be performed until 3 consecutive negative results are obtained after Visit 13 (Day 90).

12. Patients enrolled at University of Massachusetts Chan Medical School will have an additional ELISpot collection at Day -1, 15, 22, 30, 45, 60, 75, and 90.

13. ELISpot collection at Day 38, 52, 68, and 82 is only for University of Massachusetts Chan Medical School UMass Chan patients.

14. Full Spine MRI (inclusive of Cervical, Thoracic and Lumbar Spine). Cervical Spine MRIs should be completed based on Cervical Spine Protocol.

SCHEDULE OF ASSESSMENTS

Table 2 Schedule of Assessments for Day 97 to Day 1800 (Month 60)

Activity/Assessment	Day 97, Day 104, and Day 111	Day 120 (M4)	Day 150 (M5)	Day 180 (M6)	Day 270 (M9)	Day 360 (M12)	Day 450 (M15)	Day 540 (M18)	Day 630 (M21)	Day 720 (M24)	Day 1080 (M36)	Day 1440 (M48)	Day 1800/EOS (M60)
<i>Visit Window</i>	±2 days	±2 days	±2 days	±7 days	±2 days	±7 days	±2 days	±7 days	±2 days	±7 days	±30 days	±30 days	±30 days
<i>Visit Number</i>	Visits 14 to 16	Visit 17	Visit 18	Visit 19	Visit 20	Visit 21	Visit 22	Visit 23	Visit 24	Visit 25	Visit 26	Visit 27	Visit 28
<i>Visit Type</i>	Remote ¹	Clinic	Remote ¹	Clinic	Clinic	Clinic	Remote ²	Clinic	Remote ²	Clinic	Clinic	Clinic	Clinic
Vital Signs		X		X	X	X		X		X	X	X	X
Height and Weight		X		X	X	X		X		X	X	X	X
Electrocardiogram		X		X		X		X		X			
Brief Physical Examination		X		X	X	X		X		X	X	X	X
Neurological Examination		X		X	X	X		X		X	X	X	X
Electrophysiological Exam		X		X		X				X			
Somatosensory Evoked Potentials						X							
MRI of the Full Spine ^{3,9}				X		X							
MRI of the Brain ³				X		X		X		X	X	X	X
MRI of the Cervical Spine ⁹						X				X			
Blood Chemistry	X	X	X	X	X	X		X		X	X	X	X
Hematology		X	X	X	X	X		X		X	X	X	X
Urinalysis				X		X				X			
Neutralizing Antibody Titer Testing		X	X	X	X	X							
Enzyme-linked Immunosorbent Spot		X	X	X	X	X							
Plasma Biomarkers ⁸				X		X				X	X	X	X
Lumbar Puncture and Cerebrospinal Fluid Collection for Biomarkers ⁴						X				X			
Viral Shedding ⁵		X	X	X	X	X							
Extended Disability Status Score				X	X	X		X		X			
SSPROM				X	X	X		X		X			
Balance Evaluation System Test (MiniBEST)				X		X		X		X	X	X	X
2-minute Walk Test, 5 Times Sit-To-Stand Test, and Timed Up and Go ^{6,7}				X	X	X	X	X	X	X	X	X	X
6-minute Walk Test and Dual-task Timed Up and Go ⁶				X		X		X		X	X	X	X
Postural Body Sway ^{6,7}				X		X		X		X	X	X	X
Clinical Global Impression and CGI-C				X	X	X	X	X	X	X	X	X	X
MSWS-12, MSQoL-54, PGI Scales, and Urinary and Bowel Questionnaire				X		X		X		X	X	X	X
CentrePoint Watch Data Collection				X		X				X	X	X	X
Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse Events Monitoring	X	X	X	X	X	X	X	X	X	X	X	X	X
Continuous data collection (everyday)													

Phase 1/2 Clinical Research Protocol SBT101-CT101

SwanBio Therapeutics, Ltd.
Version 9.0; 13 December 2023

Abbreviations: M = month; MRI = magnetic resonance imaging; MSQoL-54 = Multiple Sclerosis Quality of Life-54; MSWS-12 = Multiple Sclerosis Walking Scale; PGI = Patient Global Impression; SSPROM = Severity Score System for Progressive Myelopathy.

1. A qualified professional will conduct a home healthcare visit for sample collection in conjunction with the site staff, who will conduct assessments virtually. Patients will be contacted by the clinical site to review and record concomitant medications, AEs and functional progress since the last visit. The phone calls will be conducted until the patient has completed immunosuppression regimen tapering.
2. Patients will be contacted by the study site staff to conduct assessments virtually.
3. With and without contrast.
4. Patients should refrain from air travel at least 2 days after cerebrospinal fluid collection.
5. Viral shedding (serum, saliva, urine) assessments are to be performed until 3 consecutive negative results are obtained after Visit 13 (Day 90).
6. Completed using the Mobility Lab (OPAL) sensors.
7. The qualified professional will not routinely conduct these assessments with the patients during home healthcare visits.
8. Patients should be fasted.
9. Full Spine MRI inclusive of cervical, thoracic and lumbar Spine. Cervical Spine MRIs should be completed based on Cervical Spine Protocol.

1. BACKGROUND INFORMATION AND STUDY RATIONALE

1.1 Overview of the Target Disease

Adrenoleukodystrophy (ALD) is an inherited progressive neurodegenerative disorder caused by a mutation in the adenosine triphosphate (ATP)-binding cassette transporter subfamily D member 1 (*ABCD1*) gene. This gene is localized to the X chromosome and encodes a peroxisomal ATP-binding cassette half-transporter responsible for transporting very long chain fatty acids (VLCFAs) from the cytosol into the peroxisome for degradation.¹⁻² A mutation in the *ABCD1* gene results in reduced degradation and accumulation of saturated VLCFAs in tissues and body fluids (i.e., brain, nervous system, adrenal glands), which in turn leads to demyelination, central nervous system (CNS) deterioration, and neurologic defects.¹⁻³ The accumulation of VLCFAs – particularly C26:0 – is acknowledged to be the biochemical hallmark of ALD and serves as a diagnostic marker.³⁻⁴

The principal phenotypes observed in males with ALD are adrenomyeloneuropathy (AMN), the cerebral demyelinating form of ALD (cerebral ALD), and adrenal insufficiency (Addison's disease).⁵ There are marked differences in the clinical presentations of these phenotypes, including the age of onset, pathology, and progression of disease, indicating that AMN, cerebral ALD, and adrenal insufficiency are etiologically related but distinct disease states that necessitate different treatment strategies. Among these disease states, AMN is the most common. It is characterized by a slowly progressive axonopathy affecting sensory ascending and motor descending spinal cord tracts that manifests in aging patients, with 100% penetrance in men.⁵

In AMN, the largest and longest axons in the spinal cord sustain the greatest degree of damage and a dying-back pattern of axonal degeneration has been reported in the dorsal columns (gracile fasciculus, and the cuneate fasciculus) and corticospinal tracts; the large myelinated fibers appear to be the most susceptible.

Onset typically occurs between the ages of 20 and 30 years in male patients; the initial symptoms include poor balance and falls, progressive stiffness and weakness of the legs, impaired vibration and position senses, sphincter disturbances, sexual dysfunction, scarce scalp hair (alopecia), and impaired adrenocortical function.^{3,6-7} Patients with AMN typically start to use walking aids 13 to 16 years after onset of AMN symptoms.

Approximately half of patients with AMN show some degree of brain involvement on magnetic resonance imaging (MRI) or clinical examination.⁸ Abnormal MRI signals of white matter have been observed, but no gadolinium enhancement is present, indicating an intact blood brain barrier and the absence of an acute inflammatory process. In 20 to 60% of patients with AMN, brain involvement becomes severely progressive and leads to serious cognitive and behavioral disturbances that may progress to total disability and death.⁷ In two-thirds of patients with AMN, neurological disability progresses slowly and, within 10 to 15 years, motor disability becomes severe and patients require the use of walking aids.⁵

Mutation in the *ABCD1* gene affects adrenal glands, and primary adrenal insufficiency is common in males with AMN⁵. Adrenal insufficiency can be treated using steroid (prednisone/prednisolone) replacement therapy. Undiagnosed or inappropriately treated adrenal insufficiency or rapid change of steroid dose can progress to adrenal crisis, which can lead to rapid deterioration and death if left untreated.

There are no curative treatments available for AMN. Current therapeutic strategies are aimed at eliminating or ameliorating symptoms that affect functional abilities and/or impair quality of life. These include, among others, monitoring of patients to prevent the development of secondary impairment or disability, such as lumbar disc degeneration and chronic immobility caused by gait disturbances; managing spasticity through physiotherapy and the use of anti-spasticity medications; and reducing urinary symptoms through physiotherapy, anticholinergic drugs, and long-term catheterization. These therapies can somewhat improve the quality of life of patients; however, without addressing the genetic root cause of AMN, patients are left with a progressive disorder that leads to lifelong disability.

1.2 Rationale for SBT101 Gene Therapy

In recent years, there have been great biotechnological advances in the field of gene therapy through the development of viral vector gene therapies for inherited diseases caused by genetic defects. These therapies utilize modified viruses, usually recombinant adeno-associated virus (AAV) vectors or adenovirus vectors, as drug-delivery vehicles to introduce specific deoxyribonucleic acid (DNA) gene sequences into cells to produce functional copies of the gene product, thereby correcting the biochemical defects caused by gene mutations. In 2017, the United States (US) Food and Drug Administration (FDA) approved the first viral vector gene therapy, Luxterna[®], for the treatment of inherited retinal diseases and, in 2019, Zolgensma[®] was approved for the treatment of the genetic disease of spinal muscular atrophy.

In vivo gene therapy offers the possibility of a one-time treatment for an inherited disease, with the prospect of life-long benefits.⁹ Recombinant AAV gene therapies directed at the CNS, particularly recombinant AAV serotype 9 (AAV9), have produced promising pre-clinical results, indicating great potential in recombinant AAV9 gene therapy as treatment for AMN, and are increasingly being translated from the bench to clinical trials,¹⁰ and even marketing authorization (e.g., Zolgensma[®]).

SwanBio Therapeutics, Ltd (SwanBio) is developing SBT101, a recombinant AAV9 vector encoding the functional human *ABCD1* gene (*hABCD1*; *AAV9-hABCD1*), for use as a single dose administered via intrathecal (IT) infusion to treat AMN by correcting for the underlying genetic defect in the CNS. Efficacy has been demonstrated in preclinical trials conducted by the Sponsor. A dose-dependent increase in *ABCD1* protein expression in the spinal cord and statistically significant improvement in mitochondrial deoxyribonucleic acid (mtDNA) were demonstrated in male *Abcd1* knockout (*Abcd1*^{-/-}) mice treated with SBT101 compared to untreated littermate controls. These findings are in line with reports in the literature, where functional reduction of VLCFA accumulation as well as improved behavior and neurological presentation were demonstrated in *Abcd1*^{-/-} mice injected with recombinant AAV9-*hABCD1*, with no observable toxicity.¹¹⁻¹³ In another preclinical trial by the Sponsor, a single IT administration of low dose SBT101 was well tolerated by non-human primates (NHPs), indicating a promising safety profile (see the Investigator's Brochure for more details).

While safety and efficacy have been demonstrated in animal studies, SBT101 has not yet been evaluated in humans. The purpose of this first-in-human (FIH) Phase I/II study is to evaluate the safety, tolerability, and efficacy of one-time IT administration of SBT101 on disease progression in adult patients with AMN. Efficacy will be evaluated by comparing disease progression in SBT101 vs. control patients, and by comparing data from patients enrolled to SBT101-CT101 vs.

data from patients with AMN enrolled in SBTNHX-CT901. SBTNHX-CT901 is an ongoing 5-year observational study on the progression of disease conducted by the Sponsor.

1.3 Anticipated Risks

Anticipated risks have been identified based on the following:

- Risk profiles associated with IT delivery of recombinant AAV9-mediated gene therapies
- Known class effects of AAV-mediated gene therapies
- Nonclinical data on SBT101
- Natural history data and cases on AMN
- Scientific literature reviews on IT delivery of AAV-mediated gene therapies.

Anticipated risks include elevated transaminases (liver toxicity), transient thrombocytopenia, cardiac adverse events (AEs), adverse inflammatory responses in the spine and the dorsal root ganglia (DRG), viral shedding, insertional mutagenesis, and the development of an immune response against the vector and/or transgene.¹⁴⁻¹⁶ The course of AMN-related disabilities over time is poorly or incompletely understood due to a limited number of patients and lack of treatments; some patients with AMN may progress to an inflammatory cerebral disease (cALD).

Precautionary measures to minimize these anticipated risks in enrolled patients have been built into the protocol, such as the exclusion of patients with liver impairments and/or cardiac conditions, implementation of an immunosuppressive regimen, and routine monitoring of inflammatory responses and viral shedding in biological fluids.

2. STUDY OBJECTIVES AND ENDPOINTS

2.1 Objectives

- To characterize the safety and tolerability of one-time IT administered SBT101 in adults diagnosed with AMN.
- To characterize the efficacy of IT administered SBT101 in adults diagnosed with AMN.
- To assess the change in quality of life, patient abilities, and daily living activities from Baseline to Month 12 and Month 24.
- To evaluate the safety of the catheter used to infuse SBT101 intrathecally in Europe (European only objective)

2.2 Endpoints

Primary Endpoint:

- Percentage of patients with at least 1 Grade III or Grade IV AE that is at least possibly related to SBT101 at Month 24, as reported by the patients or observed by the Investigator, after SBT101 treatment.

Secondary Endpoints:

- Changes in the 6-minute Walk Test (6MWT) at Month 12 and Month 24 compared to controls, and compared to the reference changes determined by the prospective SBTNHX-CT901 study.
- Changes in the 2-minute Walk Test (2MWT) at Month 12 and Month 24 compared to controls, and compared to the reference changes determined by the prospective SBTNHX-CT901 study.
- Changes in the 5 Times Sit-to-Stand Test (5XSST) at Month 12 and Month 24 compared to controls, and compared to the reference changes determined by the prospective SBTNHX-CT901 study.
- Changes in postural body sway amplitudes at Month 12 and Month 24 compared to controls, and compared to the reference changes determined by the prospective SBTNHX-CT901 study.
- Proportion of patients with Expanded Disability Status Score (EDSS) of ≥ 5.5 at Month 12 and Month 24 compared to controls, and compared to the reference changes determined by the prospective SBTNHX-CT901 study.
- Proportion of patients with increased anti-spasticity- medication dose at Month 12 and Month 24 compared to controls, and compared to the reference changes determined by the prospective SBTNHX-CT901 study.
- Changes in Timed Up and Go (TUG) at Month 12 and Month 24 compared to controls, and compared to the changes observed in the prospective SBTNHX-CT901 study.
- Changes in Dual-task TUG (TUG-DT) at Month 12 and Month 24 compared to controls, and compared to the changes observed in the prospective SBTNHX-CT901 study.
- Changes in Balance Evaluation System Test (miniBESTest) at Month 12 and Month 24 compared to controls, and compared to the changes observed in the prospective SBTNHX-CT901 study.
- Changes in Clinical Global Impression (CGI; performed by a qualified professional) score at Month 12 and Month 24 compared to controls.

- Changes in Patient Global Impression of Severity (PGI-S), Patient Global Impression of Severity – Balance (PGI-S Balance), and Patient Global Impression of Change – Balance (PGI-C Balance) scores at Month 12 and Month 24 compared to controls, and compared to the changes observed in the prospective SBTNHX-CT901 study.
- Percentage of patients with at least 1 Grade III or Grade IV AE that is at least possibly related to SBT101 at Month 60, as reported by the patients or observed by the Investigator, after SBT101 treatment.

Exploratory Endpoints:

- Changes in the Severity Score System for Progressive Myelopathy (SSPROM) score at Month 12 and Month 24 compared to controls, and compared to the changes observed in the prospective SBTNHX-CT901 study.
- Changes in the Multiple Sclerosis Quality of Life-54 (MSQoL-54) score at Month 12 and Month 24 compared to controls, and compared to the changes observed in the prospective SBTNHX-CT901 study.
- Changes in the Multiple Sclerosis Walking Scale (MSWS-12) score at Month 12 and Month 24 compared to controls, and compared to the changes observed in the prospective SBTNHX-CT901 study.
- Changes in the Urinary and Bowel Questionnaire at Month 12 and Month 24 compared to controls, and compared to the changes observed in the prospective SBTNHX-CT901 study.
- Changes in the conduction velocity, distal latency, and F-wave- of peroneal compound motor action potential compared to controls, and compared to the changes observed in the prospective SBTNHX-CT901 study.
- Changes in somatosensory evoked potentials at Month 12 and 24 compared to controls
- Changes in the daily life profile, activity bouts, and sleep parameters, as measured by the Centropoint Watch, compared to controls, and compared to the changes observed in the prospective SBTNHX-CT901 study.
- Clinical Global Impression-Change by visit
- Changes in the following parameters at Month 12 and Month 24 compared to controls:
 - Plasma and/or cerebrospinal fluid (CSF) neurofilament light chain (NfL), lysophosphatidylcholine (LPC) VLCFAs, and VLCFAs.
 - Cervical spine MRIs (diffusion tensor imaging [DTI], magnetization transfer [MT], and spectroscopy parameters).
- T-cell reactivity to the capsid by visit from pre-IMP procedure through to Day 90.

Safety Endpoints:

- Incidence of treatment-emergent adverse events (TEAEs).
- Changes in the amplitude of sural nerve sensory action potential at Month 1 and Month 6 compared to Baseline.
- Changes in the latency of H-reflex of soleus muscle at Month 1 and Month 6 compared to Baseline.
- Changes in somatosensory evoked potentials at Month 3 compared to Baseline.
- Incidence of abnormal values in clinical laboratory assessments, vital signs, and electrocardiogram (ECG) parameters posttreatment.
- Changes in clinical laboratory assessments, vital signs, physical and neurological examinations, and ECG parameters post-treatment compared to Baseline.

- Frequency and severity of adverse events related to the catheter used for infusion of SBT101 intrathecally in Europe (European only endpoint)

3. STUDY DESIGN

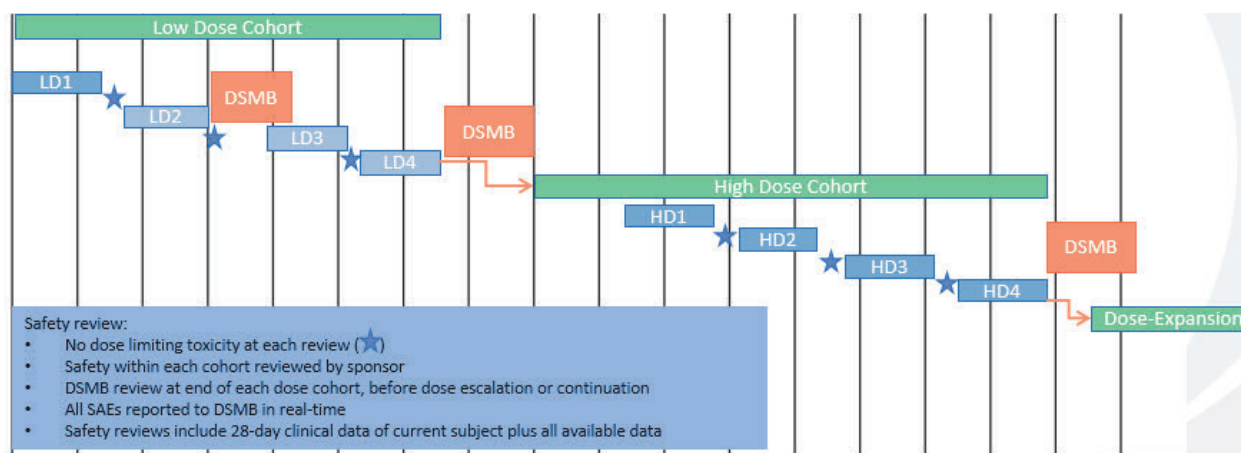
3.1 Overall Study Design and Plan

This is a Phase1/2 randomized, blinded, sham-controlled, multicenter, dose escalation study to evaluate the safety and efficacy of one-time IT administration of SBT101 in a minimum of 16 adult male patients with AMN aged 18-65 years. During dose escalation, Dose Level 1 and Dose Level 2 patients will receive open-label SBT101 to establish safety and the maximum tolerated dose (MTD). After confirmation of safety, the MTD will be selected for the randomized, blinded Cohort 3 dose expansion phase. The study consists of 2 parts:

- Part 1: A 24-month core study period to evaluate the safety and potential impact of SBT101 on disease progression. Part 1 will consist of 2 phases:
 - Dose Escalation Phase (open-label): Two (2) doses of SBT101 will be evaluated to establish the maximum tolerated dose (MTD)
 - Dose Level 1 Cohort – 1.0E14 vector genomes (vg)
 - Dose Level 2 Cohort– 3.0E14 vg
 - Dose Expansion Phase (randomized, blinded): Additional patients will be enrolled to receive SBT101 at MTD
- Part 2: An unblinded 3-year long-term safety follow-up period with annual follow-up visits to evaluate the safety of SBT101 and disease progression.

During the Dose Escalation Phase, a minimum of 8 patients will be sequentially enrolled, first to the Dose Level 1 Cohort followed by the Dose Level 2 Cohort, as illustrated in [Figure 1](#). A minimum of 4 patients at Dose Level 1 will receive open-label SBT101, and a minimum of 4 patients Dose Level 2 will receive open-label SBT101.

Figure 1 Staggered Enrollment and Dose Escalation with Safety Review Plan During Part 1 of the Study



Abbreviations: DSMB = Data and Safety Monitoring Board.

During the Dose Escalation Phase, Dose Level 1 Cohort and Dose Level 2 Cohort will be open label. The Dose Expansion Phase will be blinded per [Table 6](#). The Sponsor will review the available safety data each time a patient completes the Day 30 Visit. The review of safety data will be completed before the next patient receives SBT101, as detailed in [Section 5.2.2](#). After

completion of a minimum of 2 patients in each Dose Level Cohort, the independent DSMB will review the available safety data of all patients in the cohort that complete the Day 30 visit. The decision to proceed with the next Dose Level Cohort or to initiate the Dose Expansion Phase will be made after the independent DSMB and the Sponsor have reviewed the safety data.

During the Dose Expansion Phase, an additional 8 patients will be enrolled and randomized to SBT101 (at MTD) or control in a 3:1 ratio. The Sponsor, in consultation with the DSMB, may decide to investigate an additional dose of SBT101 in a subset of patients in the Dose Expansion Phase different than Dose Level 1 or Dose Level 2. Additionally, the Sponsor, in consultation with the DSMB, may elect to increase the number of patients in a Dose Level Cohort during the study.

Patients who received SBT101 will continue on to Part 2 for long-term safety follow-up after Month 24. Patients who in the control arm will have the option to be treated with open-label SBT101. Patients who opt to be treated with open-label— SBT101 must continue to meet all exclusion and inclusion criteria (except for inclusion criterion #1 and #3 and exclusion criterion #3) related to severity of AMN disease). If they choose to be treated with open-label they will restart all procedures at Day -1 and will be followed for another 60 months.

After appropriate information about the study and SBT101 has been provided to the patient and informed consent has been obtained, patients will be evaluated for study eligibility at the Screening Visit (Visit 1) according to inclusion/exclusion criteria ([Section 4](#)). Screening evaluations will be conducted as detailed in [Section 6.1](#). After confirmation of eligibility, patients in Dose Level 1 Cohort and Dose Level 2 Cohort will receive SBT101 and patients in Dose Expansion will be randomized to the SBT101 or control arm.

On Day -1 (Visit 2), patients will be admitted to the study site and remain at the site for a minimum of 3 days. All patients will be initiated on an immunosuppressive regimen to prevent the development of an immune response to the AAV9 capsid and transgene for a minimum of 3 months. Induction will be achieved with methylprednisolone sodium succinate for injection (from here on referred to as methylprednisolone) and maintenance will be achieved with oral prednisone/prednisolone ([Section 5.2.7.1](#)). The details of the prednisone/prednisolone regimen and tapering schedule are provided in [Appendix 1](#).

On Day 1 (Visit 3), patients will undergo the investigational medicinal product (IMP) procedure. In this protocol, “IMP procedure” refers to SBT101 infusion in patients randomized to the SBT101 arm and the imitation (sham) procedure in patients randomized to the control arm. Patients randomized to SBT101 will undergo a lumbar puncture and 12-16 cc of CSF will be collected for laboratory assessments; SBT101 will then be instilled into the CSF by slow and continuous delivery over approximately 5 hours at a rate of up to 8 mL/hour. Patients randomized to control will undergo an imitation (sham) procedure consisting of a lumbar puncture for collection of CSF (12-16 cc) for laboratory assessments. The infusion bag for control patients will contain normal saline 0.9% (Placebo_{IMP}), which will not be instilled into the CSF, but instead collected in a Collection EVA bag attached to the skin below the lumbar puncture site. Both SBT101 and control patients will have a Collection EVA bag attached below the lumbar puncture site to maintain the blind ([Section 5.2.5](#)). Patients will be allowed to take breaks and encouraged to walk once every hour during the infusion.

Patients may be discharged from the study site on Day 2 (Visit 4), after completing a 24-hour observation period following the IMP procedure.

Safety follow-up and efficacy assessments will be completed at clinic visits or remote visits per the Schedule of Assessments (**Table 2** and **Table 3**).

Remote visits will be conducted by a qualified professional and a study team member virtually. At the remote visits indicated in the Schedule of Assessments, patients will use The Mobility Lab (OPAL) sensors to complete the 2MWT, 5XSST, postural sway parameters, and TUG.

Patient-reported outcomes (PROs), including the MSWS-12, MSQoL-54, PGI-S, PGI-S Balance, PGI-C Balance, and Urinary and Bowel Questionnaire, will be completed electronically as detailed in the Schedule of Assessments (**Table 2** and **Table 3**).

Patients enrolled at the University of Massachusetts Chan Medical School will undergo an additional blood draw to assess the T-cell reactivity to the AAV capsid, as measured by enzyme-linked immunosorbent spot (ELISpot), per the Schedule of Assessments (**Table 2** and **Table 3**).

The End of Study (EOS) Visit will be conducted at the Month 60 Visit or if patient withdraws according to the assessments listed in **Table 3**.

The duration of study participation for patients will be up to 69 months, including up to 4 months for Screening, up to 3 months for re-screening (if necessary), the 24-month core study period, and 36 (± 2) months for long-term safety follow-up.

For control arm patients who choose to receive open-label SBT101 after completing Month 24 and the cohort primary analysis has been completed, the duration of study participation will be a maximum of 93 months.

3.2 Considerations Due to the COVID-19 Pandemic

Due to the Coronavirus disease 2019 (COVID-19) pandemic, adjustments to visits or schedule may need to be made to accommodate safety concerns and other restrictions. Any changes to the study and monitoring plans that could impact patients will be communicated to study patients.

All protocol deviations due to the COVID-19 pandemic will be documented as such.

3.3 Discussion of Study Design

3.3.1 Rationale for Study Design

As this is a FIH study, two dose levels of SBT101 will be evaluated to establish the MTD during the Dose Escalation Phase. To ensure patient safety, the safety data of the first patient in a cohort will be reviewed before treating the second patient in that cohort, and the safety data of the second patient will be reviewed before escalating the dose to the Dose Level 2 or treating the remaining patient(s) in the cohort. The safety data of all patients in a cohort will also be reviewed by the independent DSMB before treating patients at the next dose level or proceeding to Dose Expansion at MTD.

Part 1 will be 24-months to ensure changes in the progression of disease following SBT101 administration can be captured within the study period. In studies evaluating the progression of axonopathy in patients with AMN, it was reported that significant changes in the EDSS, SSPROM, TUG, and postural body sway parameters (i.e., sway amplitude and sway path) could be detected over a period of 24 months; changes in the 6MWT could be detected over 12 months.¹⁷⁻¹⁸ Patients will also be evaluated for safety, including the development of an

immune response to the AAV capsid and transgene, which in other studies has been reported to have onset approximately 2-3 weeks post-infusion.¹⁹⁻²⁰

SBT101 has the potential to address a significant clinical need for patients with AMN, for whom there are no curative treatment options available. For ethical purposes, control patients will have the option to be treated with open-label SBT101 at the end of the cohort primary analysis, pending adequate safety findings. Patients who opt to be treated with open-label SBT101 will restart all procedures from Day -1 (Visit 2) onward. Throughout Part 1 and Part 2 of the study, efficacy and safety data will be collected through in clinic- and remote visits to reduce the study time commitments and burden on study patients.

The design of this study will allow unbiased evaluation of the long-term safety and efficacy of SBT101, enable all study patients to receive SBT101 treatment, and reduce the time commitments of the study to circumvent attrition.

3.3.2 Rationale for Choice of Endpoints

The endpoints of the study were chosen based on variables associated with AMN and/or demonstrated to capture severity and progression of disease.

Disturbances in gait, balance, and postural sway are part of the clinical presentation in patients with AMN. Outcome measures that assess these parameters, such as postural sway and the miniBESTest, have been reported to be reliable and valid tools that detect fall status, gait difficulties, and postural control deficits in patients with AMN and/or patients with balance deficits.^{18,21-23} Functional outcome measures, such as the EDSS, SSPROM, TUG, and 6MWT have been reported to distinguish between ambulatory groups (i.e., unaffected walking, affected but unaided walking, and walking with aids) and detect progression of disease at follow-up, indicating good clinical validity in assessing disability in AMN.¹⁷ Patient-reported outcomes, such as the MSWS-12, MSQoL-54, and Patient Global Impression (PGI) have been widely used to assess walking function (MSWS-12), quality of life (MSQoL-54), and efficacy of treatment (PGI) in patients with mobility issues or disability, such as the elderly, multiple sclerosis, and/or Parkinson's disease, among others.²⁴⁻²⁶

The accumulation of VLCFAs is acknowledged to be the biochemical hallmark of ALD and serves as a diagnostic marker; lysophosphatidylcholine-VLCFAs (LPC-VLCFAs) are elevated in dried blood spots from ALD males and used for ALD newborn screening.^{3-4,27} The brain derived protein NfL is a marker for neuroaxonal damage that are released into the CSF, and further into blood, following neuronal injury.²⁸⁻³⁰ These biomarkers have been reported to have predictive value in the progression and/or severity of degenerative disorders.³⁰⁻³⁴

Nerve conduction studies (NCS) will be used to evaluate motor and sensory nerve function of the lower extremities. Abnormal NCS results have been reported in AMN, as well as correlation with VLCFAs, suggesting predictive value of NCS in evaluating treatments.³⁵

Brain and Full Spine (cervical, thoracic, and lumbar) MRIs, with and without contrast, and Cervical Spine MRI including DTI, MT, and spectroscopy parameters will be used as surrogate outcome measures to monitor the progression of axonopathy and DRG function. These measures were found to predict progression of myelopathy in the cervical spinal cord over a period of approximately 2 years, as well as differentiate asymptomatic patients with AMN from healthy controls, and correlated with functional outcome measures, such as the EDSS, SSPROM, and TUG.^{17,36}

3.3.3 Rationale for Choice of Study Population

This Phase 1/2 study is designed to evaluate the efficacy of IT administration of SBT101 in adult male patients with AMN using outcome measures of gait and balance that have been reported to have good clinical validity in assessing disability in AMN (see [Section 3.3.2](#)).

As the progression of AMN disease cannot be predicted in asymptomatic patients, the eligibility criteria of this study were set to ensure that symptomatic patients that show evidence of motor tract involvement are enrolled, based on an EDSS score between 1 and 4.5. This range of EDSS scores will also ensure that enrolled patients can perform all of the study assessments, as patients with advanced AMN are often unable to perform certain balance assessments, such as those that require standing with feet together and eyes closed.

While AMN results in severe disability, advances in technology have made it possible for patients with advanced disease to remain functional and independent despite their disabilities. Nevertheless, poor posture and use of assistive devices such as cane, walker, etc., especially in patients with advanced disease, may result in spinal stenosis or narrowing of the spinal canal, which in turn may affect CSF circulation and interfere with SBT101 administration via IT infusion. As such, patients with advanced disease or a history of brain or spinal cord disease that would interfere with lumbar puncture procedures and CSF circulation will be excluded from the study.

Additionally, patients with advanced disease are less likely to benefit from SBT101 treatment. In AMN, the largest and longest axons in the spinal cord sustain the greatest degree of damage and a dying-back pattern of axonal degeneration has been reported in the dorsal columns (gracile fasciculus, and the cuneate fasciculus) and corticospinal tracts; the large myelinated fibers appear to be the most susceptible.³⁷ However, examinations of the DRG in patients with AMN do not demonstrate neuronal loss; instead, the large neurons of the DRG only undergo atrophy, which makes therapeutic intervention a realistic possibility, especially in the early stages of the disease. DRG pathology results in sensory ataxia and impaired balance in patients with AMN, and balance assessments are key outcome measures of the study. Consequently, patients with the milder disease are likely to have less irreversible neuronal loss, making them better candidates for the study, whereas patients with advanced disease are less likely to benefit from SBT101 treatment, as these patients are likely to have more DRG degeneration.

3.3.4 Justification for SBT101 Dose Levels

The SBT101 dose levels for this study were selected based on safety, efficacy, and biodistribution data from preclinical studies. *In vitro* data and *in vivo* pharmacology, biodistribution, and toxicology findings from studies conducted in mice and NHPs demonstrated that SBT101 is efficient and can be safely administered via IT infusion. Overall, IT administration of AAV-hABCD1 vectors was well tolerated in mice and NHPs.

Efficacy was demonstrated in *in vivo* pharmacology studies conducted in *Abcd1*^{-y} mice and *Abcd1/Abcd2* double knockout mice by determination of hABCD1 protein expression, distribution of AAV9 vector genomes, mtDNA levels, and VLCFA levels. These data were analyzed to determine exposure-based safety margins. In a study of *Abcd1*^{-y} mice, there was a dose dependent- increase in ABCD1 protein levels in the spinal cords of all *Abcd1*^{-y} mice treated with SBT101, and mice receiving 2.0E10 vg/animal had a statistically significant improvement in mtDNA at 8 weeks post-administration compared to untreated littermate controls and animals

treated at doses $\leq 6.0 \times 10^9$ vg/animal. In another study, *Abcd1*^{-y} mice were treated with recombinant AAV9-h*ABCD1* at doses ranging from 5.5×10^{10} to 5.5×10^{11} vg/animal. The mid dose of 1.6×10^{11} vg/animal resulted in a statistically significant reduction of VLCFA in the spinal cord. At 5.5×10^{10} vg/animal, a trend of lowering levels of VLCFA was observed. Similar findings of VLCFA reduction and improvement in behavior were reported in the literature.³⁸⁻³⁹ Based on these molecular changes, the minimum effective dose (MED) in mice was established to be 2.0×10^{10} vg/animal. The MED is also supported by the functional improvement observed in mouse study NCP-MOU-101-05, a 20-month dose-finding proof-of-concept study. Compared to control mice, administered AAV9-Null, there was an increased grip strength at 7-8 months post administration of SBT101 to *Abcd1*^{-y}/*Abcd2*^{-/-} mice at 3.3×10^{10} vg/an and 3.3×10^{11} vg/an, equal to the grip strength of wild-type mice. The grip strength in *Abcd1*^{-y}/*Abcd2*^{-/-} mice was observed to be maintained up through 10 months post administration, compared to *Abcd1*^{-y}/*Abcd2*^{-/-} mice administered AAV9-Null vector. The increase in grip strength occurred together with a dose dependent decrease of VLCFA compared to *Abcd1*^{-y}/*Abcd2*^{-/-} animals administered AAV9-Null vector. The lowest dose (3.3×10^{10} vg/an) in mice at which functional improvement and reduction in VLCFA were observed translates to a human dose of 1.1×10^{14} vg/person.

To determine how many cells need to express ABCD1, 2 criteria were used. In one study, 40% of thoracic DRG neurons stained positive for ABCD1 expression in a healthy human donor.⁴⁰ In addition, women with a single mutated copy of the *ABCD1* gene demonstrate variable expression of the X-linked ALD disease, offering insight into the degree of ABCD1 required to modify the course of disease. Many women are asymptomatic for much, if not all, of their lifetimes, and neither adrenal insufficiency nor cerebral disease have been observed.

Epidemiological studies have shown that the onset of spinal cord disease in women with ALD usually occurs much later in life, and the progression of disease is considered slower compared to men with AMN. Due to the random process of X inactivation, the X chromosome with a mutant allele is inactivated in some cells in female carriers, while the X chromosome with a normal allele is inactivated in other cells (mosaic pattern). As a result of this random inactivation of the X chromosome, 23% to 86% of fibroblasts and white blood cells in women carrying an *ABCD1* mutation have been reported to lack the ABCD1 protein.⁴¹⁻⁴² This variability in ABCD1 expression does not appear to result in differences in the clinical manifestation of the disease in these patients; most of the women in these studies were asymptomatic, suggesting that this residual level of expression is sufficient to avert the majority of disease. Therefore, it can be hypothesized that the progression of AMN disease in humans can be altered by addition of *ABCD1* to at least 23% of cells. This level of transduction can be achieved with the doses proposed in this study. In a nonclinical biodistribution study conducted by the Sponsor (NCP-NHP-101-01), single IT infusions of the AAV9 vector containing a green fluorescent protein reporter gene at doses of 1.2×10^{13} vg and 3.4×10^{13} vg (corresponding to 1.4×10^{14} and 3.9×10^{14} vg/person, respectively; **Table 4**) in cynomolgus monkeys resulted in expression of the reporter gene in 25% to 100% of spinal cord and DRG neurons (as measured by immunohistochemistry).

Safety was demonstrated at similar and higher doses in NHPs; in study NCP-NHP-101-05, single IT administrations at doses up to 7.6×10^{13} vg/animal (corresponding to a human dose of 8.3×10^{14} vg/person; **Table 4**) were well tolerated by NHPs. Based on efficacy, biodistribution, and safety data, and taking into consideration differences in CSF volumes across species, a dose of SBT101 was calculated (see dose conversion for humans in **Table 4** and dose ranges across species in **Figure 2**). As SBT101 is intended for IT administration, the scaling of doses between

species was based on CSF volume. The proposed doses in patients, 1.0E14 and 3.0E14 vg/person, fall within dose ranges that have been demonstrated to be associated with activity/efficacy in mice, and translate into doses that efficiently transduce spinal cord and DRG neurons in NHPs. These doses have also been shown to be safe in both mice and NHPs, with a safety margin for the high dose of approximately 2.75-fold.

Further details on *in vivo* and *in vitro* pharmacology, toxicology, and safety, and efficacy studies are provided in the Investigator’s Brochure.

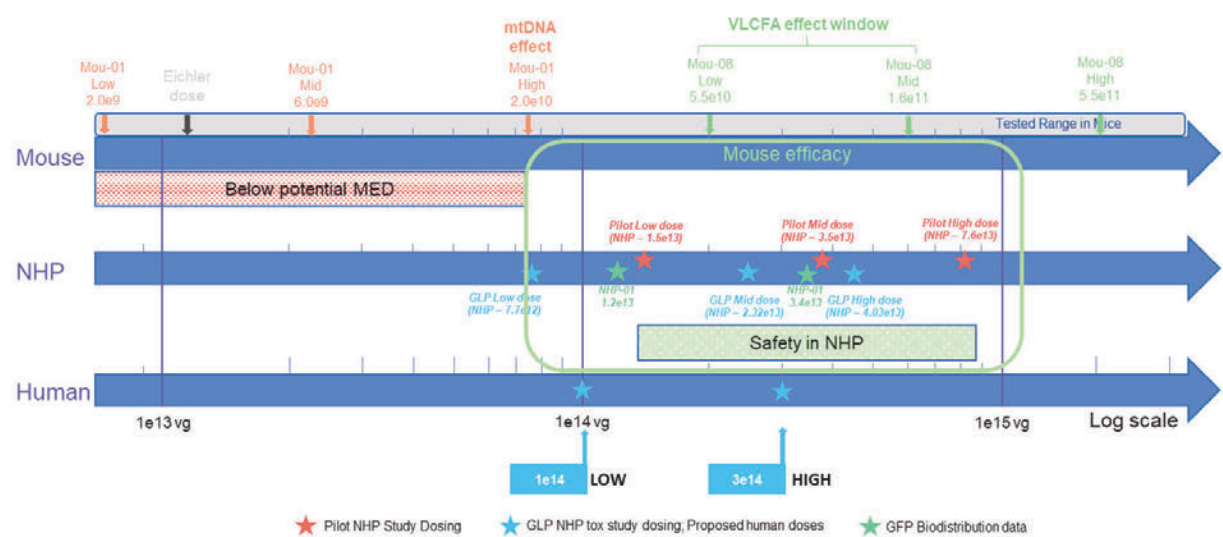
Table 3 CSF Volumes in Different Species and Comparison of Doses Between Species

Species	CSF Volume (mL)	Relative to Humans	Dose (vg)	Equivalent Dose in Humans (vg)
Mouse	0.04 ⁴³⁻⁴⁴	0.000267	mtDNA effect (MED): 2.0E10	MED: 7.50E13
			Grip effect: 3.3E10	1.24E14
			VLCFA effect: 5.5E10	2.06E14
NHP	13 ⁴⁵	0.086	1.26E13	1.45E14
			1.50E13 (low)	1.73E14 (low)
			3.38E13	3.90E14
			7.62E13 (high)	8.79E14 (high)
Human	150 ⁴³⁻⁴⁶	1		

Calculation of equivalent human dose used non-rounded conversion factors

Abbreviations: CSF = cerebrospinal fluid; MED = minimum effective dose; mtDNA = mitochondrial deoxyribonucleic acid; NHP = non-human primate; vg = vector genomes; VLCFA = very long chain fatty acid

Figure 2 Dose Ranges Across Species



Abbreviations: GFP = green fluorescent protein; GLP = Good Laboratory Practice; MED = minimum effective dose; mtDNA = mitochondrial deoxyribonucleic acid; NHP = non-human primate; VLCFA = very long chain fatty acid; vg = vector genomes.

3.3.5 Rationale for Immunosuppression

Activation of the immune system in the brain following gene therapy has been reported in studies for CNS disorders, raising concerns about neuroinflammation, decreased transgene expression, and decreased longevity of transgene expression.^{15,47-48} Humoral immune responses derived from antibody-producing B-cells can develop against the AAV, and some anti-AAV antibodies are neutralizing, preventing AAV from infecting cells and thereby impeding therapeutic efficacy.^{10,15} However, several other studies have shown no evidence of neuroinflammation, underscoring the need to compare study designs across clinical trials. In studies of AAV-mediated gene therapy delivered directly into the CNS in conditions such as Parkinson's disease,⁴⁹ Canavan disease,⁵⁰ late infantile neuronal ceroid lipofuscinosis,⁵¹ and giant axonal neuropathy,⁵² AAV vector administration was associated with little to no detectable immune response against the capsid or transgene in serum and peripheral blood mononuclear cells.

The risk of developing an immune response with SBT101 is anticipated to be low, as SBT101 is administered via the IT route directly into the CNS, which is known to be an immune privileged compartment of the body due to immune response limiting adaptations, such as the blood brain barrier and blood CSF barrier, and the CSF generally contains lower anti-AAV neutralizing antibody (nAb) levels compared to the systemic circulation. Robust and localized expression throughout relevant neuronal cell types in the CNS can be achieved, thereby reducing potential neurotoxicity from transgene expression in nontarget cell populations.

To ensure the safety of patients, prevent the potential development of an immune response to the AAV capsid and transgene, and protect transgene expression, all patients will receive an immunosuppressive regimen consisting of corticosteroids (methylprednisolone and prednisone/prednisolone). Maintenance therapy will continue until Day 90, at a minimum, or longer in the event of increased T-cell reactivity or ongoing inflammatory response.

Corticosteroids are highly effective in the treatment of immune reactions, including myelitis and severe autoimmune hepatitis, a disease in which the liver is infiltrated by cytotoxic lymphocytes directed against hepatocytes that display epitopes against which the immunocytes have been sensitized.

4. PATIENT SELECTION AND WITHDRAWAL CRITERIA

4.1 Patient Inclusion Criteria

Patients with AMN will be eligible for this study if they meet all the following criteria:

1. Male adults aged ≥ 18 and ≤ 65 years
2. Diagnosed with X-linked ALD, including proven mutation in the *ABCD1* gene through confirmatory genetic testing, and supported by historically elevated VLCFA.
3. Clinical evidence of spinal cord involvement with EDSS score between 1 and 4.5 and a pyramidal function score of ≥ 1 in the Functional System Score of the EDSS. Signs of pyramidal tract dysfunction don't include hyperreflexia.
4. Must agree to use double-barrier contraception methods (e.g., condom and 1 other form of contraception for female sexual partners that are of childbearing potential, such as diaphragm, intrauterine device, spermicidal jelly, and/or hormonal contraceptive) and to not donate sperm for at least 6 months following the IMP procedure.
5. For patients who are receiving any other treatment for ALD, including off-label medications and/or supplements (e.g., antioxidants, Lorenzo's oil, statins, etc.) or physical rehabilitation support, such treatments must have been at a stable dose and/or frequency for ≥ 4 weeks prior to Screening and patients must agree to continue at the same dose and/or frequency through Part 1 of the study.
6. The patient provided written informed consent prior to any study procedures being performed.

4.2 Patient Exclusion Criteria

All patients will be excluded from this study if they meet any of the following criteria:

1. Presence of inflammatory cerebral disease, established by radiographic review of brain MRI demonstrating a Loes score ≥ 0.5 on the 34-point scale, except for the abnormalities that can be observed in patients with AMN without inflammatory cerebral demyelination with Loes score ≤ 4 .
2. Pathological changes identified on brain MRI, including all lesions from previous diagnosis of inflammatory cerebral disease identified on brain MRI as contrast-enhancing (gadolinium-enhancing) lesion with Loes score ≥ 0.5 on the 34-point scale, except for the abnormalities that can be observed in patients with AMN without inflammatory cerebral demyelination with Loes score ≤ 4 .
3. 15 years or more have elapsed since the initial onset of myeloneuropathy manifestations, such as walking or running difficulties, bladder dysfunction, increased muscular tone, spasticity, weakness, balance problems, etc.
4. Contraindications for MRI procedure and/or contrast materials.
5. History of a brain or spinal cord disease that would interfere with lumbar puncture procedures, CSF circulation, and/or safety assessments.
6. Contraindication to steroids, sirolimus, tacrolimus, and/or anesthetic medications.
7. Contraindication to SBT101 and/or any of its ingredients.
8. Unstable adrenal function (e.g., untreated or inappropriately treated adrenal insufficiency).
 - a. Adrenal function will be evaluated through laboratory assessments of cortisol, plasma adrenocorticotrophic hormone (ACTH), plasma renin, aldosterone, sodium,

- and potassium levels at Screening. Monitoring of replacement therapy will be mainly clinical. Determination of inappropriately treated adrenal insufficiency will be made by the Investigator in consultation with the Medical Monitor.
- b. Patients who meet the following criteria will be considered to have adrenal insufficiency: (i) basal cortisol concentration <275 nmol/L (10 µg/dL) in the morning (i.e., 6 AM to 10 AM) and (ii) plasma ACTH $>2 \times$ upper limit of normal (ULN).
9. Positive for human immunodeficiency virus (HIV) type 1 or 2 (HIV1, HIV2), hepatitis B virus (HBV), or hepatitis C virus (HCV).
 - a. Patients who have been vaccinated against HBV (e.g., HBV surface antibody-positive) who are negative for other markers of prior HBV infection (e.g., HBV core antibody-negative) are eligible.
 - b. Patients who are positive for anti-HCV antibodies are eligible, as long as they have a negative HCV load as measured by quantitative polymerase chain reaction (qPCR).
 10. Presence of clinically significant active bacterial, viral, fungal, parasitic, or prior associated infection.
 11. History of diabetes or abnormal fasting serum glucose (≥ 126 mg/dL) or hemoglobin A1C $\geq 6.5\%$.
 12. Patients who have received a gene therapy.
 13. Current use of medications that could potentially lead to changes in intracranial pressure (e.g., levothyroxine, vitamin A supplementation, oral contraceptives, tetracycline, acetazolamide [Diamox]).
 14. Patients who are currently using strong CYP3A4 inducers or strong CYP3A4 inhibitors and are unwilling or unable to stop prior to and during the immunosuppression regimen.
 15. Patients who are currently receiving or have received an investigational drug or procedure within 3 months prior to Screening. The use of investigational drugs is prohibited throughout Part 1 of the study.
 16. Patients with unstable, clinically significant neurologic (other than AMN), psychiatric, cardiovascular, ophthalmologic, pulmonary, hepatic, renal, metabolic, gastrointestinal, urologic, immunologic, hematopoietic, and/or endocrine disease (other than adrenal insufficiency) and/or other abnormality that may impact the ability to participate in the study or that may potentially confound the study results. It is the responsibility of the Investigator to assess clinical significance; however, consultation with the Sponsor's Medical Monitor may be warranted.
 17. Patients who, in the opinion of the Investigator, have any other medical or psychological condition or social circumstance that would impair their ability to participate reliably in the assessments, or who may increase the risk to themselves or others by participating.
 18. Patients who are an immediate family member, study site employee, or are in a dependent relationship with a study team member involved in the conduct of this study (e.g., spouse, parent, child, sibling).

4.3 Patient Withdrawal/Removal from Study Participation

Patients have the right to withdraw from the study at any time and for any reason. In the event of a withdrawal, the reason will be documented in the electronic case report form (eCRF).

If a study site identifies a patient who did not meet the eligibility criteria and was inadvertently enrolled, the Sponsor must be notified immediately. If the Sponsor identifies a patient who did not meet the eligibility criteria and was inadvertently enrolled, the study site will be notified immediately. A discussion may occur between the Sponsor and the Investigator to determine whether the patient may continue in the study.

The Investigator or designee may decide to discontinue or withdraw a patient from the study. The Investigator may also discontinue the IMP procedure if any patient exhibits dose-limiting toxicities (DLTs), as evidenced by the Dosing Suspension Criteria outlined in [Section 6.19](#). In any of these events, every effort must be made to immediately notify the Sponsor's Medical Monitor.

Reasons for patient withdrawal may include any of the following:

- The patient may request to discontinue from the study for any reason
- The Sponsor or designee discontinues the study or discontinues a patient's participation in the study for medical, safety, regulatory, and/or any other reasons consistent with applicable laws, regulations, and Good Clinical Practice (GCP)
- Patient noncompliance
- The patient has an intercurrent illness that would affect assessment of clinical status to a significant degree, per the Investigator's judgement
- The patient exhibits an unacceptable toxicity (including a clinically significant laboratory abnormality) that meets any of the following criteria:
 - Necessitates discontinuation from study participation
 - Compromises the patient's ability to continue study-specific procedures, per the Investigator's judgement
- It is not in the patient's best interest to continue in the study (Any other condition or circumstance that would jeopardize the welfare of the patient if he were to continue in the trial)

For patients who have received SBT101 and wish to withdraw from the study, a request will be made to, at minimum, follow the patient for safety monitoring. The date and reason for any patient discontinuation or withdrawal will be recorded in the eCRF. The eCRF and other study-related source documents must be completed/collected up to the point of withdrawal and will be retained by the Sponsor. Patients who withdraw from the study prior to the EOS Visit at Month 60 will have an early EOS Visit with all the procedures performed as shown in the [Section 6.16](#).

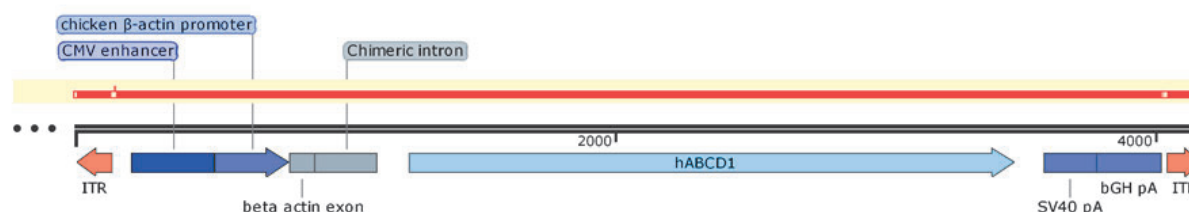
5. STUDY TREATMENT

5.1 Study Products

5.1.1 Description

SBT101 is a non-replicating recombinant AAV9 gene therapy vector bioengineered to contain a complementary deoxyribonucleic acid (cDNA) copy of the functional *hABCD1* gene that can be used for expression of the protein by transduced cells. SBT101 is generated by transiently transfecting human embryonic kidney (HEK)293 cells with cis-, trans-, and helper-plasmids. The viral particle contains a single-stranded DNA vector genome and a capsid comprised of 3 structural Cap proteins (viral protein-1, -2 and -3). The vector genome consists of AAV2 inverted terminal repeats (ITRs) flanking the transgene expression cassette, a chicken β -actin promoter, a human cytomegalovirus enhancer, a chimeric intron, the cDNA encoding *hABCD1*, and 2 polyA signals. A schematic of SBT101 vector genome is presented in [Figure 3](#).

Figure 3 SBT101 Vector Genome Structure



Abbreviations: bGH = bovine growth hormone; CMV = cytomegalovirus; *hABCD1* = human *ABCD1* gene; ITR = inverted terminal repeat; pA = polyA; SV40 = simian virus 40.

SBT101 works by delivering copies of the *hABCD1* gene to the recipient's spinal cord cells through transduction by the recombinant AAV9 vector. The recipient's cell machinery will use the *hABCD1* gene to produce functional copies of the ABCD1 protein, thereby correcting the biochemical defects underlying AMN.

The drug product SBT101 is formulated as a colorless solution provided in a single-use 2 mL Crystal Zenith[®] vial closed with a chlorobutyl stopper and an aluminum flip-off seal with a polypropylene disk. The drug product is frozen at $\leq -65^{\circ}\text{C}$. Once thawed, the fill volume of each vial allows for withdrawal of 1 mL of the drug product for a target concentration of $1.0\text{E}13$ vg/vial. Multiple vials will be combined to prepare the appropriate dose level per patient (see the SBT101-CT101 Pharmacy Manual for further details).

A diluent will be provided for preparation of the appropriate dose volumes across all Dose Level Cohorts. The diluent matches the drug substance formulation buffer and contains potassium chloride, potassium dihydrogen sulfate, sodium chloride sodium phosphate dibasic (anhydrous), and polaxamer (Kolliphor P188). The diluent will be provided in 10R glass vials containing 10 mL of the diluent and sealed with a stopper and flip-off seal.

Each SBT101 and diluent vial will be labelled according to US FDA and European Union (EU) regulations. The lot numbers used will be recorded in the study source documentation and the Interactive Voice/Web Response System (IXRS).

Labels for SBT101 and diluent will include, at a minimum, the following information in English:

- Protocol number
- Sponsor name and address
- Product name and strength
- Dosage form and route of administration
- Directions for use
- Patient identification (ID) number
- Site number
- Lot number
- Storage instructions
- Disposal instructions
- Caution statements, including the following:
 - Keep out of the reach of children
 - For clinical trial use only
 - Caution: New drug – limited by Federal Law to investigational use (for US sites or similarly worded statement as applicable for non-US sites)

Labels affixed to SBT101 vials will at minimum include the protocol number, patient ID, vial number, and lot number.

5.1.2 Drug Preparation and Administration

The procedures for preparing and handling SBT101 and Placebo_{IMP} infusion bags are outlined in the SBT101-CT101 Pharmacy Manual.

SBT101 will be administered via IT infusion by trained site staff (e.g., neuroradiologists, neurosurgeons, or anesthesiologists) using fluoroscopic guidance. A catheter will be inserted at the lower lumbar area (e.g. L3-L4 interspace) and forwarded up at the T8-T10 level. A volume of 12-16 cc of CSF will be collected via gravity for assessment of biomarkers. SBT101 will then be instilled into the CSF by slow and continuous delivery over approximately 5 hours at a rate of up to 8 mL/hour using the CADD Solis 2110 Ambulatory infusion pump. It is estimated that the infusion will involve up to 40 cc.

Patients randomized to control will undergo an imitation (sham) procedure comparable to that of the SBT101 infusion. A lumbar puncture will be performed and 12-16 cc of CSF will be collected for biomarker assessments. Catheter tubing will be attached to a Collection EVA bag placed below the lumbar puncture site. The infusion bag for control patients will contain normal saline 0.9% (Placebo_{IMP}) at a comparable volume to infusion bags prepared for SBT101 patients. Placebo IMP will not be instilled into the CSF but collected in the Collection EVA bag through the tubing. The duration of the imitation (sham) procedure will be comparable to SBT101 infusion; approximately 5 hours.

Further details are provided in the SBT101-CT101 Pharmacy Manual.

5.1.3 Storage and Stability

Single-use vials of SBT101 will be shipped within a suitable container in dry ice with a temperature monitoring device. The SBT101 vials must be stored frozen at $\leq -65^{\circ}\text{C}$ until the vials are thawed and prepared for administration.

Diluent vials must be stored refrigerated at 2-8°C and allowed to come to controlled (ambient) room temperature prior to preparing for administration.

Further details are provided in the SBT101-CT101 Pharmacy Manual.

5.1.4 Source of Study Drug and Diluent

SBT101 and the diluent will be provided to study sites by the Sponsor.

5.2 Treatment Administration

5.2.1 Treatment Dose and Schedule

Patients will undergo the IMP procedure on Day 1 (Visit 3) according to their assigned Dose Level Cohort. Patients receiving SBT101 will be administered 1.0E14 vg (Dose Level 1) or 3.0E14 vg (Dose Level 2); patients randomized to control will undergo an imitation (sham) procedure consisting of a lumbar puncture for collection of CSF samples, but no IMP will be infused into the CSF.

5.2.2 Dose Level Cohorts

Part 1 of the study consists of 2 phases – a Dose Escalation Phase, aimed at establishing the MTD, and a Dose Expansion Phase, during which additional patients will be enrolled and treated at the MTD.

During the Dose Escalation Phase, a minimum of 8 patients will sequentially be assigned to a Dose Level Cohort. A minimum of the 4 patients will be assigned to the Dose Level 1 Cohort (1.0E14 vg). At the time of the DSMB Meeting, if no DLTs are observed and treatment of patients at the next dose level is recommended by the DSMB, the next patients (minimum of 4 will be assigned to the Dose Level 2 Cohort (3.0E14 vg); see [Section 6.19.2](#) for a list of DLTs. The DSMB, in consultation with the Sponsor, may recommend increasing the number of patients in a given Dose Level Cohort. Dose Levels will be studied sequentially; only one Dose Level will be available for patient assignment at a given time.

Table 4 Steps for Dose Level Allocation

Step	Procedure
1	Patients are treated according to their assigned dose based on their Dose Level Cohort.
2	After each patient has completed the Day 30 visit, the Sponsor's Medical Monitor reviews the patient's safety data.
3	If DLTs ¹ are observed for any patient, proceed to Step 8. If no DLTs ¹ are observed, proceed to Step 4 or Step 6, depending on which dose level is currently being treated
4	Repeat Steps 1 through 3 for all patients in Dose Level Cohort 1.
5	After all patients have been dosed in Dose Level Cohort 1, complete Step 9.
6	Repeat Steps 1 through 3 for all patients in Dose Level Cohort 2.
7	After all patients have been dosed in Dose Level Cohort 2, complete step 9.
8	The DSMB reviews patient data for all patients through Day 30 of follow-up and makes a decision on whether to treat additional patients within the Dose Level Cohort or suspend further enrollment.

Table 4 Steps for Dose Level Allocation

Step	Procedure
9	The DSMB reviews patient data for all patients through Day 30 of follow-up and makes a decision on whether to treat additional patients within the Dose Level Cohort, proceed to the next Cohort, or suspend further enrollment.

Abbreviations: DLT = dose-limiting toxicity; DSMB = Data Safety Monitoring Committee.

¹ DLTs are outlined in [Section 6.19.2](#)

Once the MTD has been established during the Dose Escalation Phase, 8 additional patients will be enrolled at the Dose Expansion Phase and randomized to SBT101 or control.

Once all patients in a cohort have completed the Day 720 (Month 24) Visit, patients who were randomized to the control arm will have the option to be treated with open-label SBT101. Patients who opt to be treated with open-label must continue to meet all exclusion and inclusion criteria (except for inclusion criterion #1 and #3 and exclusion criterion #3). If they choose to be treated with open-label SBT101, open-label restart all procedures at Day 1 and will be followed for another 5 years.

The Sponsor, in consultation with the DSMB, may decide to investigate an additional dose of SBT101 in the Dose Expansion Phase different than Dose Level 1 and Dose Level 2. Additionally, the Sponsor, in consultation with the DSMB, may elect to increase or decrease the number of patients in a Dose Level Cohort during the study.

5.2.3 Randomization

During Part 1 of the study, patients in the Dose Expansion Phase will be randomized to SBT101 or control in a 3:1 ratio at the Screening Visit (after confirmation of study eligibility) using IWRS. 8 patients in the Dose Expansion Cohort will be randomized; 6 patients randomized to SBT101 at MTD and 2 controls (imitation (sham) procedure)

5.2.4 Duration of Treatment

Patients will be admitted to the study site the day before the IMP procedure and will remain in at the site through Day 2 ([Table 2](#)). Patients will undergo the IMP procedure on Day 1 (Visit 3), which will last 5 hours. Patients will be followed for 2 years to evaluate the safety and potential impact of SBT101 on disease progression, and then for another 3 years to evaluate long-term safety of SBT101 and disease progression.

5.2.5 Blinding

Part 1 of the study will be blinded per [Table 6](#). The treatment will not be blinded to the study patients during Dose Level 1 and Dose Level 2 of the study. During Dose Expansion, study patients will be blinded to their treatment assignment throughout Part 1 of the study. Patients randomized to control (imitation procedure) who opt to receive open-label SBT101 at the end of Part 1 will be unblinded to treatment assignment. Blinding of other team members during the study is outlined in [Table 6](#)

Table 6 Blinding of Dose Levels

	Dose Level 1 and 2 Cohort Dose Escalation Phase	Dose Expansion Phase
Blinded	Not Applicable	Study patients, Study Team conducting efficacy assessments, Sponsor, and Medical Monitor
Unblinded	Study patients and Study Team conducting efficacy assessments; and all other team members, including the Sponsor and Medical Monitor, as well as the DSMB	All other team members and the DSMB
Abbreviations: DSMB = Data and Safety Monitoring Board.		

Infusion bags with SBT101 and diluent or normal saline 0.9% (Placebo_{IMP}) will be labelled such that only the unblinded study site Pharmacist or designee will know the contents of the infusion bags. The unblinded study site Pharmacist or designee will prepare the blinded infusion bag for each patient, affix the blinded label to the infusion bag, and send the blinded infusion bags to the infusion center for administration. The IMP procedure will be performed by the unblinded Investigator. During the Dose- Expansion Cohort, patients randomized to both the SBT101 and control arms will have a Collection EVA bag placed below the infusion site. Placebo_{IMP} will not be instilled into the CSF of control patients, but collected in the Collection EVA bag, as described in [Section 5.1.2](#).

5.2.6 Unblinding (Dose Expansion Cohort)

Breaking the blind during Part 1 of the study will only be permitted in the event of a serious adverse event (SAE), unexpected adverse drug reaction, or medical emergency for which knowing the treatment assignment is required for making therapeutic decisions.

If the Investigator deems it necessary to break the study blind in the interest of the patient's medical safety, the Investigator must make every effort to contact the Sponsor's Medical Monitor or designee before the blind is broken. If the study site is unable to contact the Medical Monitor or designee prior to breaking the blind, the Medical Monitor or designee must be contacted within 24 hours following disclosure of treatment assignment. Any premature unblinding should be promptly documented; the Investigator must note the date, time, and reason for unblinding.

Emergency unblinding for AEs may be performed through IXRS, which may supplement or take the place of emergency codes generated by a computer drug-labeling system. This option may ONLY be used if the patient's well-being requires knowledge of their treatment assignment.

In the event of a suspected unexpected serious adverse reaction (SUSAR), the blind will be broken by the Sponsor only for the patient who had the SUSAR. The blind will be maintained for study team members responsible for the ongoing conduct of the study (see [Table 6](#)). Unblinded data will only be accessible to those who need to be involved in safety reporting to health authorities, applicable regulatory authorities, and Institutional Review Board (IRB)/Ethics Committee (EC).

Blinded team members will only have access to blinded data, unless granting access to unblinded data is deemed necessary for safety reasons.

5.2.7 Concomitant Medications and Other Treatments

5.2.7.1 Immunosuppression

Starting on Day -1 (Visit 2), patients will receive an immunosuppressive regimen for at minimum until Day 90. All patients, including control patients, will be administered 1 g methylprednisolone via intravenous (IV) infusion over the span of 2 ($\pm$ 1) hours on Day -1 (Visit 2), Day 1 (Visit 3), and Day 2 (Visit 4), as indicated in the Schedule of Assessments ([Table 2](#)).

Thereafter, patients will receive oral prednisone/prednisolone at a dose of 0.5 mg/kg/day, starting on Day 3 until Day 90 at a minimum, this can be extended as needed per clinical judgement. If clinically indicated (i.e., based on clinical symptoms, liver enzyme elevations or worsening of neurological/spinal cord function), prednisone/prednisolone dose and duration can be modified based on discussions between the Investigator and the Medical Monitor.

A standard clinical tapering regimen will be initiated after the patient is clinically evaluated (Brief Physical and Neurological examination, at a minimum) by the Investigator Day 90 or later, and considered stable to discontinue prednisone/prednisolone treatment or to reduce the dose to a level necessary to treat underlying adrenal insufficiency (Addison's disease). LFTs will also be monitored biweekly until 4 weeks after the prednisone/prednisolone dose has been tapered down to a daily dose of 10 mg. Once prednisone/prednisolone has been discontinued or tapered down to a dose <10 mg/day, biweekly chemistry laboratory assessments (including ALT, AST, GGT, CPK and troponins) will be conducted to ensure stability of these analytes.

During the taper, the Investigator should monitor patients for potential corticosteroid withdrawal symptoms, worsening of neurological symptoms, or appearance of an immune response. The Investigator should also monitor patients for signs and symptoms of adrenal insufficiency.

The details of the prednisone/prednisolone regimen and an example of a tapering schedule are provided in [Appendix 1](#).

Patients in both treatment and control arms will receive the same protocol of prednisone/prednisolone to ensure all patients are appropriately blinded to the treatment assignment. Open-label steroid dosing and taper eliminate the risk of inadequate dosing and allows the clinician to make clinical decisions in real time tailored to the individual patient's situation. More importantly, the patient's cooperation to identify early signs of adrenal crisis is a requisite in weaning off the prednisone dose to avoid life-threatening adrenal crisis. Hence, an unblinded taper of prednisone will reduce the risk of adrenal crisis.

If patients develop suspected cellular immune responses to the gene therapy, indicated by clinical signs and symptoms and/or T-cell reactivity as measured by ELISpot, the Medical Monitor should be consulted. Such patients may be treated with sirolimus or another immune suppressor based on clinical indication as adjuvant to the ongoing prednisone/prednisolone regimen.

If any patient exhibits an elevation of hepatic transaminases, worsening neurological/spinal cord function, or signs of an immune response against SBT101, the Medical Monitor should be consulted and altering the corticosteroid regimen should be discussed. If the modified corticosteroid management plan is insufficient to resolve the elevation of hepatic transaminases for any patient or signs of an immune response against SBT101, additional immunosuppressive

regimens should be considered on a patient-by-patient basis, based on discussions between the Investigator and the Medical Monitor.

If a patient cannot tolerate prednisone/prednisolone (e.g. severe gastric pain or any evidence of bleeding, excessive jitteriness), the Medical Monitor should be consulted and the patient may be treated with sirolimus or another immune suppressor until Day 90 at a minimum, based on clinical indication.

See [Appendix 2](#) for details on the approach to the use of sirolimus.

While on immunomodulatory regimens and tapering, all patients will be closely monitored for AEs, including opportunistic infections. Antibiotics and/or other medications to minimize the risk of opportunistic infections, and vaccinations may be prescribed at the discretion of the Investigator.

All events related to the use of immunomodulatory drugs (e.g., hyperglycemia, weight gain, infections, worsening or changes in neurological symptoms) will be recorded as AEs connected to their use.

5.2.7.1.1 Methylprednisolone

Methylprednisolone sodium succinate for injection is a corticosteroid medication formulated for IV administration.

Details on the preparation of methylprednisolone infusion bags, conditions for use, storage, and handling instructions are provided in the SBT101-CT101 Pharmacy Manual.

5.2.7.1.2 Prednisone/Prednisolone

Prednisone/prednisolone is a corticosteroid medication that will be supplied to the patient in tablet form for oral administration. Please refer to the medication package insert or summary of product characteristics (EU countries) for the physical description, and details on storage conditions and AEs. The details of the prednisone/prednisolone regimen and an example of a tapering schedule are provided in [Appendix 1](#).

5.3 Treatment Compliance

5.3.1 Study Drug Dispensing and Accountability

The unblinded Pharmacist or designee is responsible for ensuring appropriate storage in accordance with SBT101 labels, as well as adequate accountability of all used and unused study drug vials. This includes acknowledgment of receipt of each shipment of study drug (quantity and condition), patient dispensing records, and returned or destroyed IMP. Dispensing records will document quantities received from the Sponsor or designee and quantities dispensed to patients, including lot number, date dispensed, patient ID, and the initials of the person dispensing the medication. All study drug supplies, and associated documentation will be periodically reviewed and verified by the Study Monitor over the course of the study.

Unused, partially used, and empty SBT101 containers will be destroyed at study sites according to institutional practice, standard operating procedures for the destruction of hazardous materials, and applicable local regulations.

Further details are provided in the SBT101-CT101 Pharmacy Manual.

5.3.2 Assessment of Treatment Compliance

Since all patients will undergo SBT101 infusion/sham procedure at the study site under the supervision of the study team, treatment compliance will be well documented, with infusion details and lot numbers recorded in the source documentation and eCRFs.

6. STUDY CONDUCT

Note: *It is recommended that all gait and balance assessments be completed at approximately the same time of day for each visit. Patients should hold anti-spasticity medications until completion of gait and balance assessments.*

6.1 Screening Visit (Clinic Visit)

- Obtain informed consent for SBT101-CT101, and optional biomarker research prior to any study procedures
- Review inclusion/exclusion criteria
- Record patient demographics
- Record medical history, including date and type of diagnosis
- Full physical examination
- Neurological examination
- Assessment of adrenal function (see [Section 7.2.24.1](#))
- Conduct testing for HIV, HBV, and HCV
- Conduct genetic testing for the *ABCD1* gene mutation to confirm ALD diagnosis
- Record vital signs, height, and weight
- Perform ECG
- Conduct electrophysiological (EP) examination (NCS)
- Conduct Somatosensory evoked potentials (SSEP)
- Perform and review MRIs of the brain (with and without contrast) and Full spine (with and without contrast),
- Perform and review MRIs cervical spine (diffusion tensor imaging [DTI], magnetization transfer [MT], and spectroscopy parameters).
- Collect laboratory samples per the SBT101-CT101 Laboratory Manual
- Conduct the following assessments using The Mobility Lab (OPAL) sensors: 2MWT, 6MWT, 5XSST, postural body sway, TUG, and TUG-DT
- Conduct the EDSS, miniBESTest, and SSPROM
- Conduct the CGI
- Record concomitant medications
- Ensure the following PROs are completed ± 5 days of the visit: MSWS-12, MSQoL-54, PGI-S, PGI-S Balance, PGI-C Balance, and Urinary and Bowel Questionnaire
- Complete IXRS after eligibility is confirmed
- Note: Re-screening is possible after consultation with the Medical Monitor. Re-screening may increase the total study participation by 3 months.

6.2 Day -1 Visit (Admission; Clinic Visit)

- SBT101 must be received by the study site prior to initiation of any study related procedures
- Review inclusion/exclusion criteria to confirm patient eligibility
- Brief physical examination
- Height and weight
- Collect laboratory samples per the SBT101-CT101 Laboratory Manual
- ***For patients at University of Massachusetts Chan Medical School only***– collect additional ELISpot blood sample

- Administer IV methylprednisolone; administration should be completed at least 6 hours prior to the IMP procedure. Methylprednisolone infusion should not begin until all other inclusion/exclusion criteria have been completed.
- Record concomitant medications and AEs

6.3 Day 1 Visit (Study Drug Infusion Day; Clinic Visit)

The following procedures must be performed **before** the IMP procedure:

- Record vital signs
- After a catheter has been inserted for IT infusion, and prior to the IMP procedure (i.e., SBT101 infusion or imitation (sham) procedure), collect CSF via gravity for laboratory assessment of CSF biomarkers
- Record concomitant medications and AEs

Perform the IMP procedure

- The IMP procedure will be performed by trained site staff (e.g., neuroradiologists, neurosurgeons, or anesthesiologists) using fluoroscopic guidance
- Procedure for treatment with SBT101
 - Complete a lumbar puncture followed by slowly infusing SBT101 into the spinal canal over the course of 5 hours (reference Pharmacy Manual for infusion set up). The lumbar puncture will be done under fluoroscopy guidance. The catheter will be inserted at the lower lumbar area (e.g. L3-L4 interspace) and forwarded up at the T8-T10 level.
- Patients randomized to the control (imitation (sham)) arm
 - Complete a lumbar puncture for collection of cerebrospinal fluid samples.
 - The procedure will be comparable to that of SBT101; instead of the catheter being inserted, it will be attached to the Collection EVA bag for collection. The collection EVA bag will be attached placed below the injection site on the skin. The SBT101 infusion bag will contain saline.
- All patients will have the infusion site, tubing, and collection bags covered throughout the infusion so that the patients and site stay blinded.
- Further details of the procedure can be found in the Pharmacy Manual.
- Record AEs

Conduct the following **after** the IMP procedure:

- Administer IV methylprednisolone approximately 2 hours after completion of the IMP procedure
- Record concomitant medications and AEs

6.4 Day 2 Visit (Clinic Visit; Discharge)

- Record vital signs
- Perform ECG
- Brief physical examination
- Collect laboratory samples per the SBT101-CT101 Laboratory Manual
- Record concomitant medications and AEs
- Administer IV methylprednisolone

- Dispense oral prednisone/prednisolone for Day 2 through Day 30 and instruct the patient to start treatment on Day 3
- Dispense CentrePoint Insight watch and instruct the patient on how and when to use the device during the study

6.5 Day 4 Visit (Phone Call)

- Record concomitant medications and AEs

6.6 Day 8 Visit (Clinic Visit)

- Record vital signs
- Perform ECG
- Brief physical examination
- Collect laboratory samples per the SBT101-CT101 Laboratory Manual
- Record concomitant medications and AEs

6.7 Day 15, Day 22, Day 45, Day 75, Day 97, Day 104, Day 111, and Day 150 Visits (Remote Visits)

- A qualified professional will visit the patient at home to collect laboratory samples per the SBT101-CT101 Laboratory Manual, concomitant medications, and AEs, as indicated in the Schedule of Assessments [Table 2](#). The qualified professional will also collect the additional ELISpot blood sample for patients at University of Massachusetts Chan Medical School, as indicated in the Schedule of Assessments.
- The clinical site study team member will contact the patient to review and record concomitant medications, AEs and functional progress since the last visit.

6.8 Day 30 and Day 90 Visits (Clinic Visits)

- Record vital signs, height and weight
- Perform ECG
- Brief physical examination
- Neurological examination
- Perform EP examination (NCS)
- Collect laboratory samples per the SBT101-CT101 Laboratory Manual
- Perform and review MRIs of the brain (with and without contrast)
- Dispense sufficient oral prednisone/prednisolone until next in-clinic visit; review dosing instructions with the patient to ensure proper and timely self-administration of the drugs by the patient
- Record concomitant medications and AEs
- ***For patients at University of Massachusetts Chan Medical School only –***
 - Collect additional ELISpot blood sample
- ***Day 90 Visit only:***
 - Conduct Somatosensory evoked potentials (SSEP)
 - Perform and review MRIs of the Full spine (with and without contrast)

6.9 Day 38, Day 52, Day 68, Day 82 Visits (Remote ELISpot UMass only)

- A qualified professional will visit the patient at home to collect an ELISpot blood sample, as indicated in the Schedule of Assessments ([Table 2](#)).

6.10 Day 60 and Day 120 Visits (Clinic Visits)

- Record vital signs, height and weight
- Perform ECG
- Brief physical examination
- Neurological examination
- Perform EP examination (NCS)
- Collect laboratory samples per the SBT101-CT101 Laboratory Manual
- Record concomitant medications and AEs
- Dispense sufficient oral prednisone/prednisolone until taper; review dosing instructions with the patient to ensure proper and timely self-administration of the drugs by the patient.

6.11 Day 180 Visit (Clinic Visit)

- Record vital signs, height, and weight
- Perform ECG
- Brief physical examination
- Neurological examination
- Perform EP examination (NCS)
- Perform and review MRIs of the brain (with and without contrast) and Full Spine (with and without contrast)
- Collect laboratory samples per the SBT101-CT101 Laboratory Manual
- Conduct the following assessments using The Mobility Lab (OPAL) sensors: 2MWT, 6MWTS, 5XSST, postural body sway, TUG, and TUG-DT
- Conduct the EDSS, miniBESTest, and SSPROM
- Conduct the CGI, CGI-C
- Ensure the following PROs are completed ± 5 days of the visit: MSWS-12, MSQoL-54, PGI-S, PGI-S Balance, PGI-C Balance, and Urinary and Bowel Questionnaire
- Record concomitant medications and AEs

6.12 Day 270 Visit (Clinic Visit)

Patients will be contacted by a study team member to conduct the following procedures:

- Record vital signs, height, and weight
- Brief Physical Examination
- Neurological Examination
- Collect laboratory samples per the SBT101-CT101 Laboratory Manual
- Conduct the following assessments using The Mobility Lab (OPAL) sensors: 2MWT, 5XSST, postural body sway, and TUG
- Record concomitant medications and AEs

6.13 Day 360 and Day 720 Visits (Clinic Visits)

- Record vital signs, height, and weight
- Perform ECG
- Brief physical examination
- Neurological examination
- Perform EP examination (NCS)
- Perform and review MRIs of the brain (with and without contrast)
- Perform and review MRIs cervical spine (diffusion tensor imaging [DTI], magnetization transfer [MT], and spectroscopy parameters).
- Conduct Somatosensory evoked potentials (SSEP)
- Collect laboratory samples per the SBT101-CT101 Laboratory Manual
- Perform a lumbar puncture for assessment of CSF biomarkers
- Conduct the following assessments using The Mobility Lab (OPAL) sensors: 2MWT, 6MWT, 5XSST, postural body sway, TUG, and TUG-DT
- Conduct the EDSS, miniBESTest, and SSPROM
- Conduct the CGI, CGI-C
- Ensure the following PROs are completed ± 5 days of the visit: MSWS-12, MSQoL-54, PGI-S, PGI-S Balance, PGI-C Balance, and Urinary and Bowel Questionnaire
- Record concomitant medications and AEs
- **Day 360 Visit only:**
 - Perform and review MRIs of the Full Spine (with and without contrast)
 - Dispense Mobility Lab Opal sensors and Clario eCOA tablet and instruct the patient on how and when to use these devices during the study

6.14 Day 450 and Day 630 Visits (Remote Visits)

Patients will be contacted by a study team member to conduct the following procedures:

- Conduct the following assessments using The Mobility Lab (OPAL) sensors: 2MWT, 5XSST, postural body sway, and TUG
- Record concomitant medications and AEs

6.15 Day 540 Visit (Clinic Visit)

- Record vital signs, height, and weight
- Perform ECG
- Brief physical examination
- Neurological examination
- Perform and review MRIs of the brain (with and without contrast)
- Collect laboratory samples per the SBT101-CT101 Laboratory Manual
- Conduct the following assessments using The Mobility Lab (OPAL) sensors: 2MWT, 6MWT, 5XSST, postural body sway, TUG, and TUG-DT
- Conduct the EDSS, miniBESTest, and SSPROM
- Conduct the CGI, CGI-C
- Record concomitant medications and AEs

6.16 Day 1080 (Month 36), Day 1440 (Month 48), and Day 1800 (Month 60)-End Of Study Visits (Clinic Visits)

- Record vital signs, height, and weight
- Brief physical examination
- Neurological examination
- Collect laboratory samples per the SBT101-CT101 Laboratory Manual
- Perform and review MRIs of the brain (with and without contrast)
- Conduct the following assessments using The Mobility Lab (OPAL) sensors: 2MWT, 6MWT, 5XSST, postural body sway, TUG, and TUG-DT
- Conduct the miniBESTest
- Conduct the CGI, CGI-C
- Ensure the following PROs are completed ± 5 days of the visit: MSWS12, MSQoL54, PGI-S, PGI-S Balance, PGI-C Balance, and Urinary and Bowel Questionnaire
- Record concomitant medications and AEs

6.17 Remote Visit Follow-up Phone Call

In follow-up to remote visits, scheduled and unscheduled, patients will be contacted by a study team member to collect the following:

- Functional progress since the last visit, including but not limited to ambulation, strength, and sensation.
- Record concomitant medications and AEs

The remote visit follow-up phone calls will be conducted until the patient has completed immunosuppression regimen tapering.

6.18 Unscheduled Visit

Additional clinic visits may be added if deemed necessary by the Investigator. Activities at these visits will be directed by the circumstances; at minimum, these should include:

- Assess compliance with protocol restrictions
- Record concomitant medications and AEs
- Measure body temperature
- Additional tests or procedures, as appropriate

The reason for the unscheduled visit and the results of any tests or procedures will be recorded in the patient's eCRF.

6.19 Treatment Discontinuation and Dosing Suspension Criteria**6.19.1 Prior to Methylprednisolone Infusion on Day -1**

Patients will be considered to be screen failures if they do not meet the study eligibility criteria on Day -1 (Visit 2).

If a patient meets all eligibility criteria but the IMP for the patient has not been received by the study site, the study site should reschedule the Day -1 Visit procedures for the patient.

Methylprednisolone infusions will not be performed until it is confirmed that the IMP for the patient has been received by the study site.

6.19.2 Dosing Suspension Criteria

Dosing in this study may be suspended at any time for safety reasons. It will be the responsibility of the DSMB to make a recommendation to the Sponsor if they believe there is reasonable cause to suspend dosing. In such an event, the Sponsor will promptly inform the Investigators, individual study sites' IRB/EC, regulatory authorities, and other appropriate institutional regulatory bodies. While dosing is suspended, no new IMP procedures will be initiated; however, patients who have already undergone the IMP procedure will continue to be followed in the study.

If a patient experiences an AE of $\geq$ Grade III toxicity, the event will be investigated in real time and further dosing will be suspended while the event is under investigation after consultation with the DSMB. Additionally, any of the following DLTs will result in suspension of further dosing while the events are under investigation after consultation with the DSMB:

- Any patient develops study drug-related or probably study drug-related Grade III or Grade IV toxicity, excluding elevated transaminases
- Any patient develops study drug-related or possibly study drug-related Grade III or Grade IV elevated transaminases that fail to improve or resolve within 4 weeks on the immunosuppressive regimens
- Any patient develops study drug-related or possibly study drug-related Grade IV (>10 -fold) elevated transaminases
- Any patient develops contrast enhancement on MRI of their spinal cord in the first 6 months, contrast enhancement of their DRGs in the first 3 months, or $>50\%$ drop in sensory amplitudes in the first 3 months after administration of SBT101
- If a patient shows signs of inflammatory cerebral disease (contrast enhancement) within 6 months following the IMP procedure, the DSMB will be informed and a MRI of the brain will be repeated in 1-3 months. Dosing will be postponed until the DSMB agrees on the action plan.
- Any patient experiences a medically important event that warrants further evaluation
- Should AAV vector-mediated insertional mutagenesis occur, it is not likely to be observed in the first year after the IMP procedure; therefore, the Sponsor intends to conduct long-term safety monitoring of patients in the extension study.
- Any pregnancies that occur in study patients' partners will be reported on the Pregnancy Form after consent has been provided.

6.17.3 Study Stopping Rules

If a patient experiences any study drug-related death during the study or a Severe Adverse Event (SAE) included or not in the dosing suspension rules with a possible prolonged or permanent disability not expected as the natural progression of AMN, the event will be investigated in real time and further enrollment will be suspended. The DSMB will be informed, and an ad-hoc meeting will be conducted. Additional information will be provided in the DSMB charter

An event of insertional mutagenesis will be handled as a serious adverse event (SAE) and reported in the time frame as outlined in [Section 7.3.8](#) (Reporting of Serious Adverse Events).

The DSMB will review data relevant to the event and will receive input from the Sponsor and/or Investigator before providing appropriate recommendations. The event and the DSMB's recommendation will be discussed with the United States (US) Food and Drug Administration (FDA) and other regulatory authorities. All patients who were administered SBT101 will continue to comply with the follow-up schedule according to the protocol.

7. STUDY MEASUREMENTS AND ASSESSMENTS

7.1 Timing of Assessments

The assessments should be completed at the visits indicated in the Schedule of Assessments ([Table 2](#)).

7.2 Assessments

7.2.1 2-minute Walk Test (2MWT)

The 2MWT is a measure of self-paced walking and functional capacity that assesses how much distance patients can cover walking in 2 minutes.⁵³ Patients should walk in a marked 7-meter hallway back and forth for 2 minutes. The patient should rest for at least 20 minutes before the 2MWT.

The Mobility Lab (OPAL; see [Section 7.2.13](#)) will be used in this assessment.

Further details are provided in the SBT101-CT101 Training Manual.

7.2.2 6-minute Walk Test (6MWT)

The 6MWT assesses the distance walked over 6 minutes as a sub-maximal test of aerobic capacity and endurance.⁵⁴ Patients should walk in a marked 12-meter hallway back and forth for 6 minutes. Patients may take as many rests as needed and all assistive devices used must be documented.

The Mobility Lab (OPAL; see [Section 7.2.13](#)) will be used in this assessment.

Further details are provided in the SBT101-CT101 Training Manual.

7.2.3 5 Times Sit-to-Stand Test (5XSST)

The 5XSST assesses sit-to-stand ability and is a measure of lower extremity strength, balance control, and fall risk.⁵⁵⁻⁵⁸ This test measures the time taken to stand 5 times from a sitting position as quickly as possible.

The Mobility Lab (OPAL; see [Section 7.2.13](#)) will be used in this assessment.

Further details are provided in the SBT101-CT101 Training Manual.

7.2.4 Postural Body Sway

The postural body sway protocol consists of 2 series of measurements in 7 conditions with a fixed sequence:

- Eyes closed, feet apart
- Eyes open, feet apart
- Eyes closed, feet together
- Eyes open, feet together
- Eyes closed, feet together, foam surface
- Tandem stance
- Single limb stance

The Mobility Lab (OPAL; see [Section 7.2.13](#)) will be used in this assessment.

Further details are provided in the SBT101-CT101 Training Manual.

7.2.5 Timed Up and Go (TUG)

The TUG is an assessment of basic mobility skills that measures the time in seconds that patients require to rise from a standard chair, walk 3 meters, turn, return to the chair, and sit down again; the patient is allowed to have a practice try that is not scored.⁵⁹

The Mobility Lab (OPAL; see [Section 7.2.13](#)) will be used in this assessment.

Details on how to complete the assessment are provided in the SBT101-CT101 Training Manual.

7.2.6 Dual-task Timed Up and Go (TUG-DT)

The TUG-DT is a variation of the TUG that measures the time in seconds that patients require to rise from a standard chair, walk 3 meters, turn, return to the chair, and sit down again while simultaneously performing a cognitive task. The patient will be given a number (e.g., 100) and instructed to count backwards by intervals of 3 while performing TUG.

The Mobility Lab (OPAL; see [Section 7.2.13](#)) will be used in this assessment.

Details on how to complete the assessment are provided in the SBT101-CT101 Training Manual.

7.2.7 Balance Evaluation System Test (miniBESTest)

The miniBESTest evaluates dynamic balance by assessing 4 different balance control systems: anticipatory postural adjustments, reactive postural control, sensory orientation, and dynamic gait.⁶⁰ It includes TUG-DT, a variation of the TUG assessment that measures functionality in patients attempting to combine a motor and cognitive task.⁶¹⁻⁶² The miniBESTest assessment has 14 items that are scored on a 3-level ordinal scale with a total possible score of 28.

Specific training is required and can be found in the SBT101-CT101 Training Manual.

7.2.8 Expanded Disability Status Score (EDSS)

The EDSS is a widely used assessment that provides a single number measure of disability.⁶³ It includes Functional Systems Scores and Disability Status Scores. The EDSS will be conducted by a neurologist or qualified healthcare professional that will carry out the neurological examination to assess several distinct “functional systems”, including pyramidal, cerebellar, brain stem, sensory, bowel and bladder, visual, and mental function. The qualified healthcare professional will examine the systems and then complete the EDSS score based on an ordinal rating scale ranging from 0 to 10.

Further details are provided in the SBT101-CT101 Training Manual.

7.2.9 Severity Score System for Progressive Myelopathy (SSPROM)

The SSPROM is a measure of the severity of myelopathy that covers the following domains: motor disability reported and “examined”, “sphincter dysfunction”, “presence of spasticity and hyperreflexia”, and “sensory losses”.⁶⁴

Motor disability and sphincter dysfunction are patientive domains that rely on the information provided by the patient. The sphincter dysfunction domain is adapted from the related sub-score of the Functional Systems Score, which is a part of the EDSS. The other domains are related to quantitative assessment of the standardized neurological examination. All items are weighted with the following contributions from each system: 50% motor strength, 10% tonus and stretch reflexes, 20% sphincter function, and 20% sensory system. All of these domains are summed to obtain a final score (0 to 100), where lower scores indicate higher degree of impairment.

Further details are provided in the SBT101-CT101 Training Manual.

7.2.10 Clinical Global Impression (CGI) and Clinical Global Impression – Change (CGI-C) – Physician

The CGI is a brief assessment of the Physician's view of a patient's global functioning prior to and after initiating a treatment; it provides a summary measure while taking into account the patient's medical history, psychosocial circumstances, symptoms, behavior, and the impact of the symptoms on the patient's ability to function.⁶⁵⁻⁶⁶

For the CGI-Change (CGI-C) treatment response ratings should take account of therapeutic efficacy.

Further details are provided in the SBT101-CT101 Training Manual.

7.2.11 Concomitant Medications and Therapies

Details of all concomitant medication/therapy use, including all medications administered for the treatment of AEs, must be recorded in the patient's eCRF. Any questions should be discussed with the Medical Monitor.

7.2.12 Patient-reported Outcomes (PROs)

7.2.12.1 Patient Global Impression (PGI) Scale

The PGI is a patient-completed scale that assesses symptom severity.⁶⁷ Three (3) PGI sub-scales will be used:

- PGI-S: Measures the patient's perception of the severity of disease (i.e., AMN)
- PGI-S Balance: Measures the severity of balance impairments
- PGI-C Balance: Measures changes in balance-related symptoms compared to the previous visit at which postural body sway was evaluated.

Further details are provided in the SBT101-CT101 Training Manual.

7.2.12.2 Multiple Sclerosis Walking Scale (MSWS-12)

The MSWS-12 is a self-reported 12-item scale that measures the impact of disease on a patient's ability to walk in the previous 2 weeks.⁶⁸ Each item is rated on a scale of 1 to 5 (1 = not at all; 2 = a little, 3 = moderately, 4 = quite a lot, 5 = extremely). The overall score is calculated by adding item scores for a total score from 0 to 100; higher scores indicate greater impact on walking.

Further details are provided in the SBT101-CT101 Training Manual.

7.2.12.3 Multiple Sclerosis Quality of Life-54 (MSQoL-54)

The MSQoL-54 is a 54-item scale that evaluates quality of life with sub-scales for physical function, physical role limitations, emotional role limitations, pain, emotional well-being, energy, health perceptions, social function, cognitive function, health distress, and sexual function.⁶⁹

Further details are provided in the SBT101-CT101 Training Manual.

7.2.12.4 Urinary and Bowel Questionnaire

This PRO questionnaire is used to understand the urinary and bowel symptoms in adults with AMN.

Further details are provided in the SBT101-CT101 Training Manual.

7.2.13 The Mobility Lab (OPAL)

The Mobility Lab uses a multi-sensor device called OPAL, which is applied to the patient's feet and lower back, to evaluate disease progression. The Mobility Lab (OPAL) sensors will be used for in-clinic assessments of the 6MWT and TUG-DT, and for remote visit assessments of the 2MWT, 5XSST, Postural Body Sway, and TUG.

Further details are provided in the SBT101-CT101 Training Manual.

7.2.14 CentrePoint Insight Watch

The Bluetooth® LE 5 smart CentrePoint Insight Watch features ActiGraph's validated 3 axis accelerometer and data filtering technology, and displays the date, time, device wireless mode, and battery status. Patients will wear the Sponsor-provided activity monitor 24 hours a day throughout Part 1 of the study. Data will automatically be transferred to the CentrePoint technology platform in the cloud via the use of an installed telephone application. The CentrePoint Insight Watch will collect overall activity and sleep data. The watch will need to be charged monthly and the patient should acknowledge their understanding of its use during the screening visit.

Further details are provided in the SBT101-CT101 Training Manual.

7.2.15 Physical Examination

Physical examinations will be performed by the Investigator or designee at the time points indicated in the Schedule of Assessments ([Table 2](#)).

A full physical examination will be conducted at the Screening Visit. This will include examinations of all major body/organ systems, including skin, head, eyes, ears, nose, throat, neck, thyroid, lungs, heart, abdomen, lymph nodes, and extremities, as well as height, weight, and calculation of BMI. Measurements of height and weight should be taken with the patient in light clothing or underwear and without shoes.

Brief physical examinations will be conducted at the time points indicated in the Schedule of Assessments ([Table 2](#)). These will include examinations of general appearance, cardiovascular and respiratory systems, abdomen, extremities, and skin. Additional full, brief, or targeted physical examinations (e.g., targeted to any new signs or symptoms) may be performed at any time at the discretion of the Investigator or designee, for example, to evaluate an AE. All

clinically significant physical examination findings that are new or have worsened since the last physical examination must be recorded as AEs in the patient's eCRF and source documentation.

7.2.16 Vital Signs

Vital sign measurements will be conducted at the time points indicated in the Schedule of Assessments (**Table 2**) and will include systolic blood pressure, diastolic blood pressure, pulse rate, respiratory rate, and body temperature.

Vital signs will be measured in the supine position following a 5-minute rest. All blood pressure measurements should be taken on the same arm throughout the study. Vital signs may be measured using automated vital signs monitor, although manual measurements of blood pressure are preferred. All vital sign measurements must be done using a calibrated instrument. For each patient, the same method of measurement should be used at all visits. All vital sign values, whether measured manually or via an automated monitor, will be recorded in the patient's eCRF and source documentation.

7.2.17 Electrocardiogram (ECG)

Electrocardiograms will be conducted at the time points indicated in the Schedule of Assessments (**Table 2**).

All ECG recordings should be 12-lead and should be performed after patients have rested in a supine position for at least 5 minutes and, if applicable, prior to study drug dosing and/or blood sample collection. During the collection of ECGs, patients should be in a quiet setting without distractions and should refrain from talking or moving the arms or legs.

The following parameters and intervals will be assessed: heart rate, RR interval, PR interval, QRS complex, uncorrected QT interval, and corrected QC interval by Fredericia (QTcF). The occurrence of depolarization or repolarization disorders, arrhythmic disorders, and/or other abnormalities will be noted. A designation of clinical significance will also be noted.

It is important that the leads be placed in approximately the same positions at each recording in order to achieve accurate ECG recordings. In some cases, it may be appropriate to repeat an abnormal ECG to rule out improper lead placement.

All ECG results must be reviewed by the Investigator or designee. In the event of abnormal results, the Investigator or designee must evaluate the ECG abnormalities promptly. Any clinically significant finding must be reported as an AE.

All ECG parameters and assessments must be recorded in the patient's eCRF and/or stored with the patient's source documentation.

7.2.18 Neurological Examination

A neurological examination will be performed by the Investigator or designee at the time points indicated in the Schedule of Assessments (**Table 2**) and will include the following:

- Mental status (e.g., orientation to person, place, and time)
- Cranial nerve examination (e.g., extraocular movements, facial muscles [raise eyebrows, eye closure, and smile])
- Motor examination (e.g., bulk, tone, and strength of upper and lower extremities)

- Reflexes (e.g., plantar [Babinski], muscle stretch reflexes of biceps, triceps, patellar, and ankles)
- Coordination
- Sensory examination (e.g., touch, pain, vibration, and position)
- Gait

7.2.19 Electrophysiological Examination

The EP examinations will be conducted at the time points indicated in the Schedule of Assessments (**Table 2**) and will include NCS of motor and sensory peripheral nerves of the upper and lower extremities. The essential parameters evaluated during peripheral NCS are amplitudes, peak/distal latency, and conduction velocity of sensory and motor nerves. Soleus muscle H-reflexes and F-waves of all tested motor nerves will also be assessed. Evaluation of nerves by ultrasonographical imaging of the peripheral nervous system can be performed together with NCS, if possible.

EP examinations will be reviewed by an independent reviewer prior to the start of the immunosuppression taper. The independent review will be documented and completed at a frequency per the Electrophysiological Examination and Somatosensory Evoked Potentials Review Charter.

7.2.20 Somatosensory Evoked Potentials

The SSEP examinations will be conducted at the time points indicated in the Schedule of Assessments (**Table 2**). The central afferent functions are studied with somatosensory evoked potentials (SSEP). At a minimum, potentials of the median nerve at the wrist and posterior tibial nerve at the ankle will be required. For the median nerve SSEP, the peak latency of N13 and the inter-peak latency of N13–N20 will be measured, and for the tibial nerve SSEP, the N21 peak latency and the N21–P38 inter-peak latency will be measured.

SSEP examinations will be reviewed by an independent reviewer prior to the start of the immunosuppression taper. The independent review will be documented and completed at a frequency per the Electrophysiological Examination and Somatosensory Evoked Potentials Review Charter.

7.2.21 Imaging Studies

MRIs of the Brain, full spine (Cervical, Thoracic, and Lumbar), with and without contrast, and Cervical Spine MRIs inclusive of DTI, MT, and spectroscopy parameters performed at the time points indicated in the Schedule of Assessments (**Table 2**).

All MRIs will be assessed and a Loes score will be assigned to any brain MRI performed by the clinical site by an independent reviewer. If a patient is diagnosed with or suspected of a cerebral disease by the clinical site, blinded independent experts will assess/confirm the presence of the cerebral disease. The Imaging Review Charter, which describes the procedures for image blinding, reading, and scoring, will be used by the central reviewers.

Image acquisition is included in the SBT101-CT101 Imaging Manual. Additional advanced imaging techniques may be performed on an exploratory basis to better characterize the degree of spinal disease; refer to SBT101-CT101 Imaging Manual for additional details.

7.2.22 Lumbar Punctures

Lumbar punctures will be performed at the time points indicated in the Schedule of Assessments (**Table 2**) for assessment of CSF biomarkers (**Section 7.2.24.3**).

Lumbar punctures may cause severe headaches in some patients. As such, prophylactics for severe headaches, such as blood patches or increased caffeine intake, may be recommended at the discretion of the Investigator.

7.2.23 Laboratory Assessments

Safety laboratory assessments will be conducted at the time points indicated in the Schedule of Assessments (**Table 2**) and will include blood chemistry (sodium, potassium, calcium, creatinine), hematology (thrombocyte count), prothrombin time), troponin, vitamin B12 levels, liver function tests (LFTs; i.e., alanine aminotransferase [ALT], aspartate aminotransferase [AST], total bilirubin), and urinalysis.

Prior to the IMP procedure, troponin, thrombocyte count, vitamin B12 level, and liver function will be assessed by laboratory testing (e.g., AST, ALT, total bilirubin, and prothrombin time). Troponin, thrombocyte count, and LFTs will be monitored for at least 3 months after the IMP procedure and until results are unremarkable (i.e., values within normal ranges and no Grade III or Grade IV elevated transaminases that are related or possibly related to SBT101). In addition, LFTs will be monitored biweekly for 4 weeks after the prednisone/prednisolone dose has been tapered down to a daily dose of 10 mg.

7.2.24 Other Laboratory Assessments

Other laboratory assessments include evaluation of adrenal function, genetic testing for confirmation of *ABCD1* mutation, biomarkers, and monitoring for viral shedding and immunogenicity. These assessments will be conducted at the time points specified in the Schedule of Assessments (**Table 2**).

7.2.24.1 Adrenal Function

Adrenal function will be assessed at the Screening Visit by laboratory assessments of cortisol, plasma ACTH, plasma renin, aldosterone, sodium, and potassium levels. Monitoring of replacement therapy will be mainly clinical. Determination of inappropriately treated adrenal insufficiency will be made by the Investigator in consultation with the Medical Monitor.

In addition, patients who meet the following criteria will be considered to have adrenal insufficiency and excluded from the study:

- Basal cortisol concentration <275 nmol/L (10 µg/dL) in the morning (i.e., 6 AM to 10 AM)
- Plasma ACTH >2 × ULN

7.2.24.2 Genetic Testing

Blood samples will be collected from consented patients to confirm underlying mutation of the *ABCD1* gene at the Screening Visit.

7.2.24.3 Biomarkers

Plasma and CSF samples will be collected at the time points indicated in the Schedule of Assessments (**Table 2**) to measure NfL, LPC-VLCFAs, and VLCFAs and, where available. For all VLCFA collection timepoints (**Table 2**) the subject should fast.

Samples for CSF biomarker assessments on Day 1 (Visit 3) will be collected via gravity.

Samples for CSF biomarker assessments on Day 360 and Day 720 will be collected by lumbar puncture.

Details on collection and handling of biomarker samples are provided in the SBT101-CT101 Laboratory Manual.

7.2.24.3.1 Optional Exploratory Blood and CSF Biomarkers

Where local regulations and IRB/ECs allow, additional blood and CSF samples will be collected from patients who consented to optional blood/CSF collection for future research on Day 1 (Visit 3) prior to the IMP procedure (**Table 2**). These samples will only be used for investigations related to ALD and gene therapy or class of drugs under study in the context of this clinical trial. The development of anti-hABCD1 antibodies may also be evaluated in these samples. Samples will not be used for broad exploratory unspecified disease or population genetic analyses.

Collected samples will be coded with the patient ID and retained for up to 5 years after the last patient has completed the last study visit. The samples and any data generated from the samples will be anonymized and only linked to the patient ID. Only the Investigator and authorized study team members will be able to link back the samples (and associated data) to the patient. The duration of retention will allow the Sponsor to respond to regulatory requests related to the study drug.

After 5 years, the samples will be destroyed in accordance with local regulations. Patients may request to have their samples destroyed at any time.

7.2.24.4 Viral Genome Detection/Vector Shedding

Sampling of serum, saliva, and urine to determine vector DNA levels will be performed at specific time points post-IMP procedure, as specified in the Schedule of Assessments (**Table 2**). Sampling should continue for an individual patient and for a specific matrix until 3 consecutive negative samples have been detected for the patient for that particular type of matrix.

7.2.24.5 Immunogenicity

Blood samples will be collected to analyze total antibodies and NAb titers for AAV9.

Assessments of T-cell immunity to AAV9 and hABCD1 will be performed by ELISpot at the time points specified in the Schedule of Assessments (**Table 2**).

Assessments of T-cell reactivity will be performed with ELISpot and by-patient and by-visit data listings of ELISpot results will be generated. These listings will include positive and negative

results and the number of spot forming units (SFU) per 1.0E6 peripheral blood mononuclear cells (PBMC) for each peptide pool and control (positive and negative) stimulation. Positive and negative results with SFU/1.0E6 PBMC will be evaluated for correlations with changes in clinical chemistry laboratory assessments or AEs, as appropriate for the data collected. Additional ELISpot testing may be performed at the discretion of the site Investigator; based on the test results, the immunosuppression regimen may be altered.

7.2.24.5.1 Additional ELISpot Collection

All patients at University of Massachusetts Chan Medical School will have an additional ELISpot collection at the time points specified in the Schedule of Assessments (**Table 2**). The collection time points may be expanded beyond the 90 days after consultation between the site and the Medical Monitor.

The additional ELISpot assay will be conducted by a research laboratory. Results will be used to characterize the cellular immune response to gene therapy and inform the need for intensified or altered immunosuppressive regimens, including use of sirolimus. Based on test results, the patient's immunosuppressive regimen may be altered.

7.3 Adverse Events

The Investigator is responsible for the detection and documentation of all AEs and SAEs.

7.3.1 Definition of Adverse Events

7.3.1.1 Adverse Events

Adverse events must be assessed for the investigational product in this study. An investigational product is defined as a pharmaceutical form of an active ingredient or placebo being tested or used as a reference in the study, whether blinded or unblinded.

The International Council for Harmonisation (ICH) of Technical Requirements for Pharmaceuticals for Human Use Guideline for GCP E6 defines an AE as the following:

An AE is any untoward medical occurrence in a patient administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can, therefore, be any unfavorable and unintended sign (including an abnormal finding), symptom, or disease temporarily associated with the use of an IMP, whether or not related to the IMP.

The following are considered to be AEs:

- Any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug; does not imply any judgment about causality
- Any new disease or exacerbation of an existing disease
- Recurrence of an intermittent medical condition (e.g., headache) not present at baseline
- Laboratory test or other clinical test (e.g., ECG or X-ray) with a clinically significant abnormality (as defined below)
- An effect of the study medication, including comparator

- Any dose of medication (study drug or other concomitant medication) that is taken at a dose higher than the prescribed dose (i.e., an overdose). An overdose should be reported as an AE whether or not it is associated with any symptoms or signs

The following are not considered to be AEs:

- Medical or surgical procedures (e.g., surgery, endoscopies, tooth extraction, transfusion, etc.) – the condition which leads to the procedure is the AE
- Preexisting diseases or conditions or laboratory abnormalities present or detected prior to the screening evaluation that do not worsen
- Situations where an untoward medical occurrence has not occurred (e.g., hospitalization for elective surgery, social and/or convenience admissions, etc.)

Clinically significant changes in objective findings (e.g., physical examination, neurological examination) should be considered AEs only if they meet one or more of the following criteria:

- Associated with accompanying symptoms
- Require medical/surgical intervention
- Lead to discontinuation from the study
- Lead to significant additional concomitant drug treatment, or other therapy
- Lead to any of the outcomes included in the definition of an SAE
- Are considered clinically significant by the Investigator

Whenever possible, the etiology of the abnormal finding (rather than the abnormal finding itself) should be documented as the AE. Repeated additional tests and/or other evaluations required to establish the significance and etiology of an abnormal result should be obtained when clinically indicated.

Any abnormal test result that is determined to be an error does not require reporting as an AE.

Surgical procedures themselves are not AEs but are therapeutic measures for conditions that require surgery. The condition for which the surgery is required may be an AE, if it occurs or is detected during the study period. Planned surgical measures permitted by the clinical study protocol (if any) and the condition(s) leading to these measures are not AEs, if the condition(s) was (were) known before the start of the study treatment and documented in the patient's medical record. In the latter case, the condition should be reported as part of the patient's medical history.

All patients who have AEs, whether considered to be associated with the use of the investigational product or not, must be monitored to determine the outcome of the event(s). The clinical course of the AE will be followed according to accepted standards of medical practice, even after the end of the period of observation, until a satisfactory explanation is found, or the Investigator considers it medically justifiable to terminate follow-up.

7.3.1.2 Serious Adverse Events

All SAEs must be evaluated as potential SAEs. If an event is not an AE per the definition in [Section 7.3.1.1](#), it cannot be an SAE, even if serious conditions are met.

An SAE is any untoward medical occurrence that occurs at any dose and meets at least 1 of the following criteria:

- Results in death
- Is life-threatening (i.e., the patient was at immediate risk of death from the AE as it occurred; this does not include an event that, had it occurred in a more severe form or was allowed to continue, may have caused death)
- Requires inpatient hospitalization or prolongation of existing hospitalization for the AE
 - The following types of hospitalizations are not considered SAEs:
 - Hospitalization(s) for planned (pre-scheduled) medical procedures known at the time of screening
 - Protocol-specific hospital admission
 - Respite care
 - Admission for the treatment of pre-existing condition (known at the time of screening) not associated with the development of a new AE or with the worsening of the pre-existing condition
 - Observation/same day/ambulatory procedure
- Is a persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions
- Is a congenital anomaly/birth defect
- Is an important medical event or reaction

7.3.2 Adverse Event Reporting Period

Adverse events include events that have appeared or worsened during the clinical trial. They may also include pre- or post-treatment complications that occur because of protocol-mandated procedures (e.g., invasive procedures, such as venipuncture).

Any AE (i.e., a new event or an exacerbation of a preexisting condition) with an onset date after the patient provides informed consent through the patient's EOS Visit will be recorded as an AE on the appropriate eCRF page(s).

All SAEs, regardless of cause or relationship, occurring up to and including the EOS Visit must be documented and reported.

Follow-up of SAEs will continue through the last day of the study or until the event has resolved or stabilized to the satisfaction of the Investigator and the Sponsor's Medical Monitor or designee. Investigators are not obligated to actively seek out SAEs beyond the follow-up period.

7.3.3 Adverse Event Reporting Requirements

The Investigator is responsible for the detection and documentation of AEs and SAEs that occur while the patient is enrolled in the study. Adverse events may be spontaneously reported by a patient or his/her representative or noted during questioning and examination of a patient. All AEs will be assessed by the Investigator and documented regardless of apparent relationship to the study drug. For each AE documented, the Investigator will evaluate and report the date of onset and resolution, outcome, severity, relationship to the study drug, action taken, additional treatments required to manage the event, and determination of seriousness.

All identified study drug-related AEs and SAEs occurring during the trial must be fully recorded and described on the appropriate eCRF page. The AE should be reported in standard medical

terminology. Whenever possible, the AE should be evaluated and reported as a diagnosis, rather than as individual signs or symptoms. A cluster of signs and symptoms that results from a single cause should be reported as a single AE (e.g., fever, elevated white blood cells, cough, abnormal chest X-ray, etc. can all be reported as “pneumonia”).

If a definitive diagnosis is not possible, the individual symptoms and signs should be recorded. Documentation must be supported by an entry in the patient’s medical record.

The relationship to the study drug should be assessed using the definitions in [Section 7.3.5](#).

7.3.4 Severity of Adverse Events

The severity of all AEs and SAEs will be graded according to the version of the Common Terminology Criteria for Adverse Events (CTCAE) that is current at the time of study conduct. The Investigator or designee should determine the severity of the AE based on the overall clinical importance or significance of the finding for that individual patient.

If an AE that was reported during the study increases or decreases in severity, then the resolution date and time for that AE will be recorded, and a new record will be initiated with the new severity. If the severity of an AE remains the same, the AE will be kept open through to resolution.

7.3.5 Relationship to Study Drug

The Investigator must assess the likelihood that the study drug caused or contributed to each AE, and document this assessment assigning one of the following relatedness criteria to each AE:

- **Unrelated:** In the opinion of the Investigator, there is no association between the study drug and the reported event.
- **Unlikely:** In the opinion of the Investigator, it is unlikely that there is an association between the study drug and the reported event.
- **Possible:** In the opinion of the Investigator, treatment with the study drug may have caused or contributed to the reported event, but it could also have been caused by other factors (i.e., the event follows a reasonable temporal sequence from the time of study drug administration and/or follows a known response pattern to the study drug but is also known to be caused by other factors).
- **Probable:** In the opinion of the Investigator, it is likely that the study drug caused or contributed to the reported event, based on a reasonable temporal sequence of the event with the study drug and the Investigator’s clinical judgement.
- **Definite:** In the opinion of the Investigator, it is definite that the study drug caused or contributed to a reported event, and other conditions (e.g., concurrent illness, progression/expression of disease state, or concurrent medication reaction) do not explain the event.

For the purposes of determining expedited reporting status to Health Authorities, the Sponsor considers the assessments of “unrelated” and “unlikely” as unrelated to the study drug and the assessments of “possible”, “probable”, and “definite” as related to the study drug.

In addition, for any analyses of AE data in which only two categories of “related” and “unrelated” are used, the assessments of “unrelated” and “unlikely” will be combined into the

category of “unrelated”, and the assessments of “possible”, “probable”, and “definite” will be combined into the category of “related”.

7.3.6 Follow-up of Unresolved Adverse Events

All AEs should be followed until resolved, until the Investigator assesses them as stable, or until the patient’s participation in the study ends.

7.3.7 Pregnancies

In the event of any pregnancies in study patients’ partners, consent will be requested to follow the mother and child after delivery.

All pregnancies that occur or become confirmed during the study must be reported in a Pregnancy Form and submitted to the Sponsor or designee within 1 business day of awareness of the pregnancy. Pregnancies should be reported in the Pregnancy Form and follow SAE reporting methods.

All pregnancies should be followed and discussed with the Medical Monitor as follows:

- The Investigator will follow-up with the patient or the patient’s female partner and report to the Sponsor or designee using the Pregnancy Form after delivery. Generally, follow-up will only be required for 6 weeks beyond the delivery date.
- The outcome of the pregnancy will be reported to the Sponsor or designee using the Pregnancy Form. Any termination of pregnancy will be reported, regardless of fetal status (i.e., presence or absence of anomalies) or indication for the procedure.

Any SAEs occurring in the child (e.g., congenital anomalies or other conditions present at birth, whether genetically inherited or occurring *in utero*) must also be documented on the Pregnancy Form.

7.3.8 Reporting of Serious Adverse Events

The Sponsor has requirements for expedited reporting of safety events in accordance with specific requirements of worldwide regulatory authorities; therefore, any SAEs and/or pregnancies that occur during the study must be immediately notified to the Sponsor.

All SAEs, including those not suspected to be related to the study drug, must be immediately reported to the Sponsor. The procedures for reporting SAEs and pregnancies, regardless of relationship to the study drug, are as follows:

SAEs

- Record the SAE within 24 hours of awareness of the event by logging into the EDC system and completing the initial SAE Report
- This will trigger an email notification to the Pharmacovigilance Team and Sponsor distribution lists
- The Investigator must update the SAE Report as soon as any additional information becomes available
- The Investigator must also report SAEs to the IRB/EC per local regulatory requirements

Pregnancies

- Complete the Pregnancy Form in EDC
- This will trigger an email notification to the Pharmacovigilance Team and Sponsor distribution lists

Contact Information

The contact information for reporting SAEs and pregnancies is provided below:

SAE CONTACT
In the event of a system failure or for questions about completing the forms in EDC:

For fatal or life-threatening events, copies of hospital discharge reports, autopsy reports, and other documents must be provided, as applicable. The Sponsor may request additional information from the Investigator to ensure timely and accurate completion of safety reports.

If a SUSAR occurs during the study, in accordance with 21 CFR 312.32 and local authorities, the Sponsor will submit SUSARs to the FDA within 15 calendar days; fatal or life-threatening SUSARs will be submitted within 7 calendar days.

8. STATISTICAL METHODS

8.1 Sample Size Calculation

A minimum of sixteen (16) patients will be enrolled in this study. This sample size is considered sufficient to assess initial safety and tolerability of 2 dose levels of SBT101, and no formal sample size calculation was performed for this study.

8.2 Definition of Analysis Populations

The Full Analysis Set (FAS) will consist of all patients who signed informed consent and were treated or randomized.

The Safety Analysis Set will consist of all patients who received any amount of SBT101 or had the imitation (sham) procedure performed as part of this study.

The Modified Intent to Treat (mITT) Analysis Set is a subset of the FAS and will consist of all patients for whom the full amount of study medication (or imitation (sham) procedure) was administered, with at least one post-baseline efficacy measure.

The SBTNHX-CT901 study external control group will consist of the FAS of study SBTNHX-CT901, with additional restrictions to align with the key inclusion criteria of this study: age at baseline ≤ 65 years, EDSS at baseline ≤ 4.5 .

8.3 Analyses

No formal inferential statistical analyses will be performed. All presentations will be descriptive in nature. Continuous variables will be summarized with descriptive statistics including the number of non-missing values, mean, standard deviation, median, minimum, and maximum. In some cases, the standard error of the mean and/or confidence intervals may be presented. Categorical variables will be summarized by number, percent of patients and, if applicable, the number of events. All collected data will be listed for the FAS.

In the Part 1 analyses, treatment groups will be assigned according to the actual procedure performed rather than the randomized group. Furthermore, the SBT101 patients will be grouped by the dose level received (Dose Level 1, Dose Level 2). In the Part 2 analyses, control patients who received open-label SBT101 will be included with the SBT101 group, counting the day of SBT101 administration as their baseline.

Details regarding the statistical methods and definitions will be provided in the Statistical Analysis Plan (SAP). The SAP will be finalized before the interim database lock.

Statistical analyses will be performed using SAS[®] Version 9.4 or higher (SAS Institute, Cary, North Carolina 27513).

8.3.1 Patient Disposition

The data on patient disposition and informed consent will be listed, containing details on treated and completed patients, as well as information regarding withdrawal reasons or protocol deviations.

A summary will be prepared showing the number of patients randomized, patients who have undergone the IMP procedure, patients completing Month 12 and Month 24 (Year 2) Visits,

control patients who crossover to open-label SBT101, and patients completing each year of follow-up in Part 2.

8.3.2 Demographic and Baseline Characteristics

Baseline data will be summarized for the Safety Analysis Set, and (if different) for the mITT Set. These summaries will include the SBTNHX-CT901 group, for which the baseline is defined by the start of the prospective data collection.

Separate Part 2 summaries will be prepared with the crossover patients considered having their baseline at the time of SBT101 infusion.

Medical history will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) and listed.

8.3.3 Investigational Product Exposure

Data collected about SBT101 infusion/imitation (sham) procedure (for cross-over patients, both the original imitation (sham) procedure and SBT101 infusion at Month 24) will be listed.

8.3.4 Prior and Concomitant Medications

All medications will be coded using World Health Organization (WHO) Drug.

Prior medications are defined as medications which stopped before initiation of the study drug administration/imitation (sham) procedure. Concomitant medications are defined as medications taken at any time during or after the study drug administration/imitation (sham) procedure. Prior and concomitant medication usage will be summarized by Anatomical Therapeutic Chemical (ATC) class and preferred term

Medications designated as planned immunosuppressive medications ([Section 5.2.7.1](#)) will be summarized separately from the other prior and concomitant medications. For these medications, duration and dose of each medication will be summarized.

8.3.5 Safety Analyses

All AEs will be listed and coded using MedDRA and graded for severity according to the version of the CTCAE that is current at the time of study conduct.

Treatment emergent AEs will be defined as those AEs which started during or after the administration of SBT101/imitation (sham) procedure or the immunosuppressant pre-medication.

An overall summary of TEAEs will be prepared, showing incidence of any TEAE, serious TEAEs, TEAEs related to SBT101/imitation (sham) procedure, TEAEs related to immunosuppressant medications, serious related TEAEs, and deaths.

TEAEs will be summarized by system organ class and preferred term presenting the number of patients with events and the number of events. This summary will be repeated with a breakdown by maximal severity of the events. Separate summaries will be made for selected AE categories.

All TEAE summaries will be performed for the Safety Analysis Set and will include SBT101 and control (imitation (sham) procedure) arms, as well as the dose level breakdown for SBT101 (if the study proceeds with the dose escalation). The Part 1 analysis will include summaries

through Month 12 (Year 1) and Month 24 (Year 2). The Part 2 analysis will include all TEAEs with possible breakdowns by milestone time after SBT101.

Summary statistics for the baseline and change from baseline at each scheduled visit for continuous variables will be tabulated for laboratory, vital signs, and ECG assessments. In listings, data outside of the normal reference range will be flagged.

All data from MRI monitoring of signs of inflammatory cerebral disease will be listed. The number of patients with signs of inflammatory cerebral disease at Day 30 and the number of patients with resolved signs of inflammatory cerebral disease within 1-3 months will be tabulated.

8.3.6 Efficacy Analysis

Efficacy data will be summarized using descriptive statistics by nominal visit; analysis windows will not be defined.

Summaries of the 2MWT and 6MWT will only utilize data where the same walking aids were used between baseline and post-baseline. Changes in walking aids during the study will be listed.

During Part 1, continuous efficacy endpoints will be summarized descriptively for the variables' absolute value, change from baseline, and, where appropriate, percent change from baseline. The groups to be included are SBT101 (Dose Level 1, Dose Level 2), control, and SBTNHX-CT901 group.

If important baseline characteristics are found to be different between patients and the SBTNHX-CT901 group, techniques such as propensity score weighting may be employed to reduce bias in the summary statistics for the SBTNHX-CT901 group.

During Part 2, patients who crossover to open-label SBT101 will have their baseline re-defined in reference to the SBT101 administration and be included with the SBT101 group. Descriptive statistics will be produced for absolute value, change from baseline and percent change from baseline. The SBT101 group will be presented, possibly separating Dose Level 1 and Dose Level 2.

Individual patient data will be plotted over time for select variables, using spaghetti plots to show progression over time. Means with standard error bars will also be plotted over time.

For selected variables, progression over time post-SBT101 treatment may be characterized via modeling. Further details will be presented in the SAP.

8.3.7 Neutralizing Antibody Assay

Data from NAb assays will be listed. Exploratory analyses may be conducted to evaluate association between NAb positivity and efficacy endpoints. Further details will be presented in the SAP.

8.4 Interim Analysis Plan

An interim analysis is planned at the completion of Part 1 by all patients. This interim analysis will include all data collected up to and including Month 24 Visit (but prior to administration of SBT101 for control patients who receive open-label SBT101 at the end of Part 1). Additional interim analyses are planned at the end of one year of follow-up of treated patients.

8.5 Data Safety Monitoring Board

An independent DSMB will be established to review safety data throughout the study. Members will include at least 3 independent physicians with expertise in neurological diseases and/or gene therapy and an independent statistician.

The DSMB will review the safety data of the patients completing the Day 30 visit within each Dose Level Cohort. The decision to proceed to the next Dose Level Cohort and/or to initiate the Dose Expansion Phase will be made after review of safety data by the independent DSMB and the Sponsor.

Further details will be provided in the DSMB Charter.

9. QUALITY CONTROL AND QUALITY ASSURANCE

9.1 Routine Monitoring

The Investigator is responsible for the validity of all data collected at the study site and must accept the various monitoring procedures employed by the Sponsor. The purpose of monitoring is to verify that the rights and well-being of human patients are protected; that trial data are accurate, complete, and verifiable with source data; and that the trial is conducted in compliance with the protocol, GCP, and the applicable regulatory requirements.

A Study Monitor assigned by the Sponsor is responsible for monitoring various aspects of the study, including routine review of the eCRFs and source documents at regular intervals throughout the study to verify adherence to the protocol and the completeness, consistency, and accuracy of data entered in the eCRFs. Visits will usually take place within a predetermined interval, but this may vary during the study. The Investigator must agree to allow the study monitor and authorized representatives of the Sponsor to inspect all eCRFs and corresponding source documents, such as original medical records, patient records, and laboratory raw data, as well as agree to assist with their activities if requested. The Investigator should provide adequate time and space for monitoring visits and ensure that any problems detected in the course of these monitoring visits are resolved.

The Monitor will query any missing or spurious data with the Investigator or designee, which should be resolved in a timely manner. A Monitoring Visit Log will be maintained, recording each visit, the reason for the visit, the Monitor's signature, and Investigator or designee's confirmation signature.

9.2 Site Audits

For the purpose of compliance with GCP and regulatory agency guidelines, it may be necessary for Sponsor-authorized Quality Assurance personnel and/or authorized personnel from an external regulatory agency to conduct an audit/inspection of a study site. The purpose of an audit is to assess the quality of data regarding accuracy, adequacy, and consistency, and to assure that studies are in accordance with GCP and Regulatory Agency guidelines. Having the highest quality data from studies is an essential aspect of drug development.

The Investigator will be given sufficient notice to prepare for such visits, which are usually planned to take approximately 2 days and may be conducted at any stage during the study. The audit will involve the review of all study-related documentation, which is required by GCP to be maintained by each site, and review of source documents against the eCRFs to assure the adequacy and accuracy of the information that has been recorded, including the verification of any AEs that have occurred.

In the event of the site being notified of a regulatory inspection, the Sponsor will help with the preparation; it is essential that the Sponsor be immediately notified of the inspection.

10. DATA HANDLING AND RECORD KEEPING

10.1 Case Report Forms

An EDC will be used in this study. All data collected during the study will be recorded in individual, patient-specific eCRFs developed for the study. An eCRF must be completed for each patient who signs an informed consent form (ICF). Study sites will maintain a separate source for data entered by the study site into the EDC.

Data managed by a central vendor, or any data for which electronic documentation is provided by the patient, will be stored electronically in the central vendor's database system. Any data from paper source documentation provided by the patient will be documented by each study site in that site's study file.

All data should be entered in the eCRFs within 5 days of the patient visit.

10.2 Source Documentation and Medical/Study Records

The patient ID and date of study start, along with the protocol number, should be recorded in the patient's medical/study records. At minimum, the Investigator or designee should also record confirmation of written and oral consent, the patient's clinical status/disease being treated, dates of study visits, and medications in the medical/study records; copies of all relevant records should be included.

10.3 Access to Source Documentation

The Investigator and staff must agree to allow the Study Monitor and authorized Sponsor representatives to inspect all eCRFs and corresponding source documents (e.g., original medical records, patient records, and laboratory raw data) and to have access to the clinical supplies, as well as dispensing and storage areas. The Investigator and staff must also agree to assist with Study Monitor activities, if requested, and should provide adequate time and space for monitoring visits.

Study patients will have access to safety laboratory assessment results upon request but should be aware that these are typically not immediately available.

10.4 Documentation of Screen Failures

Investigators must account for all patients with a signed ICF. If a patient is found to be ineligible to participate in the study after the Screening Visit, the Investigator will mark the patient as a screen failure and complete the required pages of the eCRF.

Patients may be re-screened after consultation with the Sponsor.

10.5 Protocol Deviations

Protocol deviations will be documented and assessed on a case-by-case basis. Significant protocol deviations must be reported to the IRB/EC according to local regulations.

Any protocol deviations due to COVID-19 will be documented as such (see [Section 3.2](#)).

10.6 Data Generation and Analysis

Data generation and analysis will be specified and detailed in the SAP.

10.7 Retention of Data

The Investigator or designee must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. These documents should be classified into at least 2 separate categories, as follows:

- Investigator study file
- Patient clinical source documents

The Investigator study file will contain the protocol and amendments, eCRFs and query forms, IRB/EC and governmental approval forms with correspondence, informed consent, staff *curricula vitae* and authorization forms, and other appropriate documents and correspondence.

Patient clinical source documents (usually defined by the protocol-specified procedures and data collection requirements in advance to record key efficacy and safety parameters independent of the eCRFs) include, but are not limited to, patient hospital/clinic record, physician and nurse notes, appointment book records, MRI reports, special assessment reports, consultant letters, and screening and enrollment logs.

All clinical study documents must be retained by the Investigator for at least 2 years after the last approval of a marketing application in an ICH region (i.e., US, Europe, or Japan) and until there are no pending or contemplated marketing applications in an ICH region; or, if no application is filed or if the application is not approved for such indication, until 2 years after the investigation is discontinued and regulatory authorities have been notified. Investigators may be required to retain documents longer if required by applicable regulatory requirements, local regulations, or as agreed upon with the Sponsor. The Investigator or designee must contact the Sponsor prior to destroying any records associated with the study. The Sponsor will notify the Investigator when the trial records are no longer needed.

If an Investigator withdraws from the study (e.g., relocates, retires, or dies), the records shall be transferred to a mutually agreed upon designee (e.g., another Investigator or IRB/EC). Notice of such transfer will be given in writing to the Sponsor. If the Investigator cannot guarantee this archiving requirement at the study site for any or all of the documents, special arrangements must be made between the Investigator and the Sponsor to store these documents in sealed containers outside of the site such that these documents can be returned sealed to the Investigator in the event of a regulatory audit. Where source documents are required for the continued care of the patient, appropriate copies should be made for storage outside of the site.

11. ETHICS

11.1 Institutional Review Board (IRB)/Ethics Committee (EC)

The study protocol and amendments, ICFs and other study-relevant forms, information about payments and compensation available to patients, and documentation evidencing the Investigator's qualifications should be submitted by the Investigator to the IRB/EC for review and approval according to local regulations prior to the start of the study. The IRB/EC written approval should identify all documents reviewed by name, version, and the date on which the IRB/EC met and granted the approval.

Any modifications to IRB/EC-approved documents must be submitted to the IRB/EC for approval before implementation.

11.2 Ethical Conduct of the Study

11.2.1 The study will be conducted in accordance with the following:

- The principles of the ICH Guideline E6 for GCP (CPMP/ICH/135/95 Jan 1997)
- European Directive 2001/20/EC
- Standard operating procedures and/or guidelines
- The US FDA regulations
- The Declaration of Helsinki
- All applicable federal, state, and local laws, rules, and regulations relating to the conduct of the study

11.3 Patient Information and Consent

In obtaining and documenting informed consent, the Investigator must comply with the applicable regulatory requirements and adhere to ICH GCP (E6). Each patient must be adequately informed, in a language that they can understand and read, of the aims, methods, anticipated benefits, potential hazards and discomforts, and their right to abstain from participating in the study and withdraw their consent at any time without affecting their medical care. If important new information is incorporated in the ICF and approved by the IRB/EC, all patients actively participating in the study must be re-consented.

Written informed consent from adult patients must be personally signed and dated by the study patient and by the Investigator or designee who conducted the informed consent discussion. The Investigator or designee must provide all enrolled patients with a copy of their signed informed consent. The study Monitor will inspect the original, signed ICFs of all enrolled patients.

11.4 Patient Confidentiality

The Investigators, Sponsor, and its designees will preserve the confidentiality of all patients taking part in the study in accordance with GCP, local regulations and, to the extent applicable, the Health Insurance Portability and Accountability Act of 1996 ("HIPAA"). Subject to the requirement for source data verification by the study personnel by reference to the patient's notes, confidentiality of all patient identities will be maintained. Only the year of birth and study number will be used on the eCRF and in all study correspondence, as permitted. No material bearing a patient's name will be kept on file by the Sponsor.

Data protection and privacy regulations will be observed in capturing, forwarding, processing, and storing patient contact details and data. Patients will be informed accordingly and requested to give their consent on contact details regarding data handling procedures in accordance with national regulations. In order to secure data privacy protection, patient contact details will be sent to a dedicated unit in charge of scheduling and setting up the interviews independently from the study Sponsor, Monitor, data management and data analysis structures, and other study stakeholders.

12. STUDY TERMINATION

Study sites will be closed upon study completion. A trial site is considered closed when all required documents and study supplies have been collected and a study site closure visit has been performed.

The Investigator may initiate a study site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure by the Sponsor or Investigator may include, but are not limited, to the following:

- Failure of the Investigator to comply with the protocol, the requirements of the IRB/EC or local health authorities, the Sponsor's procedures, or GCP guidelines
- Inadequate recruitment of patients by the Investigator
- Discontinuation of further study intervention development
- The Sponsor or designee and/or the IRB/EC deems it necessary to close a study site for medical, safety, regulatory, and/or other reasons consistent with applicable laws, regulations, and GCP.

13. PUBLICATION POLICY

All information contained in this protocol and the study results are considered to be confidential. The Investigator agrees to use this information for the purpose of conducting this study. It is understood that the Sponsor may use data derived from this study for the purpose of research and development. The data may be disclosed by the Sponsor to other Investigators, the FDA, other government agencies or foreign drug regulatory authorities, and the public. No publication of the study design or results is permitted without specific Sponsor approval. To gain approval, a copy of the manuscript for review must be sent to the Sponsor 60 days prior to submission for publication.

It is the intent of the Sponsor to present the results of this study at future scientific meetings and to publish the results in leading scientific journals. The Investigator of each investigative site will be considered for authorship in conjunction with the Sponsor's Investigator(s). The Sponsor will determine additional authors. Presentations and manuscripts will be agreed to by the authors and the Sponsor.

14. REFERENCES

1. Mosser J, Douar AM, Sarde CO, Kioschis P, Feil R, Moser H, et al. Putative X-linked adrenoleukodystrophy gene shares unexpected homology with ABC transporters. *Nature*. 1993;361(6414):726-730.
2. Wiesinger C, Kunze M, Regelsberger G, Forss-Petter S, Berger J. Impaired very long-chain acyl-CoA beta oxidation in human X-linked adrenoleukodystrophy fibroblasts is a direct consequence of ABCD1 transporter dysfunction. *J Biol Chem*. 2013;288(26):19269-19279.
3. Moser HW, Smith KD, Watkins PA, Powers J, Moser AB. X-linked Adrenoleukodystrophy, in: Scriver R, Beaudet AL, Sly WS, Valle D (Eds.), *The Metabolic & Molecular Bases of Inherited Disease*, eighth ed., McGraw-Hill Book Co., New York, 2001, pp. 3257e3301.
4. Casasnovas C, Ruiz M, Schluter A, Naudi A, Fourcade S, Veciana M, et al. Biomarker identification, safety, and efficacy of high-dose antioxidants for adrenomyeloneuropathy: a phase II pilot study. *Neurotherapeutics*. 2019;16(4):1167-1182.
5. Kemp S, Berger J, Aubourg P. X-linked adrenoleukodystrophy: clinical, metabolic, genetic and pathophysiological aspects. *Biochim Biophys Acta*. 2012;1822(9):1465-1474.
6. Berger J, Forss-Petter S, Eichler FS. Pathophysiology of X-linked adrenoleukodystrophy. *Biochimie*. 2014;98(100):135-142.
7. Raymond GV, Moser AB, Fatemi A, Adam MP, Ardinger HH, Pagon RA, et al. X linked adrenoleukodystrophy. In: *Gene Reviews*. Seattle, Washington; c2018.
8. Eichler F, Mahmood A, Loes D, Bezman L, Lin D, Moser HW, et al. Magnetic resonance imaging detection of lesion progression in adult patients with X-linked adrenoleukodystrophy. *Arch Neurol*. 2007;64(5): 659-664.
9. Mingozzi F, High KA. Therapeutic *in vivo* gene transfer for genetic disease using AAV: progress and challenges. *Nat Rev Genet*. 2011;12:341–355.
10. Lykken EA, Shyng C, Edwards RJ, Rozenberg A, Gray SJ. Recent progress and considerations for AAV gene therapies targeting the central nervous system. *J Neurodev Disor*. 2018;10(1):16.
11. Gong Y, Mu D, Prabhakar S, Moser A, Musolino P, Ren JQ, et al. Adenoassociated virus serotype 9-mediated gene therapy for x-linked adrenoleukodystrophy. *Mol Ther*. 2015;23:824-834.
12. Gong Y, Mu D, Ren JQ, Moser A, Maguire CA, Eichler FS. Impact of rAAV9-ABCD1 upon behavioral outcome in a mouse model of X-ALD. *Mol Ther*. 2015;23(S1):S155.
13. Gong Y, Berenson A, Laheji F, Gao G, Wang D, Ng C, et al. Intrathecal adeno-associated viral vector-mediated gene delivery for adrenomyeloneuropathy. *Hum Gene Ther*. 2019;30(5):544-555.
14. Hordeaux J, Buza EL, Dyer C, Goode T, Mitchell TW, Richman L, et al. Adeno-associated virus-induced dorsal root ganglion pathology. *Hum Gene Ther*. 2020;31(15-16):808-818.
15. Perez BA, Shutterly A, Chan YK, Burne BJ, Corti M. Management of neuroinflammatory responses to AAV-mediated gene therapies for neurodegenerative diseases. *Brain Sci*. 2020;10(2):119.
16. Food and Drug Administration (2021, March 16) *Zolgensma*. US Food & Drug Administration. <https://www.fda.gov/vaccines-blood-biologics/zolgensma>
17. Huffnagel IC, van Ballegoij WJC, van Geel BM, Vos JMBW, Kemp S, Engelen M. Progression of myelopathy in males with adrenoleukodystrophy: towards clinical trial readiness. *Brain*. 2019;142(2):334-343.

18. van Ballegoij WJC, van de Stadt SIW, Huffnagel IC, Kemp S, van der Knaap MS, Engelen M. Postural body sway as surrogate outcome for myelopathy in adrenoleukodystrophy. *Front Physiol.* 2020;11:786.
19. Mueller C, Berry JD, McKennaYasek DM, Gernoux G, Owegi MA, Pothier LM, et al. SOD1 suppression with adenoassociated virus and microRNA in familial ALS. *N Engl J Med.* 2020;383(2):151-158.
20. Mendell JR, Sahenk Z, Lehman K, Nease C, Lowes LP, Miller NF, et al. Assessment of systemic delivery of rAAVrh74.MHCK7.microdystrophin in children with Duchenne Muscular Dystrophy: a nonrandomized controlled trial. *JAMA Neurol.* 2020;77(9):1122-1131.
21. Marques A, Almeida S, Carvalho J, Cruz J, Oliveira A, Jácome C. Reliability, validity, and ability to identify fall status of the Balance Evaluation Systems Test, Mini-Balance Evaluation System Test, and BriefBalance Evaluation Systems Test in older people living in the community. *Arch Phys Med Rehabil.* 2016;97(12):2166-2173.
22. Gobi M, Franchignoni F, Caligari M, Giordano A, Turcato AM, Nardone A. Comparison of reliability, validity, and responsiveness of the miniBESTest and Berg Balance Scale in patients with balance disorders. *Phys Ther.* 2013;93(2):158-167.
23. Godbole NP, Sadjadi R, DeBono MA, Grant NR, Kelly DC, James PF, et al. Gait difficulties and postural instability in adrenoleukodystrophy. *Front Neurol.* 2021;12:684102.
24. Martínez-Martín P, Rojo-Abuin JM, Rodríguez-Violante M, Serrano-Duenas M, Garretto N, Martinez-Castillo JC, et al. Analysis of four scales for global severity evaluation in Parkinson's disease. *NPJ Parkinsons Dis.* 2016;2:16007.
25. Goldman MD, Ward MD, Motl RW, Jones DE, Pula JH, Cadavid D. Identification and validation of clinically meaningful benchmarks in the 12-item Multiple Sclerosis Walking Scale. *Mult Scler.* 2017;23(10):1405-1414.
26. Pittock SJ, Mayr WT, McClelland RL, Jorgensen NW, Weigand SD, Noseworthy JH, et al. Quality of life is favorable for most patients with multiple sclerosis: a population-based cohort study. *Arch Neurol.* 2004;61(5):679-686.
27. Moser AB, Jones RO, Hubbard WC, Tortorelli S, Orsini JJ, Caggana M, et al. Newborn screening for X-linked adrenoleukodystrophy. *Int J Neonatal Screen.* 2016;2(4):15.
28. Gisslén M, Price RW, Andreasson U, Norgren N, Nilsson S, Hagberg, et al. Plasma concentration of the neurofilament light protein (NfL) is a biomarker of CNS injury in HIV infection: a cross-sectional study. [published correction appears in *EBioMedicine.* 2016;7:287-288]. *EBioMedicine.* 2015;3:135-140.
29. Kawata K, Liu CY, Merkel SF, Ramirez SH, Tierney RT, Langford D. Blood biomarkers for brain injury: what are we measuring? *Neurosci Biobehav Rev.* 2016;68:460-473.
30. Zeitlberger AM, ThomasBlack G, Garcia-Moreno H, Foiani M, Heslegrave AJ, Zetterberg H, et al. Plasma markers of neurodegeneration are raised in Friedreich's Ataxia. *Front Cell Neurosci.* 2018;12:366.
31. Islas-Hernandez A, Aguilar-Talamantes HS, Bertado-Cortes B, Mejia del Castillo GDJ, Carrera-Pineda R, Cuevas-Garcia CF, et al. BDNF and Tau as biomarkers of severity in multiple sclerosis. *Biomark Med.* 2018;12(7):717-726.
32. Schöll M, Maass A, Mattsson N, Ashton NJ, Blennow K, Zetterberg H, et al. Biomarkers for tau pathology. *Mol Cell Neurosci.* 2019;97:18-33.

33. Weinhofer I, Rommer P, Zierfuss B, Altmann P, Foiani M, Heslegrave A, et al. Neurofilament light chain as a potential biomarker for monitoring neurodegeneration in X-linked adrenoleukodystrophy. *Nat Commun.* 2021;12(1):1816.
34. van Ballegoij WJC, van de Stadt SIW, Huffnagel IC, Kemp S, Willemse EAJ, Teunissen CE, et al. Plasma NfL and GFAP as biomarkers of spinal cord degeneration in adrenoleukodystrophy. *Ann Clin Transl Neurol.* 2020;7(11):2127-2136.
35. Chaudhry V, Moser HW, Cornblath DR. Nerve conduction studies in adrenomyeloneuropathy. *J Neurol Neurosurg Psychiatry.* 1996;61(2):181-185.
36. Politi LS, Castellano A, Papinutto N, Mauro E, Pareyson D, Henry RG, et al. Longitudinal quantitative magnetic resonance imaging in adrenomyeloneuropathy. *Eur J Neurol.* 2019;26(10):1341-1344.
37. Powers JM, Deciero DP, Cox C, Richfield EK, Ito M, Moser AB, et al. The dorsal root ganglia in adrenomyeloneuropathy: neuronal atrophy and abnormal mitochondria. *J Neuropathol Exp Neurol.* 2001;60(5):493-501.
38. Gong Y, Kok R, Mu D, Gao G, Eichler F. Intrathecal delivery of rAAV9-ABCD1 by osmotic pump in a mouse model of adrenomyeloneuropathy. *Mol Ther.* 2016;24:S118-9.
39. Gong Y, Mu D, Ren J, Moser A, Maguire CA, Eichler FS. Impact of rAAV9-ABCD1 upon behavioral outcome in a mouse model of X-ALD. *Mol Ther.* 2015;23(Supplement 1):S155.
40. Höftberger R, Kunze M, Weinhofer I, Aboul-Enein F, Voigtländer T, Oezen I, et al. Distribution and cellular localization of adrenoleukodystrophy protein in human tissues: implications for X-linked adrenoleukodystrophy. *Neurobiol Dis.* 2007;28(2):165-174.
41. Watkiss E, Webb T, Bunday S. Is skewed X inactivation responsible for symptoms in female carriers for adrenoleukodystrophy? *J Med Genet.* 1993;30(8):651-654.
42. Feigenbaum V, Lombard-Platet G, Guidoux S, Sarde CO, Mandel JL, Aubourg P. Mutational and protein analysis of patients and heterozygous women with X-linked adrenoleukodystrophy. *Am J Hum Genet.* 1996;58(6):1135-1144.
43. Johanson CE, Duncan JA 3rd, Klinge PM, Brinker T, Stopa EG, Silverberg GD. Multiplicity of cerebrospinal fluid functions: New challenges in health and disease. *Cerebrospinal Fluid Res.* 2008;5:10.
44. Simon MJ, Iliff JJ. Regulation of cerebrospinal fluid (CSF) flow in neurodegenerative, neurovascular and neuroinflammatory disease. *Biochim Biophys Acta.* 2016;1862(3):442-51.
45. Bourke RS, West CR, Chheda G, Tower DB (1973) Kinetics of entry and distribution of 5-fluorouracil in cerebrospinal fluid and brain following intravenous injection in a primate. *Cancer Res.* 1973;33(7):1735-46.
46. Telano LN, Baker S. Physiology, Cerebral Spinal Fluid. In *StatPearls*. StatPearls Publishing; c2020.
47. Ransohoff RM, Brown MA. Innate immunity in the central nervous system. *J Clin Investig.* 2012;122:1164-1171.
48. Golebiowski D, van der Bom IMJ, Kwon CS, Miller AD, Petrosky K, Bradbury AM, et al. Direct intracranial injection of AAVrh8 encoding monkey-N-acetylhexosaminidase causes neurotoxicity in the primate brain. *Hum Gene Ther.* 2017;28:510-522.
49. Kaplitt MG, Feigin A, Tang C, Fitzsimons HL, Mattis P, Lawlor PA, et al. Safety and tolerability of gene therapy with an adeno-associated virus (AAV) borne GAD gene for Parkinson's disease: an open label, phase I trial. *Lancet.* 2007;369:2097-2105.
50. Leone P, Shera D, McPhee SW, Francis JS, Kolodny EH, Bilaniuk LT, et al. Longterm follow-up after gene therapy for Canavan disease. *Sci Transl Med.* 2012;4(165):165ra163.

51. Worgall S, Sondhi D, Hackett NR, Kosofsky B, Kekatpure MV, Meyzi N, et al. Treatment of late infantile neuronal ceroid lipofuscinosis by CNS administration of a serotype 2 adeno-associated virus expressing CLN2 cDNA. *Hum Gene Ther*. 2008;19:463-474.
52. National Library of Medicine [Internet]. Maryland: US National Library of Medicine; c2021. A phase I study of intrathecal administration of scAAV9/JeT-GAN for the treatment of giant axonal neuropathy. Identifier NCT02362438. 2015 Apr 24 [cited 2021 Aug 10]. Available from: <https://clinicaltrials.gov/ct2/show/NCT02362438>
53. Butland RJ, Pang J, Gross ER, Woodcock AA, Geddes DM. Two-, six-, and 12-minute walking tests in respiratory disease. *Br Med J*. 1982;284(6329):1607-1608.
54. ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories. ATS statement: guidelines for the six-minute walk test. *Am J Respir Crit Care Med*. 2002;166(1):111-117. Erratum in: *Am J Respir Crit Care Med*. 2016;193(10):1185.
55. Whitney SL, Wrisley DM, Marchetti GF, Gee MA, Redfern MS, Furman JM. Clinical measurement of sit-to-stand performance in people with balance disorders: validity of data for the Five-Times-Sit-to-Stand Test. *Phys Ther*. 2005;85(10):1034-1045.
56. Bohannon RW. Test-retest reliability of the five-repetition sit-to-stand test: a systemic review of the literature involving adults. *J Strength Cond Res*. 2011;25(11):3205-3207.
57. Lord SR, Murray SM, Chapman K, Munro B, Tiedermann A. Sit-to-stand performance depends on sensation, speed, balance, and psychological status in addition to strength in older people. *J Gerontol A Biol Sci Med Sci*. 2002;57(8):M539-M543.
58. Buatois S, Perret-Guillaume C, Gueguen R, Miget P, Vancon G, Perrin P, et al. A simple clinical scale to stratify risk of recurrent falls in community-dwelling adults aged 65 years and older. *Phys Ther*. 2010;90(4):550-560.
59. Podsiadlo D, Richardson S. The timed "Up & Go": a test of basic functional mobility for frail elderly persons. *J Am Geriatr Soc*. 1991;39(2):142-148.
60. Franchignoni F, Horak F, Godi M, Nardone A, Giordano A. Using psychometric techniques to improve the Balance Evaluation Systems Test: the mini-BESTest. *J Rehabil Med*. 2010;42(4):323-31.
61. Bohannon RW. Reference values for the timed up and go test: a descriptive metaanalysis. *J Geriatr Phys Ther*. 2006;29(2):64-68.
62. Lima LCA, Ansai JH, Andrade L, Takahashi ACM. The relationship between dual-task and cognitive performance among elderly participants who exercise regularly. *Braz J Phys Ther*. 2015;19(2):159-166.
63. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology*. 1983 Nov;33(11):1444-52.
64. Castilhos RM, Blank D, Netto CBO, Souza CFM, Fernandes LNT, Schwartz IVD, et al. Severity score system for progressive myelopathy: development and validation of a new clinical scale. *Braz J Med Biol Res*. 2012;45(7):565-572.
65. Guy W, editor. ECDEU Assessment Manual for Psychopharmacology. Rockville, MD: US Department of Health, Education, and Welfare Public Health Service Alcohol, Drug Abuse, and Mental Health Administration; 1976.
66. Busner J, Targum SD. The clinical global impressions scale: applying a research tool in clinical practice. *Psychiatry (Edmont)*. 2007;4(7):28-37.
67. ePROVIDE [Internet]. Lyon: Mapi Research Trust; c2021. Patient Global Impressions scale – change, improvement, severity (PGI-C, PGI-I, PGI-S); 2021 Jun [cited 2021 Aug 26].

Available from: <https://eprovide.mapi-trust.org/instruments/patient-global-impressions-scale-change-improvement-severity>

68. Hobart JC, Riazi A, Lamping DL, Fitzpatrick R, Thompson AJ. Measuring the impact of MS on walking ability: the 12-Item MS Walking Scale (MSWS-12). *Neurology*. 2003;60(1):31-36.
69. Vickrey BG, Hays RD, Harooni R, Myers LW, Ellison GW. A health-related quality of life measure for multiple sclerosis. *Qual Life Res*. 1995;4(3):187-206.

15. APPENDICES

15.1 Appendix 1

Prophylaxis of Immune Response

All patients will receive an immunosuppressive regimen to prevent the potential development of an immune response against AAV9 and the *hABCD1* gene and to suppress apparent immune-mediated hepatitis and/or other toxicities (see [Section 3.3.5](#); Rationale for Immunosuppression).

Immunosuppression Induction

Starting on Day -1 (Visit 2), patients will receive an immunosuppressive regimen for until Day 90 at a minimum. All patients, including control patients, will be administered 1 g methylprednisolone via intravenous (IV) infusion over the span of 2 ($\pm$ 1) hours on Day -1 (Visit 2), Day 1 (Visit 3), and Day 2 (Visit 4).

Patients should discontinue existing corticosteroid replacement therapy for adrenal insufficiency (Addison's disease).

Immunosuppression Maintenance

Patients will receive oral prednisone/prednisolone at a dose of 0.5 mg/kg/day, starting on Day 3 until Day 90, this can be extended as needed per clinical judgement. If clinically indicated (i.e., based on clinical symptoms, liver enzyme elevations or worsening of neurological/spinal cord function), prednisone/prednisolone dose and duration can be modified based on discussions between the Investigator and the Medical Monitor.

If clinically indicated (i.e., based on clinical symptoms, liver enzyme elevations or worsening of neurological/spinal cord function), prednisone/prednisolone can be reinitiated, or dose can be increased based on discussions between the Investigator and Medical Monitor. Additional immunosuppressive agents together with or in lieu of prednisone/prednisolone may also be used following agreement by the Investigator and Medical Monitor.

At the Day 90 Clinic Visit (or next Clinic Visit)

A standard clinical tapering regimen will be initiated after the patient is clinically evaluated (Brief Physical and Neurological examination, at a minimum) by the Investigator at Day 90 and considered stable to discontinue prednisone/prednisolone treatment or to reduce the dose to a level necessary to treat underlying adrenal insufficiency (Addison's disease). If the patient is not considered clinically able to initiate the tapering regimen, the evaluation will be repeated at the next planned Clinic Visit or an Unscheduled Visit at the Clinic. LFTs will also be monitored biweekly until 4 weeks after the prednisone/prednisolone dose has been tapered down to a daily dose of 10 mg.

During the taper, the Investigator should monitor patients for potential corticosteroid withdrawal symptoms, worsening neurological symptoms, or appearance of an immune response; an individualized approach may be needed for each patient. The Investigator should also ask patients to report signs and symptoms of adrenal insufficiency.

Prednisone/prednisolone will be tapered at weekly 5 mg decrements until the patient is on a dose of 20 mg/day. Thereafter, a much slower tapering schedule of weekly 2.5 mg decrements will be

needed, until the patient is on a dose of 10 mg/day. If the patient exhibits withdrawal symptoms, bimonthly (i.e., every 2 weeks) decrements should be considered. Once the patient has reached a dose of 10 mg/day, the dose of prednisone/prednisolone should be reduced or discontinued per standard of care and/or local guidelines for the management of corticosteroids appropriate for patients' pre-existing disease.

An example of a prednisone/prednisolone tapering schedule for an 80 kg patient on a daily dose of 40 mg is presented in Table A1.

Table A1 Prednisone/Prednisolone Tapering Schedule for a Patient on a Daily Dose of 40 mg

Tapering Week	Daily Dose	Dose Decrement	Monitoring Instructions
Week 0	40 mg	-	
Week 1	35 mg	5 mg/week	
Week 2	30 mg	5 mg/week	
Week 3	25 mg	5 mg/week	
Week 4	20 mg	5 mg/week	
Week 5	17.5 mg	2.5 mg/week	If withdrawal syndrome is observed, consider bimonthly decrements.
Week 6	15 mg	2.5 mg/week	If withdrawal syndrome is observed, consider bimonthly decrements.
Week 7	12.5 mg	2.5 mg/week	If withdrawal syndrome is observed, consider bimonthly decrements.
Week 8	10 mg	Per standard of care and/or local guidelines	

Once prednisone/prednisolone has been discontinued or tapered down to a dose <10 mg/day, biweekly chemistry laboratory assessments (including ALT, AST, GGT, CPK and troponins) and phone calls to the patients ([Section 6.17](#)) will be conducted twice to ensure stability of these analytes.

The final prednisone/prednisolone dose should be ascertained per standard of care or according to local guidelines for the management of corticosteroids appropriate for the patient's preexisting disease.

For patients who display any of the following at the Day 90 Clinic Visit, corticosteroid management decisions will be made based on discussions between the Investigator and Medical Monitor, and tapering will be postponed till the next planned Clinic Visit or an Unscheduled Visit at the Clinic:

- Elevation in hepatic transaminases (ALT and/or AST) by $\geq 1.5 \times$ baseline values
- Successive increases in hepatic transaminases (ALT and/or AST) during follow-up compared to baseline
- Any worsening or changes in neurological symptoms (including spinal cord function/DRG toxicities).

Based on guidelines published by the American Association for the Study of Liver Diseases (AASLD), and in the absence of an alternative etiology, treatment for vector-induced hepatitis will be instituted. This guidance can be used for other potential immune responses as shown in **Table A2**.

Table A2 Prednisone/Prednisolone Regimen for Treatment of Vector-induced Hepatitis

Schedule	Daily Dose
Prednisone/Prednisolone Regimen and Tapering	
¹ Regimen Week(s)	60 mg
Tapering Week 1	40 mg
Tapering Week 2	30 mg
Tapering Week 3	20 mg
Tapering Week 4	15 mg
Tapering Week 5	10 mg
Tapering Week 6 onward	Reduce the dose or discontinue per standard of care and/or local guidelines for the management of corticosteroids appropriate for patients' pre-existing disease.
Abbreviations: ELISpot = Enzyme-linked immunosorbent spot	
¹ Maintain dose level until transaminases and ELISpot results return to baseline levels	

If the initial corticosteroid regimen outlined in **Table A2** is insufficient to resolve the elevation of hepatic transaminases or suspected immune response, additional immunosuppressive regimens should be considered on a patient-by-patient basis based on discussions between the Investigator and Medical Monitor.

Additional immunosuppressive agents together with or in lieu of prednisone/prednisolone may also be used following agreement by the Investigator and Medical Monitor.

While on immunomodulatory regimens, all patients will be closely monitored for AEs, including opportunistic infections. Antibiotics and/or other medications to minimize the risk of opportunistic infections may be prescribed at the discretion of the Investigator. All events related to the use of immunomodulatory drugs (e.g., hyperglycemia, weight gain, infections, worsening or changes in neurological symptoms) will be recorded as AEs connected to their use.

15.2 Appendix 2

Sirolimus Treatment: Dose and Monitoring of Trough Levels

Some patients may develop cellular immune responses to AAV9 and/or the *hABCD1* gene while being treated at a regular dose of prednisone/prednisolone, as indicated by clinical signs and symptoms (e.g., rises in liver enzyme levels, reductions in nerve conduction studies, worsening of neuropathy) and/or T-cell reactivity as measured by ELISpot.

University of Massachusetts Chan Medical School only: Assessment of T-cell reactivity will be performed with ELISpot and data listings of ELISpot results by patient and by visit will be generated. These listings will include positive and negative results and the number of SFU per 1.0×10^6 PBMC for each peptide pool and control (positive and negative) stimulation. Positive and negative results with SFU/ 1.0×10^6 PBMC will be evaluated for correlations with changes in clinical chemistry laboratory assessments or AEs, as appropriate for the data collected.]

Sirolimus may be orally administered at a starting daily dose of 2 mg for patients ≥ 40 kg and 1 mg/m² for patients < 40 kg. A loading dose of approximately 6 mg can be used at the discretion of the Investigator and in consultation with the Medical Monitor.

Sirolimus trough concentrations will be maintained within 3-10 ng/mL. To this end, trough levels will be measured 7 (+/- 2) days after administration of the first dose of sirolimus and the daily dose will be adjusted, if needed. Thereafter, trough level testing and further dose adjustments, if needed, will be conducted every 7 (+/-2) days for the first 30 days, and then every 14 (+/-2) days until termination of therapy.

While on immunomodulatory regimens, all patients will be closely monitored for AEs, including opportunistic infections. Antibiotics and/or other medications to minimize the risk of opportunistic infections, and vaccinations may be prescribed at the discretion of the Investigator.

All events related to the use of immunomodulatory drugs (e.g., hyperglycemia, weight gain, infections, worsening or changes in neurological symptoms) will be recorded as AEs.