Study Title:	**ASPIRO: A Phase 1/2/3, Randomized, Open-Label, Ascending-Dose, Delayed-Treatment Concurrent Control Clinical Study to Evaluate the Safety and Efficacy of AT132, an AAV8-Delivered Gene Therapy in X-Linked Myotubular Myopathy (XLMTM) Patients**
Protocol Number:	ATX-MTM-002
Investigational Product:	AT132 (resamirigene bilparvovec)
Indication:	X-Linked Myotubular Myopathy
Sponsor:	Astellas Gene Therapies, Inc. South San Francisco, CA 94080
Development Phase:	1/2/3
Responsible Medical Officer:	*PPD*
EU CT Number:	2024-512637-32
IND Number:	017367
Protocol Version:	12.0
Date:	17 May 2024
Replaces Protocol Version:	11.0 (16 May 2023)

PROTOCOL SYNOPSIS

TITLE OF STUDY: ASPIRO: A Phase 1/2/3, Randomized, Open-Label, Ascending-Dose, Delayed-Treatment Concurrent Control Clinical Study to Evaluate the Safety and Efficacy of AT132, an AAV8-Delivered Gene Therapy in X-Linked Myotubular Myopathy (XLMTM) Patients

NAME OF TEST PRODUCT: AT132 (resamirigene bilparvovec)

NAME OF ACTIVE INGREDIENT: rAAV8-Des-hMTM1

PROTOCOL NUMBER: ATX-MTM-002

STUDY SITES PLANNED: Approximately 8 sites worldwide

PHASE OF DEVELOPMENT: Phase 1/2/3

STUDY RATIONALE:

Adeno-associated virus serotype 8 (AAV8)-mediated gene therapy offers the prospect of considerable long-term clinical benefit to X-linked myotubular myopathy (XLMTM) patients through persistent expression of the missing or damaged myotubularin protein following a single systemic therapeutic administration. Gene therapy is expected to provide persistent protein expression, corresponding long-term clinical benefit, and the potential for substantial recovery of muscle function. Substantial functional recovery and prolongation of life in both the murine (knockout) and canine (missense mutation) models of XLMTM disease, employing the same AAV8 vector, support this therapeutic strategy.

This Phase 1/2/3 study will evaluate safety and efficacy of AT132. The rarity of this disease (estimated incidence of 1 in 50,000 live male births) presents challenges to enrolling the study in a timely fashion. Enrollment of infants and young children into this first-in-human study exploring the safety of AT132 in XLMTM is justified for a number of reasons. Firstly, there are not enough adult or adolescent patients to enable a clinical study in the older population due to the rarity and early mortality of the disease. Death rates stay high throughout childhood and the majority of children do not survive into adolescence and adulthood. Therefore, there is a clear, unmet medical need in neonates and infants at high risk of death in the neonatal and infantile period. In addition, infants and younger children have fewer comorbidities, less accumulation of medical events (such as hypoxic damage and recurrent chest infections), and are not as debilitated as the few surviving patients with XLMTM, which increases the possibility of demonstrating clinical benefit. Furthermore, preexisting antibodies to adeno-associated viruses (AAVs), which could preclude administration of an AAV8 gene transfer therapy, are less prevalent in younger children compared with adults.

OBJECTIVES:

The objectives of the study are as follows:

- To determine the therapeutic dose of AT132
- To confirm the safety and efficacy of the therapeutic dose of AT132

ENDPOINTS, SAFETY ASSESSMENTS, AND ESTIMANDS:

Primary Efficacy Endpoint:

- Change from baseline in hours of ventilation support at Week 24

Key Secondary Efficacy Endpoint:

- Percentage of subjects achieving functionally independent sitting for at least 30 seconds by Week 24

Other Secondary Efficacy Endpoints:

- Time to reduction in required ventilator support to $\leq$ 16 hours a day (only in subjects who require invasive ventilation) at Week 24

- Change from baseline in Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP INTEND) at Week 24
- Change from baseline in maximal inspiratory pressure (MIP) at Week 24
- Change from baseline in quantitative analysis of myotubularin expression in the muscle biopsy at Week 24
- Change from baseline in quality of life assessments at Week 24 (ie, the Assessment of Caregiver Experience with Neuromuscular Disease [ACEND] and Pediatric Quality of Life Inventory [PedsQL])
- Number (%) of age-appropriate clinically relevant gross motor function milestones attained through Week 24
- Percentage of subjects achieving full ventilator independence at Week 24
- Survival

Safety Endpoints:

- Adverse events (AEs), serious AEs (SAEs), and findings from safety laboratory tests, 12-lead electrocardiogram (ECG), echocardiograms (ECHOs), vital signs, growth parameters, physical examinations, liver ultrasounds, antibody formation (anti-AAV8, anti-MTM1), viral shedding, annualized hospitalization rate, annualized respiratory and non-respiratory SAE rate, and length of stay per hospitalization

Exploratory Endpoints:

- Time to unassisted sitting for 30 seconds or more at Week 24
- Change from baseline in the Motor Function Measure scale (MFM-32) at Week 24
- Change from baseline in total raw score in the gross motor domain of the Bayley Scales of Infant and Toddler Development III (Bayley-III) at Week 24
- Change from baseline in total raw score in the fine motor domain of the Bayley-III at Week 24
- Change from baseline in the proportion of patients being able to feed without a gastrostomy or G-tube at Week 24
- Change from baseline in the Communicative Development Inventories scores at Week 24
- Change from baseline in the Parental Global Impression of Secretion Severity (PGIS-S) score at Week 24
- Change in the Parental Global Impression of Secretion Improvement (PGIS-I) score at Week 24
- Change from baseline in Clinical Global Impression of Severity (CGI-S) at Week 24
- Change in the Clinical Global Impression of Improvement (CGI-I) score at Week 24

Estimands:

The estimand of the primary objective is defined by the following 5 attributes:

- Treatment: Single dose of AT132
- Population: Male subjects with ventilator-dependent XLMTM, as defined by the inclusion/exclusion criteria of the study
- Endpoint: Change from baseline in hours per day of ventilation support at Week 24
- Intercurrent events and their corresponding strategies: For study discontinuation due to death or due to lack of efficacy, a composite strategy will be adopted, where subjects with the intercurrent event before Week 24 having missing observation(s) after the intercurrent event will have those missing observations(s) imputed by unfavorable values
- Population level summary: Mean change from baseline, by dose level

NUMBER OF SUBJECTS PLANNED:

Part 1: Approximately 16 subjects

Part 2: Approximately 10 subjects

DIAGNOSIS AND CRITERIA FOR INCLUSION AND EXCLUSION:

INCLUSION CRITERIA:

1. Subject has a diagnosis of XLMTM resulting from a genetically confirmed mutation in the MTM1 gene as assessed by a Sponsor-approved testing facility.*
2. Subject is male.*
3. Subject is aged less than 5 years old at dosing.*
4. Subject requires mechanical ventilatory support:
   - Part 1: Subject requires some mechanical ventilatory support (eg, ranging from 24 hours per day full time mechanical ventilation, to noninvasive support such as continuous positive airway pressure [CPAP] or bilevel positive airway pressure [BiPAP] during sleeping hours).
   - Part 2: Subject requires invasive mechanical ventilatory support ranging from 20 to 24 hours per day at screening (confirmed by daytime polysomnographic study).
5. Subject requiring invasive mechanical ventilator support is fitted with or willing to be fitted with a cuffed tracheostomy tube for some respiratory assessments.*
6. Subject has ventilator maximum positive end-expiratory pressure (PEEP) < 8 cmH_2O at screening.*
7. Signed informed consent by the parent(s) or legally authorized representative(s) (LAR) (when applicable).*
8. Subject and parent(s)/LAR(s) are willing and able to comply with study visits and study procedures.*
9. UNIQUE to France: Subject's weight is ≥ 4.8 kg.

EXCLUSION CRITERIA:

1. Subject is participating in an interventional study designed to treat XLMTM.*
2. Subject born < 35 weeks gestation who is still not term as per corrected age.
3. Subject tests positive for AAV8 neutralizing antibody with titers > 1:20 (subjects under the age of 18 months may be retested in cases where antibodies may have been maternally acquired and titers may decline in the first months of life).*
4. Subject had recent surgery (< 3 months before Day 1) or has planned surgery that may confound data collection during the first 48 weeks of the study.
5. Subject has a clinically important condition or life-threatening disease, other than XLMTM, in the opinion of the Investigator.*
6. Subject has a clinically significant underlying liver disease, defined as:
   - ≥ Grade 3 aspartate aminotransferase (AST) (> 5.0 x upper limit of normal [ULN]; Common Terminology Criteria for Adverse Events [CTCAE] v. 4.03)*
   - ≥ Grade 3 alanine aminotransferase (ALT) (> 5.0 x ULN; CTCAE v. 4.03)*
   - Hepatic peliosis or any other clinically significant structural abnormality detected by ultrasound*
7. Subject is currently experiencing a clinically important respiratory infection or other active infection.*
8. Subject has received pyridostigmine or any medication to treat XLMTM within 3 months before Day 1.*
9. Other than as required per protocol, subject has received immune-modulating agents within 3 months before Day 1 (use of inhaled corticosteroids to manage chronic respiratory conditions is allowed); use of other concomitant medications to manage chronic conditions must have been stable for at least 4 weeks before dosing.*

10. Subject has a contraindication to prednisolone.*
11. Subject has a contraindication to study drug or ingredients.*
12. Subject has previous scoliosis repair surgery/procedure, or planned/expected scoliosis repair surgery/procedure in the 12 months following Day 1 (Part 2 including any subjects enrolled under protocol v8 and beyond).
13. Subject has contractures, scoliosis, or other medical condition that would limit the potential to achieve unassisted sitting, in the opinion of the Investigator (Part 2 including any subjects enrolled under protocol v8 and beyond).
14. Subject is able to sit without assistance for at least 30 seconds at screening, in the opinion of the Investigator (Part 2 including any subjects enrolled under protocol v8 and beyond).
15. Subject has a clinically important condition, including CTCAE v4.03 Grade $\geq$ 2 anemia (< 10 g/dL hemoglobin).*
16. Subject has a contraindication to ursodiol (ursodeoxycholic acid).*
17. UNIQUE to France: Subject has a prior diagnosis or history of cardiac arrhythmias, myocarditis, or any other cardiac disease.
18. UNIQUE to France: Subject has a contraindication to general anesthesia and to muscle biopsy procedures.

* If a subject is a delayed-treatment control, this inclusion/exclusion criterion must be met before receiving AT132.

STUDY TREATMENT, DOSE, ROUTE, AND REGIMEN:

Part 1: All study subjects will be administered a single dose of AT132 by intravenous (IV) infusion (control subjects will receive delayed treatment). The first dose level will be 1.0×10^{14} vg/kg, with planned subsequent dose levels of 3.0×10^{14} vg/kg and 5.0×10^{14} vg/kg. A maximum of 3 dose levels of AT132 will be evaluated in Part 1 of the study. Dose escalations or expansions will be based on evaluations of safety data and recommendations from the Data Monitoring Committee (DMC). As implemented in protocol v5, the DMC and Sponsor determined that 3.0×10^{14} vg/kg would be evaluated in Part 2 and escalation to the 5.0×10^{14} vg/kg dose level would not occur.

Part 2 (prior to protocol v8): Subjects were randomized to receive a single dose of the optimal dose (as previously determined at the end of Part 1) of 3.0×10^{14} vg/kg AT132 by IV infusion or to receive delayed treatment following the Week 24 visit.

As of protocol v9: Following the review of benefit/risk profiles of the 1.0×10^{14} and 3.0×10^{14} vg/kg dose levels, the Sponsor, in consultation with the DMC, determined that subjects not yet dosed were to be administered the lower dose level (ie, the therapeutic dose), which is 1.3×10^{14} vg/kg AT132 as determined by the 2nd generation vg titer assay (see Section 1.4.2).

As of protocol v10 and beyond, no additional subjects will be dosed.

DURATION OF STUDY:

Approximately 10 years after AT132 administration; approximately 12 years for control subjects who are ultimately administered AT132 (at least 24 weeks to 3 years delayed treatment and an additional 10 years after AT132 administration).

REFERENCE THERAPY, DOSE, ROUTE, AND REGIMEN: None.

STUDY DESIGN:

This is a Phase 1/2/3, randomized, open-label, ascending-dose, delayed-treatment concurrent control clinical study to evaluate the safety and efficacy of AAV8-delivered gene therapy in XLMTM patients aged less than 5 years old. Subjects will receive a single dose of AT132 and be followed for safety and efficacy for 10 years, with the primary analysis occurring after all subjects have completed 24 weeks of posttreatment evaluation.

The study consists of 2 parts. Part 1 would establish the optimal dose of AT132. Part 2 would confirm the safety and efficacy of AT132 at the optimal dose level. The following describes how each part was designed and the changes as of protocol v8 and beyond.

Part 1 (fully dosed as of protocol v8):

A maximum of 3 dose levels of AT132 are planned for evaluation in Part 1 of this study (Figure 1). Four subjects will be enrolled in each dose level cohort, including 1 subject in each dose level cohort randomized to control with delayed administration of treatment. The first subject in each dose level cohort will be assigned to receive AT132 and will be treated as a sentinel subject. Subsequent subjects in that dose level cohort will be randomized (2:1) to treatment or control with delayed treatment if there are no safety concerns after at least 4 weeks of post-dose data from the sentinel subject is evaluated by the chair of the DMC. Dose escalation to the next dose level will be considered after evaluation of at least 4 weeks of data from all subjects dosed at the current dose level. Following the dose escalation portion of the study, an optimal dose will be determined in conjunction with the DMC, and control subjects will be treated at the optimal dose level.

This study's independent DMC will monitor subject safety and provide recommendations to the Sponsor regarding dose level determination, cohort expansion, and safety and study conduct matters.

Subjects who are randomized to receive AT132 may be admitted to the study clinic 1 to 2 days prior to AT132 dosing for baseline procedures, including confirmation of baseline ventilator settings, and will stay in the study clinic for at least the first overnight post-dose. If medically appropriate, subjects may be discharged from the study clinic after completion of Day 2 assessments.

Control subjects will generally have the same assessments as treated subjects but on a less frequent schedule to lessen the burden of study participation. It is anticipated that control subjects will participate for at least 24 weeks before an optimal dose is determined and the subject can be administered AT132. Following determination of the optimal dose, control subjects will undergo pretreatment baseline procedures to confirm that they remain eligible to receive treatment with AT132 at the optimal dose. Once eligible, control subjects will be dosed with AT132, and will initiate the post-dose procedures.

Treated subjects will be administered a course of concomitant prophylactic glucocorticoid (oral prednisolone) therapy commencing 1 day prior to AT132 dosing, and continuing for a period of approximately 8 weeks, then tapering from the original dose over an additional 8 weeks, per Investigator discretion, as a preventative measure for immune-mediated hepatic injury, which has been observed in gene therapy clinical studies with AAV. Tapering duration may be altered and/or supplemental administration of IV steroids (eg, methylprednisolone) or other immunosuppressive regimens may be considered in cases of potential malabsorption of oral medications.

Subjects will be followed for a total of 10 years following AT132 administration, see Section 5.2. See Figure 1 for the individual study participation timeline (Parts 1 & 2) through Week 48.

Part 2 (described herein as planned prior to protocol v8; partially dosed as of protocol v8):

In Part 2, the optimal dose of AT132 will be evaluated in an expansion cohort of 10 subjects age matched randomized to a study drug or a delayed-treatment control group (1:1 allocation ratio). A pair of subjects in each group will be prospectively best-matched based on age (±6 months) before being randomized to 1 of the treatment arms (AT132 vs. delayed-treatment control). The administration of study drug and concomitant prophylactic glucocorticoids, and the assessment of safety will be the same as described in Part 1. Following collection of their Week 24 data, control subjects will be administered AT132 and followed according to the Schedule of Events.

Part 2 as of protocol v8 and v9:

Following the review of benefit/risk profiles of the 1.0×10^{14} vg/kg and 3.0×10^{14} vg/kg dose levels, the Sponsor, in consultation with the DMC, determined that subjects not yet dosed were to be administered the lower dose level (ie, the therapeutic dose), which is 1.3×10^{14} vg/kg as determined by the 2nd generation vg titer assay (see Section 1.4.2). Dosing of the 3 enrolled delayed-treatment controls who have not yet been dosed will not be conducted as described above for Part 2, but will resume at

1.3×10^{14} vg/kg as long as each subject meets a subset of inclusion and exclusion criteria prior to dosing. If any of these control subjects is not eligible to be dosed, another eligible subject can be enrolled. Any new subject will not be considered a delayed-treatment control and therefore will not be required to wait 24 weeks before administration of study drug. Any new subject will be enrolled under all of the inclusion/exclusion criteria (including Exclusion Criteria 12-14 required for Part 2 subjects). In addition to prophylactic glucocorticoids, subjects will receive daily prophylactic ursodiol (ursodeoxycholic acid) beginning between Study Days -6 and -4. Subjects will be followed according to the Schedule of Events. **As of protocol v10 and beyond:** This study does not allow for enrollment or dosing of any future subjects. See Figure 2 for Overall Study Design.
DATA MONITORING COMMITTEE: This study will utilize an independent DMC comprising at least 3 experts with relevant expertise who will monitor subject safety and key efficacy data and provide recommendations to the Sponsor regarding expansion of dose cohorts and dose escalation or decreases. The DMC may recommend accelerated dosing of control subjects if compelling efficacy results support such action. DMC review will occur, at minimum, at the following events: • A sentinel subject has been dosed and observed for at least 4 weeks in each of the cohorts (DMC chair review) • Consideration of a dose escalation after at least 3 subjects at a dose level have been dosed and observed for at least 4 weeks following AT132 administration (full DMC review) • Dose escalation has been stopped and participating subjects have 24 weeks of post-dose follow up, at which point it is anticipated that an optimal dose can be selected (full DMC review; applicable to Part 1 of study) • Consideration of expanding enrollment at a specific dose level (full DMC review) • Review of important medical events In addition, the DMC will perform ad hoc review if requested by the Sponsor, or if stopping criteria are met. Full details of the DMC will be provided in the ATX-MTM-002 DMC charter.
DATA REVIEW COMMITTEES: There will be the following independent expert committee adjudicating endpoints and reviewing data in the study: Biopsy Review Committee • Responsible for the blinded adjudication of histopathology endpoints in muscle biopsies.
STATISTICAL METHODS: The study is no longer able to address the stated objectives, hence the statistical analysis will provide descriptive summaries by treatment groups. Data will be summarized for the following 3 treatment groups: (i) subjects treated at 1.3×10^{14} vg/kg (low dose), (ii) subjects treated at 3.5×10^{14} vg/kg (high dose), and (iii) all subjects treated with AT132 (overall; not applicable to efficacy endpoints). As of protocol v9 and beyond: The 1.0×10^{14} vg/kg dose level based on a 1st generation titer method equates to 1.3×10^{14} vg/kg as determined by a 2nd generation vg titer assay (see Section 1.4.2); and 3.0×10^{14} vg/kg dose level equates to 3.5×10^{14} vg/kg based on an analysis of the historical Study ATX-MTM-002 drug product lots using the 2nd generation vg titer assay. Number and percentage of subjects with treatment-emergent AEs (TEAEs) classified by system organ class (SOC) and preferred term (PT); by severity, causality, seriousness, and action taken with regard to study drug will be summarized. Number (%) of subjects with TEAEs leading to discontinuation of study will also be summarized. Clinical laboratory test results, vital signs, growth parameters, and ECG

findings will be summarized using descriptive statistics by time point. ECHO findings, liver ultrasounds, antibody formation (anti-AAV8, anti-MTM1), and viral shedding will be summarized.

Graphical profiles of each subject will be created for key efficacy measurements.

The primary efficacy endpoint (change from baseline in hours of ventilation support at Week 24) will be analyzed using a mixed effect model repeat measurement (MMRM) with baseline, treatment (low dose and high dose), time (Weeks 1, 4, 8, 12, 16, 24, 36 and 48) and treatment by time interactions as fixed effects and subject as random effect. The change from baseline in hours of ventilation support at Week 24 will be summarized by treatment group based on this model. The primary analysis will be conducted using the FAS.

The key secondary efficacy endpoint (percentage of subjects achieving functionally independent sitting for at least 30 seconds by Week 24) will be summarized by treatment group (low dose and high dose) using the FAS.

Other secondary or exploratory efficacy endpoints analyses of change from baseline, time to event and percentage variables will be summarized for low dose and high dose in a similar method to the analyses described above.

Details of the analysis methods will be documented in the Statistical Analysis Plan (SAP).

Analysis Population:

- Full Analysis Set (FAS) is defined as all randomized and/or enrolled subjects who received AT132 and had at least 1 postdose efficacy assessment. The FAS will be used for the primary analysis of the primary efficacy endpoint.
- Safety Analysis Set (SAF) is defined as all randomized and/or enrolled subjects who received AT132. The SAF will be used for the analysis of the safety endpoints.

Power Analysis: The following language was from protocol v9 that planned a formal analysis to compare 1.3×10^{14} vg/kg and delayed-treatment control, however given that the study will not be able to address the objectives, no such formal analysis will be conducted.

A power analysis for the primary analysis of the primary endpoint was conducted at 80% power and 0.05 level of alpha to estimate the required sample size based on Mean of 13.0 vs. 0.0 hours of reduction in ventilation need, with common SD of 6.0 by Week 24 using a t-test, resulting in at least N = 10 for a balanced 1:1 allocation between 1.0×10^{14} vg/kg AT132 (equates to 1.3×10^{14} vg/kg as determined by the 2nd generation vg titer assay) and delayed-treatment control. It is further assumed, that the same difference in means (SD), will provide at least 80% power using MMRM analysis proposed for the primary endpoint. However, it is possible that 1:1 allocation may not be feasible; 1 control subject serving as control for multiple treated subjects, or multiple control subjects being a match for a single treated subject. Therefore, all qualified control subjects will be used in the analyses to ensure adequate power is available to detect the intended difference between treated and control subjects.

STUDY SCHEMA

Figure 1: Summary of Individual Study Participation Timeline (Parts 1 & 2)

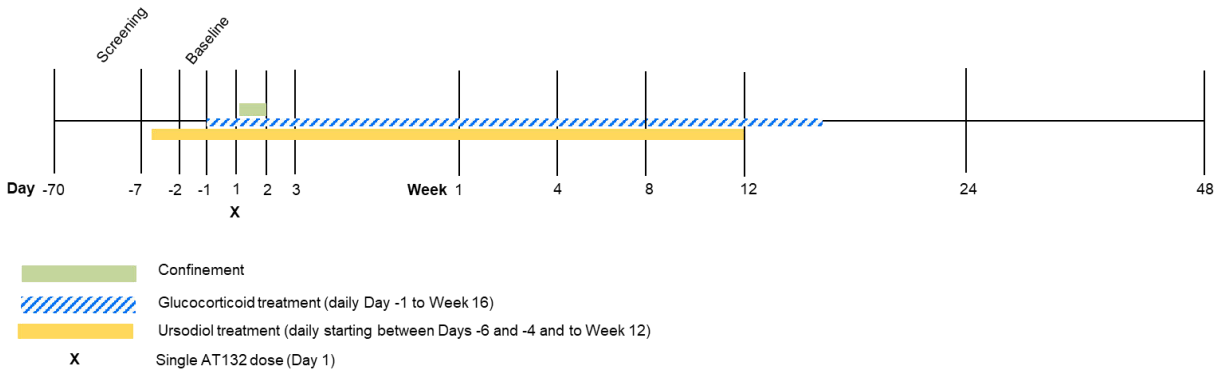
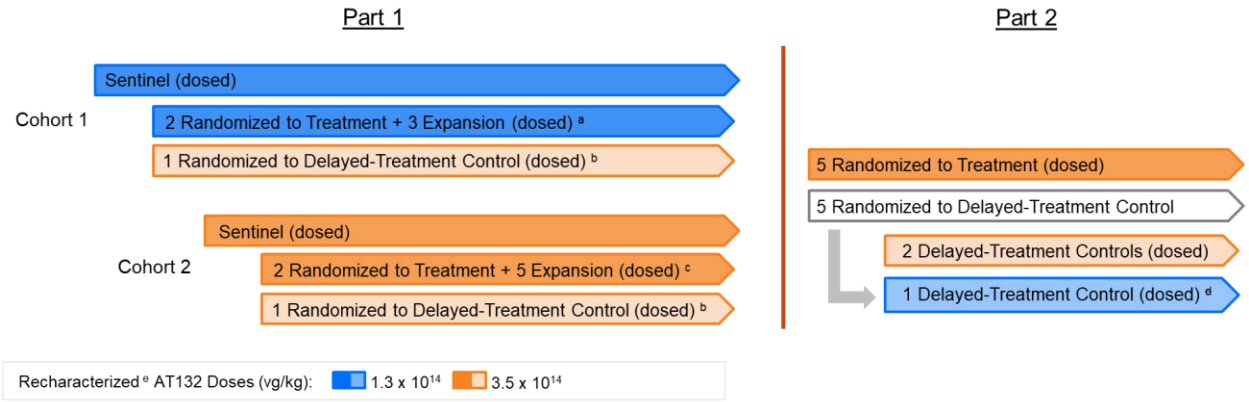


Figure 2: Overall Study Design



Notes: Delayed-treatment control subjects to be treated with AT132 after Week 24. As reflected in protocol v5, the Data Monitoring Committee (DMC) and Sponsor determined the therapeutic dose to be 3.0×10^{14} vg/kg and thus, dose escalation to the third originally planned 5.0×10^{14} vg/kg dose level (Cohort 3) did not occur.

a. An additional 3 subjects treated with 1.0×10^{14} vg/kg AT132 in Cohort 1 based on DMC recommendation.
b. Delayed-treatment control subjects from Part 1 treated with AT132 after the therapeutic dose was determined.
c. Additional subjects treated with 3.0×10^{14} vg/kg AT132 in Cohort 2 based on DMC recommendation.
d. No additional subjects will be dosed under this amendment.
e. Previously referred to as 1.0 and 3.0×10^{14} vg/kg. The 1.0×10^{14} vg/kg and 3.0×10^{14} vg/kg dose levels based on the 1st generation titer method equate to 1.3×10^{14} vg/kg and 3.0×10^{14} vg/kg, respectively, based on an analysis of all historical Study 002 drug product lots using the 2nd generation vg titer assay.

Source: Data on file.

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LIST OF ABBREVIATIONS/DEFINITION OF TERMS

AAV	adeno-associated virus
AAV8	adeno-associated virus serotype 8
ACEND	Assessment of Caregiver Experience with Neuromuscular Disease
ADL	activities of daily living
AE	adverse event
AFO	ankle-foot orthoses
AHI	Apnea-hypopnea index
ALT	alanine aminotransferase
anti-AAV8	anti-adeno-associated virus 8
AST	aspartate aminotransferase
AT	aminotransferases
AT132	rAAV8-Des-hMTM1 (resamirigene bilparvovec)
Bayley-III	Bayley Scales of Infant and Toddler Development III
BID	twice daily
BiPAP	bilevel positive airway pressure
BLOD	below the limit of detection
BNP	B-type natriuretic peptide
CDI	Communicative Development Inventories
cDNA	complementary DNA
CFR	Code of Federal Regulations
CGI-I	Clinical Global Impression of Improvement
CGI-S	Clinical Global Impression of Severity
cGMP	current Good Manufacturing Practice
CHOP INTEND	Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders
CI	Confidence intervals
CK	creatine kinase
CNM	centronuclear myopathy
CO_2	carbon dioxide
CPAP	continuous positive airway pressure
CRA	clinical research associate
CRF	case report form
CSR	clinical study report
CTCAE	Common Terminology Criteria for Adverse Events
CTL	cytotoxic T cell
DC	discharge

DMC	Data Monitoring Committee
EC	Ethics Committee
ECG	electrocardiogram
ECHO	echocardiogram
eCRF	electronic case report form
ELISpot	enzyme-linked immunosorbent spot assay
EMG	electrophysiological assessments
ENT	ear, nose and throat
$ETCO_2$	End tidal carbon dioxide
FAS	full analysis set
GCP	Good Clinical Practice
GGT	gamma-glutamyltransferase
GLP	Good Laboratory Practice
HBB2	human β globin gene second intron
HGB	Hemoglobin
HLA	human leukocyte antigen
ICF	informed consent form
ICH	International Council for Harmonisation
IFN-γ	Interferon-gamma
IgG	Immunoglobulin G
IL	interleukin
IMP	investigational medicinal product
INR	international normalized ratio
IRB	Institutional Review Board
ITR	inverted terminal repeat
ITT	Intent to Treat
IV	intravenous
Investigator	Principal Investigator or Sub-Investigator
KO	knockout
LAR	legally authorized representative
LFT	liver function test
MED	minimal effective dose
MedDRA	Medical Dictionary for Regulatory Activities
MEP	maximal expiratory pressure
MFM	Motor Function Measure
MFM-32	Motor Function Measure-32

MIP	maximal inspiratory pressure
MITT	Modified Intend to Treat
MMRM	Mixed Effect Model Repeat Measurement
MRI	magnetic resonance imaging
mRNA	messenger RNA
MTM	myotubular myopathy
MTM1	gene that codes for myotubularin (a mutation in the *MTM1* gene results in myotubular myopathy)
NAb	neutralizing antibody
NCI CTCAE	National Cancer Institute Common Terminology Criteria for AEs
NHP	nonhuman primate
NIV	noninvasive ventilation
NMD	neuromuscular disorders
NOAEL	no observed adverse effect level
NREM	non-rapid eye movement
O_2	oxygen
p.N155K	mutation found in the canine model of XLMTM
P0.1	inspiratory occlusion pressure at 0.1 seconds
pA	polyadenylation
PBMCs	peripheral blood mononuclear cells
PedsQL	Pediatric Quality of Life Inventory
PEEP	positive end-expiratory pressure
PGIS-I	Parental Global Impression of Secretion Improvement
PGIS-S	Parental Global Impression of Secretion Severity
PO_2	partial pressure of oxygen
polyA	polyadenylation
PP	Per Protocol
PSG	polysomnogram
PT	preferred term
rAAV8	recombinant adeno-associated viral vector, serotype 8
rAAV8-Des-hMTM1	non-replicating recombinant adeno-associated viral vector, serotype 8, expressing the human MTM1 cDNA under the control of the muscle-specific human desmin gene promoter
rATG	rabbit anti-thymocyte globulin (eg, thymoglobulin)
REM	rapid eye movement
RNA	ribonucleic acid
RR	respiration rate

RSI	Reference Safety Information
SAE	serious adverse event
SAF	safety analysis set
SAP	statistical analysis plan
SMA	spinal muscular atrophy
SMA-I	spinal muscular atrophy type I
SMO	supra-malleolar
SOC	system organ class
SPO_2	oxygen saturation
STIR	short tau inversion recovery
SUSAR	suspected unexpected serious adverse reaction
TAb	total antibody
TBL	total bilirubin
TCO_2	transcutaneous carbon dioxide
TEAE	treatment-emergent adverse event
TID	three times a day
TMA	thrombotic microangiopathy
TNF-α	tumor necrosis factor-alpha
TST	total sleep time
ULN	upper limit of normal
USM	urgent safety measure
vg	viral genomes
vs	versus
XLMTM	X-linked myotubular myopathy

1 INTRODUCTION

1.1 Background

X-linked myotubular myopathy (XLMTM) is a rare, severe, congenital muscle disease and is one of the “centronuclear myopathies” (CNM). It has an estimated incidence of 1 in 50,000 live male births (Orphanet, 2009; Vandersmissen, 2018), although careful, large studies attempting complete ascertainment of incidence and prevalence have not been published (Das, 2011). The only identified cause of XLMTM is a mutation in the *MTM1* gene; approximately 400 different mutations have been identified (Biancalana, 2012). The *MTM1* gene encodes a protein called myotubularin, a highly conserved, dual-specific lipid phosphatase that is involved in the development, maturation, and maintenance of skeletal muscle cells.

In normal fetal muscle, the nuclei are situated in a centrally placed chain; however, by birth, the majority of nuclei are located at the periphery of the skeletal muscle cell. Characteristic findings of XLMTM on muscle biopsy include myofiber hypotrophy, centrally nucleated myofibers, and mislocalization of organelles (Laporte, 2000; Lawlor, 2016; Pierson, 2005).

In a study of 133 different mutations in 198 unrelated families, all males had disease manifestations resulting from too little functional protein or a nonfunctional protein, 88% of whom were considered to have high morbidity (Laporte, 2000).

The majority of affected male infants with XLMTM are identified with a congenital myopathy in the perinatal period, although a definitive diagnosis of XLMTM may take several months depending on the clinical expertise available at the treating facility. There is often a prenatal history of weak or infrequent fetal movements (McEntagart, 2002) and polyhydramnios (Herman, 1999). In families without a known maternal carrier, diagnosis of XLMTM is generally based on clinical suspicion of a congenital myopathy shortly after birth, typically followed by a pathologic diagnosis from a muscle biopsy, and confirmation by subsequent genetic testing.

Although myotubularin protein is ubiquitously expressed, the most apparent consequence of *MTM1* mutations appears to be skeletal muscle pathology (Das, 2011). Genotype-phenotype correlations have occasionally been observed in XLMTM, but no definitive pattern exists. Apart from missense mutations, which manifest considerable phenotypic variation, most other mutations present with severe disease. Phenotypic variability has been observed in family members with the same mutation (Barth, 1998; Laporte, 2000) and in unrelated individuals with recurrent mutations (McEntagart, 2002).

Males present at birth with severe hypotonia, weakness, and respiratory distress (ie, the newborns are profoundly hypotonic and need rapid intervention to assist breathing), and approximately 40% have been reported to die in the first year of life (Herman, 1999; McEntagart, 2002). McEntagart, et al., have reported a median survival of 29 months. Causes of death are primarily respiratory failure or related complications such as pneumonia/respiratory tract infection, ventilator-related accidents, or cardiorespiratory failure (Herman, 1999;

McEntagart, 2002; Wallgren-Pettersson, 1995). Rarely, deaths have been reported from liver-related complications (Herman, 1999). Profound delayed motor development is a result of global weakness, with only approximately half of surviving patients able to sit unassisted and most patients never reaching ambulatory status (Herman, 1999; McEntagart, 2002). Unlike most neuromuscular diseases, XLMTM patients have no evidence of cardiac muscle pathology.

In a study of 120 XLMTM cases, 85% of patients required ventilation at birth, and 46% of patients died within 18 months (including a subset of patients who were not ventilated at birth) (McEntagart, 2002). Despite 24-hour ventilator support, 25% of patients died within the neonatal period, and only 14% of patients did not require full respiratory assistance (McEntagart, 2002). Of the proportion that survives, there is severe morbidity as well as compromised functional capability and quality of life. Despite normal or above-average intelligence, many patients have limited communication because of interference from ventilatory support aids (Herman, 1999).

Additional common clinical features and comorbidities include ophthalmoplegia and cryptorchidism (both likely related to the underlying muscle abnormality), elongated facial features and distal extremities, and high palate. More rarely reported features include hypospadias resulting from a contiguous gene deletion (Tsai, 2005), pyloric stenosis often requiring surgical intervention, spherocytosis, and hydrocephalus (Herman, 1999). There has been an increased understanding of the prevalence of hepatobiliary disease in XLMTM patients. Hepatobiliary disease has been reported in 7 to 17% of XLMTM patients in medical chart reviews and natural history studies (Gangfuss, 2021; Annoussamy, 2019; Beggs, 2018; Amburgey, 2017; Herman, 1999) and can manifest as cholestasis, jaundice, cholelithiasis, pruritus, hepatomegaly, and elevated transaminases. Additionally, the recently published literature (Dowling, 2022) based on the data from Study ATX-MTM-009 (INCEPTUS, a prospective natural history study conducted by the Sponsor) suggested that hepatobiliary disease was identified as an under-recognized comorbidity, where 91% of subjects had histories of hepatic disease and/or prospectively experienced related adverse events (AEs) or laboratory or imaging abnormalities. The more recent case series and reports (Molera, 2022; D'Amico, 2022; Neese, 2021) offered additional insight into key clinical characteristics of intrahepatic cholestasis and liver dysfunction in XLMTM patients. Presentation of liver dysfunction in XLMTM patients varies greatly among patients and within individual patients over time.

The perinatal and respiratory findings of earlier studies, and the mortality and morbidity data, were confirmed in the dataset from the Sponsor's retrospective chart review study (ATX-MTM-001 [RECENSUS]) (Beggs, 2018).

There is no known treatment or disease-modifying therapy for XLMTM, and patient management is mainly supportive, with a complicated multidisciplinary approach focused on trying to maximize functional abilities and minimize medical complications.

The Sponsor has conducted 2 patient and family meetings to better understand the humanistic impact of XLMTM (XLMTM Patient Focus Group Summary, Audentes Therapeutics, Inc. internal

report). Families report that the psychological burden of living with XLMTM is substantial. Patients with XLMTM spend a significant proportion of time in the hospital, particularly during the first few years of life; in some cases, patients remain in a hospital for the entirety of this period. Children with XLMTM are particularly affected by respiratory infections, which frequently lead to hospitalization, pneumonia, and occasionally collapsed lungs. Suctioning secretions from the throat and airways is constantly required (up to every 5-10 minutes). In addition, children must receive regular (often daily) physical therapy. Parents of children with XLMTM are not able to communicate well with their children due to speech difficulties, which is an additional frustration. In addition, families of children with XLMTM report the disease has a significant impact on routine life and can be socially isolating for both the parents and children. Overall, XLMTM leads to a substantial impact on the clinical, humanistic (including quality of life, psychological aspects, and ability to undertake activities of daily life), and economic burden of disease for patients and their families.

1.2 Description of AT132

The active substance is rAAV8-Des-hMTM1, a non-replicating recombinant adeno-associated virus serotype 8 (rAAV8), expressing the human *MTM1* complementary DNA (cDNA). The rAAV8 lacks all of the viral protein coding sequences. In this vector, transgene expression is controlled by the human desmin promoter, and ribonucleic acid (RNA) processing is mediated by the human β globin gene second intron (HBB2) and polyadenylation (pA) signal. This MTM1 expression cassette is flanked by the AAV2 inverted terminal repeats (ITRs). A schematic is shown in Figure 3.

Figure 3: Structure of the AT132 Vector Genome

hMTM1, human myotubularin cDNA; intron, engineered form of human β-globin 2nd intron; ITR, inverted terminal repeat; Pdesmin, human desmin promoter; polyA, human β-globin polyadenylation signals; SA, splice acceptor; SD, splice donor.

For further information on AT132, refer to the current Investigator's Brochure.

1.2.1 Nonclinical Experience with AT132

1.2.1.1 Nonclinical Pharmacodynamic Studies

The efficacy of gene therapy for the correction of myotubularin deficiency was evaluated in 2 animal models of XLMTM, the *MTM1* knockout (KO) mouse in the 129/PAS background (Al-Qusairi, 2009; Buj-Bello, 2002) and the p.N155K missense mutational dog model

(Beggs, 2010). In preliminary proof-of-concept studies, conducted in the *MTM1* KO mouse model, both the mouse-specific rAAV8-Des-mMTM1 and AT132 reversed the disease phenotype characteristic of XLMTM in a generally dose-dependent manner. Dose-dependent efficacy of AT132 was further corroborated in XLMTM dogs after administration of the canine-specific version of AT132 (rAAV8-Des-cMTM1), which resulted in increased survival and full correction of the MTM disease pathology and phenotype at 9 months following vector administration (≥ 2×10^{14} vg/kg) (Childers, 2014; Mack, 2017) and > 3 years following vector administration (~2×10^{14} vg/kg) (Elverman, 2016).

In the pivotal minimal effective dose (MED) study in *MTM1* KO mice, which evaluated AT132 manufactured by the clinical process, doses of 1 to 2×10^{14} vg/kg demonstrated consistent improvement in the full spectrum of motor function and XLMTM disease-related pathology assessments performed at 8 weeks following vector administration. Based on these data, the MED for AT132 was defined as 1×10^{14} vg/kg.

1.2.1.2 Nonclinical Biodistribution Studies

The biodistribution of AT132 delivered by IV injection was uniform in all tissues examined in a dose-dependent manner across species. Higher expression relative to skeletal muscle was observed in the cardiac tissue of mice and dogs, but not in nonhuman primates (NHPs). In NHPs, 8×10^{14} vg/kg AT132 resulted in vector distribution to skeletal muscles, heart, and brain, with the highest levels detected in the liver. Levels of messenger RNA (mRNA) and myotubularin protein expression were highest in the skeletal muscle, with slightly lower levels observed in the heart. Myotubularin protein levels in the liver were similar to those of control animals, supporting the specificity of the desmin promoter for expression in skeletal muscle.

1.2.1.3 Nonclinical Toxicology Studies

The doses of AT132 and species-specific vectors administered in the nonclinical safety studies ranged from 2.5×10^{13} vg/kg (KO mice) to 8×10^{14} vg/kg (NHPs). The duration of exposure to AT132 and species-specific variants ranged from 5 days in *MTM1* KO mice to > 3 years for p.N155K dogs, with safety assessments up to 9 months for dogs.

In the pivotal good laboratory practice (GLP) toxicology study in NHPs, the no observed adverse effect level (NOAEL) was defined at 8×10^{14} vg/kg. No AT132-specific adverse toxicities were observed in either the KO mouse or canine disease models up to doses of 2×10^{14} vg/kg in mice and 5×10^{14} vg/kg in dogs. Both doses corrected the XLMTM phenotype in their respective models. Consistent with historic data, administration of AT132 to NHPs resulted in induction of the humoral immune response against the AAV8 capsid, whereas no antibodies were detected against the myotubularin protein. AT132 dosing did not induce detectable AT132-specific T cell immune responses in the NHP study.

Asymptomatic cardiac lesions were observed in mice at necropsy, which were demonstrated to exhibit background cardiac lesions in the mouse strain. Although minimal to mild mononuclear

cell infiltration was observed in NHPs and dogs, no evidence of cardiac lesions was present. These data suggest there is minimal cardiac risk associated with AT132 treatment.

1.2.2 Clinical Data for AT132

This is the first-in-human study of AT132. Interim results of the ATX-MTM-002 (ASPIRO) study are summarized in the Investigator's Brochure.

1.3 Study Rationale

AT132 is being developed by the Sponsor as a gene transfer therapy for the treatment of XLMTM and would represent the first gene transfer therapy for this disease. The aim is to develop a therapy that will achieve the following:

- Improve survival
- Improve skeletal muscle function
- Improve diaphragmatic (and other respiratory muscle) function
- Improve functional outcomes (eg, speech and communication, swallowing, secretion management, developmental progression)
- Reduce the burden of disease for patients and caregivers and improve the Health-Related Quality of life for children with XLMTM

AAV8-mediated gene therapy offers the prospect of considerable long-term clinical benefit to XLMTM patients through persistent expression of the missing or damaged myotubularin protein following a single systemic therapeutic administration. Normal skeletal myofibers are long-lived, terminally differentiated, multinucleated cells in which transgene expression can be durable. Gene therapy is expected to provide persistent protein expression, corresponding long-term clinical benefit, and the potential for substantial recovery of muscle function (Beggs, 2018; Buj-Bello, 2008; Buj-Bello, 2002; Childers, 2015; Childers, 2014; Romero, 2010). Substantial functional recovery and prolongation of life in both the murine (KO) and canine (missense mutation) models of XLMTM disease, employing the same AAV8 vector, support this therapeutic strategy.

This Phase 1/2/3 study will evaluate safety and efficacy of AT132 administered to male subjects with XLMTM aged < 5 years.

1.4 Dose Selection Rationale

1.4.1 Starting Dose Selection Rationale

The proposed starting dose in Study ATX-MTM-002 was 1.0×10^{14} vg/kg, the second cohort 3.0×10^{14} vg/kg, and the third dose cohort 5.0×10^{14} vg/kg. The starting dose and subsequent doses were selected based on the results from the nonclinical studies.

In the pivotal MED study in *MTM1* KO mice, which evaluated AT132 manufactured by the clinical process, doses of 1 to 2×10^{14} vg/kg demonstrated consistent improvement in the full

spectrum of motor function and XLMTM disease-related pathology assessments performed at 8 weeks following vector administration.

In the pivotal GLP toxicology study in NHPs, the NOAEL was defined at 8×10^{14} vg/kg. Based on the nonclinical studies, safety factors of up to 8-fold were identified over the clinical starting dose of 1.0×10^{14} vg/kg.

1.4.2 Current Dose Rationale

The 1.0×10^{14} vg/kg dose level referred to herein was based on a 1st generation titer method and equates to 1.3×10^{14} vg/kg as determined by a 2nd generation viral genomes (vg) titer assay; and the 3.0×10^{14} vg/kg dose level equates to 3.5×10^{14} vg/kg based on an analysis of the historical Study ATX-MTM-002 drug product lots using the 2nd generation vg titer assay. The following rationale refers to the original nomenclature.

The first dose cohort (1.0×10^{14} vg/kg) in Part 1 was originally comprised of 4 subjects (1 sentinel subject followed by 2 randomized to receive AT132 and 1 randomized to delayed-treatment control). After evaluation of the efficacy and safety data from the 3 subjects administered AT132 at 1.0×10^{14} vg/kg from Part 1, Cohort 1 and in conjunction with the study data monitoring committee (DMC), it was decided to enroll an additional 3 subjects at the 1.0×10^{14} vg/kg dose (Cohort 1 expansion) to further evaluate the safety of this dose. After review of all 6 subjects administered AT132 in Cohort 1, in conjunction with the DMC, it was determined to be appropriate to escalate to the 3.0×10^{14} vg/kg dose cohort (Cohort 2). Following the administration of 3.0×10^{14} vg/kg AT132 to the initial 3 subjects, it was again agreed to evaluate the safety of this dose level in 3 to 5 additional subjects.

As of Study ATX-MTM-002 protocol v5, the totality of safety, efficacy, and muscle biopsy data collected was assessed to determine the dose of AT132 to carry forward into Part 2 of Study ATX-MTM-002. A statistically significant difference was not observed between dose levels (1.0×10^{14} vg/kg and 3.0×10^{14} vg/kg) in the respiratory and neuromuscular endpoints. At Week 24, myotubularin protein expression reached or exceeded normative levels in Cohort 2 subjects who all were administered 3.0×10^{14} vg/kg AT132, and there was an indication of a faster rate of histopathological improvement at this dose. AT132 was well-tolerated with a manageable safety profile across both Cohort 1 and Cohort 2 in Part 1. However, in subjects treated with 3.0×10^{14} vg/kg, there was an indication of potential dose-related adverse events (AEs) (eg, transient decrease in platelet counts, and exacerbation of preexisting hyperbilirubinemia) that could increase in frequency and magnitude should dose escalation continue. Based on these data, the Sponsor, in conjunction with the DMC, agreed that it would not be in the best interest of patient safety to continue with dose escalation beyond the 3.0×10^{14} vg/kg dose level; therefore, dose escalation to the planned 5.0×10^{14} vg/kg dose level (ie, Cohort 3) did not occur. The dose of AT132 selected to be carried forward into Part 2 was 3.0×10^{14} vg/kg.

Following the occurrence of fatal serious AEs (SAEs) in Study ATX-MTM-002, the Sponsor, in consultation with the DMC and external experts, evaluated the data and reassessed the benefit/risk profiles of the 1.0×10^{14} vg/kg and 3.0×10^{14} vg/kg dose levels. As of the data cutoff date of 08 July 2020, 5 subjects who received 3.0×10^{14} vg/kg AT132 developed AT132-related SAEs involving the hepatobiliary system. In 2 subjects, these SAEs resolved; in 3 subjects, fatal events occurred subsequent to severe cholestatic liver dysfunction. Within the 3.0×10^{14} vg/kg dose level cohort, 8 of 17 subjects (47.1%) experienced 26 SAEs considered related to AT132. In contrast, within the 6 subjects dosed at the 1.0×10^{14} vg/kg dose level, all of whom are ≥ 2 years from AT132 administration, 1 (16.7%) subject experienced 4 AT132-related SAEs, all resolved, and none involved the hepatobiliary system. In addition to a favorable safety profile observed in the subjects treated with 1.0×10^{14} vg/kg, a clinically meaningful benefit continues to be observed in this group at last follow-up. In this group, which had a mean 20.5 hours (SE 2.02) of ventilation per day at baseline (n = 5 [83.3%] with invasive ventilation; n = 1 [16.7%] with non-invasive ventilation), 5 out of 6 (83.3%) subjects were ventilatory independent at last follow-up (as of data cutoff date 08 July 2020). With respect to gross motor development, at baseline 5 of the 6 subjects did not perform any of the following major motor milestones: sit unassisted for 30 seconds, raise self to standing position, stand alone, or walk alone. At baseline, 1 subject did sit unassisted for 30 seconds but did not perform the remaining motor tasks. At last follow-up following AT132 administration at 1.0×10^{14} vg/kg, 5 out of 6 subjects performed all of these motor tasks, including walking alone. Regarding performance on the Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP INTEND), this 1.0×10^{14} vg/kg group demonstrated a baseline mean score of 37.7 (SE 2.42). At last follow-up, performance had improved to a mean score of 56.5 (3.97).

As data from Study ATX-MTM-002 subjects treated at the 1.0×10^{14} vg/kg dose level demonstrate significant and clinically meaningful benefit, an overall benefit/risk analysis by the Sponsor, in consultation with the DMC, had determined the benefit/risk profile of the 1.0×10^{14} vg/kg dose level to be favorable, and superior to the 3.0×10^{14} vg/kg dose level.

Based on feedback from the US FDA, the Sponsor improved the assay for determination of vg titer of manufactured AT132 drug product lots. Following validation, the Sponsor used this 2nd generation assay, with enhanced precision compared to the 1st generation methodology, to recharacterize the vg content of all previously administered drug product lots from Study ATX-MTM-002. This recharacterization analysis demonstrated that the 1.0×10^{14} vg/kg dose level based on the 1st generation titer method equates to 1.3×10^{14} vg/kg as determined by the 2nd generation vg titer assay. Of note, the 3.0×10^{14} vg/kg dose level equates to 3.5×10^{14} vg/kg based on this historical analysis.

As of protocol v9, subsequent subjects yet to be dosed were to be administered 1.3×10^{14} vg/kg AT132 as determined by the 2nd generation vg titer assay.

Under protocol v10 and beyond, no additional subjects will be dosed.

1.5 Overall Risks and Benefits

Key study risks are listed below:

- Subjects will have study procedure-related risks:
  - **Blood collection:** The risks of venous blood draws include discomfort at the site of puncture, possible bruising and swelling around the puncture site, rarely an infection; and, uncommonly, fainting, nausea, vomiting, and light-headedness from the procedure.
  - **Muscle biopsy:** The muscle biopsy has potential complications from the procedure that include infection at the biopsy site, bleeding and/or pain at the site of the biopsy, and psychological trauma from the scar at the site of the incision; in some cases, keloid formation can increase scarring.
  - **Cardiac or muscle magnetic resonance imaging (MRI):** The MRI scan is not associated with risks unless a subject has certain types of metal implants such as pacemakers.
  - **Procedural sedation risk**: Sedation risks in children with weak respiratory muscles varies with the medication used. Sedation risks will be explained to the family. Sedation for the imaging must be carefully administered in patients with XLMTM, since most anesthetic agents act directly on the muscle. Bulbar dysfunction, respiratory muscle weakness, gastroesophageal reflux, and scoliosis increase the risk of pneumonia. Children with respiratory and bulbar muscle weakness who are not ventilator dependent may require invasive or noninvasive ventilation after the procedure. The duration of increased ventilatory requirements would vary depending on the degree of respiratory muscle weakness. The potential side effects of specific medications administered for sedation (eg, vomiting, respiratory depression, laryngospasm, emergence reaction), and the likely duration of sedation will be discussed during informed consent for the procedure.
  - **Prophylaxis (prednisolone, ursodiol):** Subjects receive prophylactic prednisolone and ursodiol as part of the clinical study. Information regarding the risks associated with these medications can be found in the respective local prescribing information.
- Overall risks associated with rAAV gene therapy administration include hepatotoxicity, cardiac events such as myocarditis, myositis, thrombocytopenia, thrombotic microangiopathy (TMA), and sensory ganglionopathy (Arjomandnejad, 2023; FDA, 2021; Lek, 2023; Shen, 2022; Whiteley, 2023).
  - Key identified risks associated with AT132 administration observed in Study ATX-MTM-002 are listed below. Of the overall risks associated with rAAV gene therapy, thrombotic microangiopathy and sensory ganglionopathy have not been observed in ATX-MTM-002 study subjects. Long-term follow-up is important in understanding whether these AEs have long-term implications. Refer to the Investigator's Brochure for further details.
  - **Hepatobiliary events:** Many XLMTM patients have liver dysfunction and cholestasis, which places them at increased risk for hepatobiliary dysfunction or even hepatic failure with rAAV administration. A prospective natural history study (Study ATX-MTM-009) reported that overall, 91% of subjects had histories of hepatic

disease and/or prospectively experienced related AEs or laboratory or imaging abnormalities (Dowling, 2022).

- In Study ATX-MTM-002, SAEs due to hepatobiliary dysfunction occurred after AT132 administration and were characterized by hyperbilirubinemia, cholestatic liver dysfunction, transaminases elevations, ascites, and serum bile acid elevations. Fatal outcomes were observed in 4 subjects due to multifactorial causes including hepatobiliary failure: 1 subject who received the 1.0×10^{14} vg/kg dose and 3 subjects who received the 3.0×10^{14} vg/kg dose. All 4 subjects showed increases in direct and total bilirubin values (> upper limit of normal [ULN]) beginning 1 to 4 weeks after AT132 administration (Shieh, 2023). Liver findings included intrahepatocellular and canalicular cholestasis, periportal and bile ductular reaction, secondary fibrosis, and notable lack of prominent liver parenchymal inflammatory cellular infiltrates (Shieh, 2020).

- **Myocardial events:**
  - *Acute cardiac risks:* Acute myocardial events manifested as myocarditis and increases in troponins. In Study ATX-MTM-002, variable increases in troponin levels after AT132 administration were commonly observed. Two subjects experienced SAEs of myocarditis: 1 subject who received the 1.0×10^{14} vg/kg dose and 1 subject who received the 3.0×10^{14} vg/kg dose. Prolonged prednisolone administration plus addition of other immunosuppressive medications were used for treatment of myocarditis.
  - *Chronic cardiac risks:* Following prolonged immunosuppression for over 3 years, 1 subject experienced an increase in cardiac troponin levels after withdrawal of immunosuppression. Abnormalities in cardiac function have not been observed in this subject. However, long term assessment is required to understand whether there could be more significant impact on cardiac function.
- **Muscle abnormalities:** A skeletal muscle immune response to the rAAV capsid and to the transgene can occur after rAAV gene therapy administration.
  - *Acute and subacute creatine kinase (CK) elevations*: In Study ATX-MTM-002, variable increases in CK levels after AT132 administration were commonly observed, occurring soon after dosing. These CK levels generally returned to normal within the first year after AT132 administration.
  - *Chronic CK elevations and inflammatory infiltrates in skeletal muscles*: Assessment of muscle biopsies demonstrated treatment-associated inflammatory changes in muscle biopsy specimens of some subjects. No SAEs were reported in association to these findings. Improvements in neuromuscular function (respiratory and motor) have been maintained. Although the CK levels generally returned to normal within the first year after AT132 administration, chronic CK elevation over several years can occur.
- **Thrombocytopenia:** Subjects may develop transient thrombocytopenia or decreases in platelet levels from baseline after AT132 administration. In Study ATX-MTM-002, transient thrombocytopenia related to AT132 occurred within the first 2 weeks of dosing (Shieh, 2023).

Key potential study benefits include the following:

- Independent of efficacy, study subjects may receive better-coordinated and more comprehensive overall clinical care at centers of excellence.
- AT132, even if only partially effective, could cause clinically important improvements in respiratory function and mobility and a lowered risk of disease-related comorbidities, leading to greater independence for the subject and caregivers.
- Other patients may benefit from knowledge gained about XLMTM.

2 STUDY OBJECTIVES, ENDPOINTS, AND ESTIMANDS

The objectives of the study are as follows:

- To determine the therapeutic dose of AT132
- To confirm the safety and efficacy of the therapeutic dose of AT132

The endpoints of the study are as follows:

Primary Efficacy Endpoint:

- Change from baseline in hours of ventilation support at Week 24

Key Secondary Efficacy Endpoint:

- Percentage of subjects achieving functionally independent sitting for at least 30 seconds at Week 24

Other Secondary Efficacy Endpoints:

- Time to reduction in required ventilator support to ≤ 16 hours a day (only in subjects who require invasive ventilation) at Week 24
- Change from baseline in CHOP INTEND at Week 24
- Change from baseline in maximal inspiratory pressure (MIP) at Week 24
- Change from baseline in quantitative analysis of myotubularin expression in the muscle biopsy at Week 24
- Change from baseline in quality of life assessments at Week 24 (ie, the Assessment of Caregiver Experience with Neuromuscular Disease (ACEND) and Pediatric Quality of Life Inventory (PedsQL)
- Number (%) of age-appropriate clinically relevant gross motor function milestones attained through Week 24
- Percentage of subjects achieving full ventilator independence at Week 24
- Survival

Safety Endpoints:

- AEs, SAEs, and findings from safety laboratory tests, 12-lead electrocardiogram (ECG), echocardiograms (ECHOs), vital signs, growth parameters, physical examinations, liver ultrasounds, antibody formation (anti-AAV8, anti-MTM1), viral shedding, annualized hospitalization rate, annualized respiratory and non-respiratory SAE rate, and length of stay per hospitalization

Exploratory Endpoints:

- Time to unassisted sitting for 30 seconds or more at Week 24
- Change from baseline in the Motor Function Measure-32 (MFM-32) at Week 24
- Change from baseline in total raw score in the gross motor domain of the Bayley Scales of Infant and Toddler Development III (Bayley-III) at Week 24

- Change from baseline in total raw score in the fine motor domain of the Bayley-III at Week 24
- Change from baseline in the proportion of patients being able to feed without a gastrostomy or G-tube at Week 24
- Change from baseline in the Communicative Development Inventories scores at Week 24
- Change from baseline in the Parental Global Impression of Secretion Severity (PGIS-S) score at Week 24
- Change in the Parental Global Impression of Secretion Improvement (PGIS-I) score at Week 24
- Change from baseline in Clinical Global Impression of Severity (CGI-S) at Week 24
- Change in the Clinical Global Impression of Improvement (CGI-I) score at Week 24

2.1 Estimands

The estimand of the primary objective is defined by the following 5 attributes:

- Treatment: Single dose of AT132
- Population: Male subjects with ventilator-dependent XLMTM, as defined by the inclusion/exclusion criteria of the study
- Endpoint: Change from baseline in hours per day of ventilation support at Week 24
- Intercurrent events and their corresponding strategies: For study discontinuation due to death or due to lack of efficacy, a composite strategy will be adopted, where subjects with the intercurrent event before Week 24 having missing observation(s) after the intercurrent event will have those missing observations(s) imputed by unfavorable values
- Population level summary: Mean change from baseline, by dose level

3 INVESTIGATIONAL PLAN

3.1 Overall Study Design and Plan

This is a Phase 1/2/3, randomized, open-label, ascending-dose, delayed-treatment concurrent control clinical study to evaluate the safety of AAV8-delivered gene therapy in XLMTM subjects aged less than 5 years old. Subjects will receive a single dose of AT132 and will be followed for safety and efficacy for 10 years, with the primary analysis occurring after all subjects have completed 24 weeks of posttreatment evaluation.

The study consists of 2 parts. Part 1 would establish the optimal dose of AT132. Part 2 would confirm the safety and efficacy of AT132 at the optimal dose level. The following describes how each part was designed and the changes as of protocol v8 and beyond.

Part 1 (fully dosed as of protocol v8):

A maximum of 3 dose levels of AT132 are planned for evaluation in Part 1 of this study (Figure 1). Four subjects are planned to be enrolled in each dose level cohort, including 1 subject in each dose level cohort randomized to control with delayed administration of AT132. The first subject in each dose level cohort will be assigned to receive AT132 and will be a sentinel subject. Subsequent subjects at that dose level will be randomized (2:1) to treatment or control with delayed treatment if there are no safety concerns after at least 4 weeks of post-dose data from the sentinel subject is evaluated by the chair of the DMC. Dose escalation to the next dose level will be considered after evaluation of at least 4 weeks of data from all subjects dosed at the current dose level. If the data warrants, additional subjects may be included at a single dose level prior to consideration of dose escalation. Following the dose escalation portion of the study, an optimal dose will be determined in conjunction with the DMC, and control subjects will be treated at this dose.

This study's independent DMC will monitor subject safety and provide recommendations to the Sponsor regarding dose level determination, cohort expansion, and safety and study conduct matters.

Subjects who are randomized to receive AT132 may be admitted to the study clinic 1 to 2 days prior to AT132 dosing (Day -2 or Day -1) for baseline procedures, including confirmation of baseline ventilator settings, and will stay in the study clinic for at least the first overnight post-dose. If medically appropriate, subjects may be discharged from the study clinic after completion of Day 2 assessments.

Control subjects will generally have the same assessments as treated subjects, but on a less frequent schedule to lessen the burden of study participation. It is anticipated that control subjects will participate for at least 24 weeks before an optimal dose is determined and the subject can be administered AT132. Following determination of the optimal dose, control subjects will undergo pre-treatment baseline procedures to confirm they remain eligible to

receive treatment with AT132 at the optimal dose. Once eligible, control subjects will be dosed with AT132, and will initiate the post-dose procedures.

Treated subjects will be administered a course of concomitant prophylactic glucocorticoid (oral prednisolone) therapy commencing 1 day prior to AT132 dosing (Day -1), and continuing for a period of approximately 8 weeks, then tapering from the original dose over 8 weeks, per Investigator discretion (Section 4.4), as a preventative measure for immune-mediated hepatic injury, which has been observed in gene therapy clinical studies with AAV. Tapering duration may be altered and/or supplemental administration of IV steroids (eg, methylprednisolone) or other immunosuppressive regimens may be considered in cases of potential malabsorption of oral medications.

Subjects will be followed for a total of 10 years following AT132 administration, see Section 5.2. See Figure 1 for the individual study participation timeline (Parts 1 & 2) through Week 48.

Part 2 (described herein as planned prior to protocol v8; partially dosed as of protocol v8):

In Part 2, the optimal dose of AT132 will be evaluated in an additional cohort of 10 subjects. These subjects will be prospectively paired based on age (±6 months) before being randomized to study drug or delayed-treatment control (1:1 allocation). The administration of study drug and concomitant prophylactic glucocorticoids, and the assessment of safety will be the same as that described in Part 1. Following collection of their Week 24 data, control subjects will be administered AT132 and followed according to the Schedule of Events.

Part 2 as of protocol v8 and v9:

Following the review of benefit/risk profiles of the 1.0×10^{14} vg/kg and 3.0×10^{14} vg/kg dose levels, the Sponsor, in consultation with the DMC, determined that subjects not yet dosed were to be administered the lower dose level (ie, the therapeutic dose), which is 1.3×10^{14} vg/kg AT132 as determined by a 2nd generation vg titer assay (see Section 1.4.2). Dosing of the 3 enrolled delayed-treatment controls who have not yet been dosed will not be conducted as described above for Part 2, but will resume at 1.3×10^{14} vg/kg as long as each subject meets a subset of inclusion and exclusion criteria prior to dosing. If any of these control subjects is not eligible to be dosed, another eligible subject can be enrolled. Any new subject will not be considered a delayed-treatment control and therefore will not be required to wait 24 weeks before administration of study drug. Any new subject will be enrolled under all of the inclusion/exclusion criteria (including Exclusion Criteria 12-14 required for Part 2 subjects).

In addition to prophylactic glucocorticoids (Section 4.4), subjects will receive daily prophylactic ursodiol (ursodeoxycholic acid; Section 4.5) beginning between Study Days -6 and -4. Subjects will be followed according to the Schedule of Events (Sections 5.4 and 5.5). See Figure 2.

As of protocol v10 and beyond:

This study does not allow for enrollment or dosing of any future subjects.

3.2 Discussion of Study Design

Enrollment of infants and young children into this first-in-human study exploring the safety of AT132 in XLMTM is justified for several reasons:

- There are not enough adult patients to enable a clinical study in the older population due to the rarity and early mortality of the disease. It has been established that death rates remain high throughout childhood and the majority of children do not survive into adolescence and adulthood. The risk of death by 18 months is 46%, with a median survival of 29 months (McEntagart, 2002). These data have recently been confirmed in the analyses of data from RECENSUS, where high mortality (44% overall) and substantial disease burden was demonstrated, particularly in patients ≤18 months of age at last follow-up, where mortality was 64%.
- Children and infants represent the most relevant patient population for the evaluation of XLMTM. Again, due to the early mortality and severity of XLMTM, the majority of patients with XLMTM are children; therefore, this population is the most representative of subjects with XLMTM. Some patients with XLMTM do experience prolonged survival, although in these individuals the disease is often milder or the patients receive prolonged ventilation (Biancalana, 2003); therefore, the older population is not representative of infants and children, which display the more characteristically aggressive form of the disease.
- If AT132 were to be investigated in the adult population first, there would be a delay in providing a potentially life-saving therapy to infants with XLMTM; this delay may not be considered ethically appropriate. To illustrate the fragility of the patient population, 3 infant boys from INCEPTUS who were to be enrolled in ASPIRO died prior to enrollment in the study. The robust demonstration of efficacy of AT132 in mice and dogs strongly indicate that AT132 could have profound effects in patients with XLMTM and, therefore, enrollment of children could potentially save lives in this population.
- Infants and younger children have fewer comorbidities, lower accumulation of medical events (such as hypoxic damage and recurrent chest infections), and are not as debilitated as the few surviving older children or adults with XLMTM, which increases the possibility of demonstrating clinical benefit.
- Pre-existing neutralizing antibodies (NAbs) to AAV8 are less prevalent in younger children (Calcedo, 2011). Therefore, AAV8-mediated gene transfer therapy is likely to be most successful in the population of infants and young children.

The compelling nonclinical data that have been generated to date offer the prospect of direct benefit to the subject. Coupled with the high infant mortality and absence of any treatment for this ultra-orphan disease, it is appropriate to enroll infants and young children into this study. Special attention will be paid to the Protection of Human Subjects (45 Code of Federal Regulations [CFR] Part 46), Subpart D, Additional Protections for Children Involved as Subjects in Research; 21 CFR Subpart D Part 50 (50.50-50.56), Additional Safeguards for Children in Clinical Investigations; International Council for Harmonisation (ICH) E6, Good Clinical Practice (GCP); and E11, Clinical Investigation of Medicinal Products in the Pediatric Population.

3.3 Selection of Study Population

3.3.1 Inclusion Criteria

Subjects must meet all of the following inclusion criteria to be eligible for participation in this study. Subjects assigned to delayed-treatment control will be evaluated to confirm the subject still meets a subset (*) of the inclusion/exclusion criteria before receiving AT132.

1. Subject has a diagnosis of XLMTM resulting from a genetically confirmed mutation in the *MTM1* gene as assessed by a Sponsor-approved testing facility.*
2. Subject is male.*
3. Subject is aged less than 5 years old at dosing.*
4. Subject requires mechanical ventilatory support:
    - Part 1: Subject requires some mechanical ventilatory support (eg, ranging from 24 hours per day fulltime mechanical ventilation, to noninvasive support such as continuous positive airway pressure [CPAP] or bilevel positive airway pressure [BiPAP] during sleeping hours).
    - Part 2: Subject requires invasive mechanical ventilatory support ranging from 20 to 24 hours per day at screening (confirmed by daytime polysomnographic study).
5. Subject requiring invasive mechanical ventilator support is fitted with or willing to be fitted with a cuffed tracheostomy tube for some respiratory assessments.*
6. Subject has ventilator maximum positive end-expiratory pressure (PEEP) < 8 cmH_2O at screening.*
7. Signed informed consent by the parent(s) or legally authorized representative (LAR) (when applicable).*
8. Subject and parent(s)/LAR(s) are willing and able to comply with study visits and study procedures.*
9. UNIQUE to France: Subject’s weight is ≥ 4.8 kg.

3.3.2 Exclusion Criteria

Subjects who meet *any* of the following exclusion criteria are not to be enrolled in this study.

1. Subject is participating in an interventional study designed to treat XLMTM.*
2. Subject born < 35 weeks gestation who is still not term as per corrected age.
3. Subject tests positive for AAV8 neutralizing antibody with titers > 1:20 (subjects under the age of 18 months may be retested in cases where antibodies may have been maternally acquired and titers may decline in the first months of life).*
4. Subject had recent surgery (< 3 months before Day 1) or has planned surgery that may confound data collection during the first 48 weeks of the study.
5. Subject has a clinically important condition or life-threatening disease, other than XLMTM, in the opinion of the Investigator.*

6. Subject has a clinically significant underlying liver disease, defined as:
    - ≥ Grade 3 aspartate aminotransferase (AST) (> 5.0 x ULN; Common Terminology Criteria for Adverse Events [CTCAE] v. 4.03)*
    - ≥ Grade 3 alanine aminotransferase (ALT) (> 5.0 x ULN; CTCAE v. 4.03)*
    - Hepatic peliosis or any other clinically significant structural abnormality detected by ultrasound*
7. Subject is currently experiencing a clinically important respiratory infection or other active infection.*
8. Subject has received pyridostigmine or any medication to treat XLMTM within 3 months before Day 1.*
9. Other than as required per protocol, subject has received immune-modulating agents within 3 months before Day 1 (use of inhaled corticosteroids to manage chronic respiratory conditions is allowed); use of other concomitant medications to manage chronic conditions must have been stable for at least 4 weeks before dosing.*
10. Subject has a contraindication to prednisolone.*
11. Subject has a contraindication to study drug or ingredients.*
12. Subject has previous scoliosis repair surgery/procedure, or planned/expected scoliosis repair surgery/procedure, in the 12 months following Day 1 (Part 2 including any subjects enrolled under protocol v8 and beyond).
13. Subject has contractures, scoliosis, or other medical condition that would limit the potential to achieve unassisted sitting, in the opinion of the Investigator (Part 2 including any subjects enrolled under protocol v8 and beyond).
14. Subject is able to sit without assistance for at least 30 seconds at screening, in the opinion of the Investigator (Part 2 including any subjects enrolled under protocol v8 and beyond).
15. Subject has a clinically important condition, including CTCAE v4.03 Grade ≥ 2 anemia (< 10 g/dL hemoglobin).*
16. Subject has a contraindication to ursodiol (ursodeoxycholic acid).*
17. UNIQUE to France: Subject has a prior diagnosis or history of cardiac arrhythmias, myocarditis, or any other cardiac disease.
18. UNIQUE to France: Subject has a contraindication to general anesthesia and to muscle biopsy procedures.

* If a subject is a delayed-treatment control, this inclusion/exclusion criterion must be met before receiving AT132.

3.3.3 Number of Sites and Subjects

Approximately 8 sites worldwide are anticipated to participate in this study. Approximately 16 subjects in Part 1 and 10 subjects in Part 2 were planned to be enrolled under protocol amendments v5.0 through v9.0. Protocol v10 and beyond do not allow for enrollment of any future subjects.

3.3.4 Subject Identification

Each subject will be given a unique identifier. If a subject undergoes multiple screening attempts, the same unique identifier will be retained.

3.3.5 Replacement of Subjects

Subjects who withdraw prior to Week 48 may be replaced. As of protocol v8, additional subjects may be enrolled if any of the 3 enrolled delayed-treatment control subjects who are yet to be dosed do not meet a subset of inclusion and exclusion criteria prior to dosing (see Sections 3.3.1 and 3.3.2, respectively). Any new subject enrolled will not be considered a delayed-treatment control and therefore will not be required to wait 24 weeks before administration of study drug. Any new subject will be enrolled under all of the inclusion/exclusion criteria (including Exclusion Criteria 12-14 required for Part 2 subjects).

3.4 Duration of Study Participation

The expected study participation is approximately 10 years after AT132 administration; approximately 12 years for control subjects who are ultimately administered AT132 (at least 24 weeks to 3 years delayed treatment and an additional 10 years after AT132 administration).

3.5 Removal of Subjects

3.5.1 Withdrawal of Subjects

It is critical to the integrity of this study that subjects adhere to the visit schedule outlined in this protocol and to complete all scheduled procedures. As such, the Investigator and clinic staff should make every reasonable effort to convey the importance of remaining on the study to the subject or parent(s)/LAR(s). Any subject withdrawing from the study should be reported as soon as possible to the Sponsor's Medical Monitor.

Subjects may withdraw from further participation in the study at any time and for any reason. Efforts will be made to collect important data, if feasible, and if the subject agrees.

Early withdrawal may occur for any of the following reasons:

- Subject request or withdrawal of consent
- AE
- Protocol deviation (at the Sponsor's discretion)
- Investigator discretion
- Study termination by the Sponsor
- Lost to follow up

In all cases, the reason(s) for study withdrawal will be recorded in the source document and on the appropriate electronic case report form (eCRF).

3.5.2 Withdrawal Follow-Up Procedures

The Investigator will make every reasonable effort to complete the protocol-specified follow-up procedures as specified in Section 5.9, including the follow up of any unresolved AEs as described in Section 6.1.3.2. See Section 5.10 for a description of assessments for delayed-treatment control subjects who are not eligible to be dosed.

3.6 Termination of the Study

If the Investigator, Sponsor or its designee, Medical Monitor, or DMC (see Sections 7.5 and 7.6) becomes aware of conditions or events that suggest a possible hazard to subjects if the clinical study continues, then the clinical study may be terminated. The clinical study may also be terminated at the Sponsor's discretion in the absence of such a finding, at any time and for any reason.

Conditions that may warrant termination of the clinical study include, but are not limited to:

- The discovery of an unexpected, relevant, or unacceptable risk to the subjects enrolled in the clinical study
- Failure to enroll subjects at the required rate
- A decision by the Sponsor to suspend the study, or to suspend or discontinue development of the study drug, for any reason

3.7 Start and End of Study Definitions

First act of recruitment

The study start date is the date on which the clinical study will be open for recruitment of participants. The first act of recruitment is the date the first participant signs the informed consent form (ICF) and will be the study start date.

End of study

The end of the study is defined as the last visit or assessment shown in schedule of assessments (Section 5.6) for the last participant in the study. A participant is considered to have completed the study if the participant has completed all periods of the study including the last assessment shown in the schedule of assessments.

4 STUDY TREATMENT

4.1 Investigational Medicinal Product (IMP)

4.1.1 Identity of IMP

AT132 (rAAV8-Des-hMTM1) is a genetically engineered AAV vector that expresses the human *MTM1* cDNA under the control of the muscle-specific human desmin gene promoter. Viral particles are pseudotyped with AAV8 capsid, providing tropism for and transduction of muscle tissue (Wang, 2005).

The IMP will be supplied as a sterile, liquid solution with the concentration noted on each of the single-use olefin polymer vials.

The IMP packaging will comply with current Good Manufacturing Practice (cGMP), GCP, and local regulatory requirements and may include the following information: Sponsor identification, IMP name, lot number, volume, concentration, storage conditions, precautionary statements, study identification, subject ID, and use-by date.

4.1.2 Shipping and Handling of IMP

The Sponsor will coordinate a shipment of the appropriate number of AT132-containing vials to the study site pharmacy for each subject. Details regarding this process will be outlined in the study Pharmacy Manual.

At the study site, the IMP must be stored frozen at < −60°C under restricted access. All IMP must be received, inventoried, stored, and dispensed with accountability through final disposition documented according to applicable state, federal, and local regulations; ICH guidelines; GCP; and study procedures.

Institutional policy/site procedure for destruction must be reviewed and approved by the Sponsor or designee in order to destroy IMP on site.

4.1.3 IMP Accountability, Return, and Disposition

The site is responsible for maintaining accurate records (including dates and quantities) of IMP administered to each subject and excess IMP vials. The site must retain all unopened and/or expired vials of AT132 in a secure location until a Sponsor designee has confirmed accountability data unless prohibited by site/institution policy. Details regarding the expected IMP accountability process are provided in the Pharmacy Manual.

Upon documented accountability, sites with an approved destruction process may release product for destruction. A site that does not have an adequate destruction process will return unopened and/or expired IMP to the Sponsor or drug depot per the pharmacy manual instructions.

4.1.4 Dosage and Administration of IMP

All study subjects will be administered a single dose of AT132 by IV infusion (control subjects will receive delayed treatment) as specified in the study Pharmacy Manual. The starting and current dose rationales are described in Section 1.4.2.

As of protocol v9, subjects not yet dosed were to receive 1.3×10^{14} vg/kg AT132 as determined by the 2nd generation vg titer assay (see Section 1.4.2).

As of protocol v10 and beyond, no additional subjects will be dosed.

4.1.5 Treatment Compliance

Study treatment will be administered by infusion to subjects at the study site by qualified personnel. Date, time, volume, and concentration of each dose must be recorded in the IMP preparation and IMP administration source documents. In the event that administration of study treatment is incomplete, the site should record the reason and any other pertinent information in the source document.

4.2 Method of Assigning Subjects to Treatment Groups

Randomization codes will be preassigned for each subject before enrollment into the cohort. In Part 2, subjects will be matched with another subject close in age (±6 months) and will then be randomized to study treatment or delayed-treatment control. Subjects enrolled under protocol v8 and beyond will be assigned to study treatment within Part 2.

After confirmation of subject's eligibility by the Investigator, the Sponsor will provide to the Investigator the assigned dose or control designation for the subject number according to the randomization sequence and the order of subjects eligible for randomization. For subjects not assigned to the delayed-treatment control arm, the Sponsor will coordinate shipment of the appropriate number of AT132 vials to the study site pharmacy. Further details regarding this process will be outlined in the Pharmacy Manual.

4.3 Blinding

This is an open-label study. The subjects, their caregivers, the site staff, the Sponsor and delegates will be unblinded to study treatment assignment. However, the Biopsy Review Committee will be blinded to study treatment assignment or study visit per Section 7.7.

4.4 Prophylaxis with Glucocorticoids

Subjects will be administered a course of concomitant glucocorticoid therapy commencing 1 day prior to AT132 dosing, and continuing for a period of approximately 8 weeks, then tapering from the original dose over 8 weeks per Investigator discretion. This is intended to prevent any immune-mediated hepatic injury, which has been observed in subjects who have received systemic AAV gene therapy (discussed in Section 6.5.2). For subjects weighing < 60 kg, the dose will be 1 mg/kg prednisolone orally daily for 8 weeks, then tapered down for 8 weeks per

Investigator discretion. For subjects weighing ≥ 60 kg, the dose will be 60 mg prednisolone orally daily for 8 weeks, then tapered down for 8 weeks per Investigator discretion. Investigators should closely monitor all liver function (see Appendix 1) results throughout the study. Management of prophylactic prednisolone dosing in the setting of abnormal liver function test (LFT) results is discussed in Section 4.6.

The suggested prednisolone tapering regimen for subjects weighing < 60 kg is:

- Weeks 1 to 8: 1.0 mg/kg/day
- Weeks 8 to 9: 0.75 mg/kg/day
- Weeks 9 to 10: 0.50 mg/kg/day
- Weeks 10 to 11: 0.25 mg/kg/day
- Weeks 11 to 12: 0.125 mg/kg/day
- Weeks 13 to 14: 0.0625 mg/kg/day
- Weeks 15 to 16: 0.03125 mg/kg/day
- Week 16: Off

The suggested prednisolone tapering regimen for subjects weighing ≥ 60 kg is:

- Weeks 1 to 8: 60 mg/day
- Weeks 8 to 9: 45 mg/day
- Weeks 9 to 10: 30 mg/day
- Weeks 10 to 11: 15 mg/day
- Weeks 11 to 12: 7.5 mg/day
- Weeks 13 to 14: 3.75 mg/day
- Weeks 15 to 16: 1.875 mg/day
- Week 16: Off

Tapering duration may be altered and/or supplemental administration of IV steroids (eg, methylprednisolone) or other immunosuppressive regimens may be considered in cases of potential malabsorption of oral medications.

Side effects associated with oral prednisolone are well understood, and experience exists with use of steroids in pediatric populations; the most significant adverse effects (eg, growth suppression in children) usually occur after longer courses of treatment. Therefore, providing a modest dose [consistent with that used for a number of conditions, see the respective local prescribing information] of a short course of oral prednisolone is considered appropriate and an acceptable risk. UNIQUE to France: At the time of prescription there should be compliance, especially with contraindications, warnings, and precautions for use, with particular attention to drug interactions. Refer to the information available on the Public Drug Database, accessible through the Internet at: http://base-donnees-publique.medicaments.gouv.fr/.

Glucocorticoids will not be provided directly by the Sponsor but rather by a prescription from the Investigator (or another provider with notification and approval by the Investigator) and will be obtained from a local pharmacy.

4.5 Prophylaxis with Ursodiol

As prophylaxis for possible cholestatic syndromes (see Section 6.5.3.3), subjects in this study will receive 20 mg/kg/day ursodiol (ursodeoxycholic acid) administered enterally, divided either twice daily (BID) or three times a day (TID) at the Investigator's discretion. Subjects who are not already on ursodiol will begin treatment approximately 1 week before AT132 administration (see Schedule of Events in Section 5.4). Subjects who enter the study on ursodiol will continue treatment and will be adjusted to the protocol-specified dose regimen. Ursodiol treatment will continue to Week 12 and will be administered per the Investigator's discretion thereafter (see Schedule of Events in Sections 5.4 and 5.5), which may be influenced by hepatic laboratory and clinical status.

Appropriate care for an individual subject will be determined by the Investigator based on an assessment of the subject's overall medical status.

Ursodiol is generally well-tolerated and minimal side effects, most gastrointestinal in nature, have been described (see the respective local prescribing information). Therefore, a course of ursodiol as outlined in this protocol is considered appropriate and of acceptable risk.

Ursodiol will not be provided directly by the Sponsor but rather by a prescription from the Investigator (or another provider with notification and approval by the Investigator) and will be obtained from a local pharmacy.

4.6 Monitoring for and Management of Cardiac, Liver, and Neuromuscular Related Safety Events

4.6.1 Monitoring for and Management of Myocarditis

4.6.1.1 Monitoring for Myocarditis

Site Investigators are responsible for prompt review of all laboratory or procedural results. Due to the importance of accurate diagnosis and management of myocarditis for subject safety, sites must identify and establish a site pediatric cardiologist experienced in the treatment of myocarditis. The site pediatric cardiologist will assist the study site and any managing physician responsible for patient care with interpretation of abnormal cardiac test results and treatment. A high level of awareness and vigilance for potential myocarditis should be maintained in the setting of gene therapy clinical studies.

The primary monitoring strategy for identifying potential myocarditis will be by clinical assessment and scheduled (surveillance) high-sensitivity Troponin T and/or Troponin I testing. Troponin elevations can be an early signal of myocarditis, a serious cardiac condition that is critical to promptly diagnose and treat. The frequency of assessments is outlined in the

Schedule of Events (Sections 5.4, 5.5, 5.6, and 5.7). Additional assessments may be performed at the discretion of the investigator and pediatric cardiologist as outlined in Section 4.6.1.3.

4.6.1.2 Clinical Role of the Site Pediatric Cardiologist

The site pediatric cardiologist will serve as:

- Cardiology resource for the study site and clinicians who manage subjects enrolled in the ASPIRO study
- Primary reviewer of routine surveillance cardiac testing abnormalities identified by Investigators (Section 4.6.1.3)
- Specialist relative to the diagnosis and management of myocarditis or suspected myocarditis

As a member of the study site medical team, the site pediatric cardiologist will work together with the Investigator to assist with diagnosis, interpretation of cardiac testing, and treatment. In the case where the site pediatric cardiologist does not have direct access to the subject and no local cardiologist is available, the site pediatric cardiologist will provide guidance to the managing physician.

4.6.1.3 Diagnosis and Management of Myocarditis or Asymptomatic Troponin Elevations

Clinical assessment, laboratory testing, and cardiac imaging can be used to detect myocarditis associated with AT132 administration. Documentation is required for data and discussions related to diagnosis and treatment. The Investigator for the study is responsible for all study-related medical decisions.

Routine Myocarditis Surveillance

- Troponin I and/or Troponin T will be assessed at scheduled visits. Investigators will consult the site pediatric cardiologist when Troponin T and/or Troponin I are above the ULN for the laboratory reference range. All laboratory reference ranges are per the reporting of the local or central laboratory, as applicable.

Additional Testing for Myocarditis Diagnosis and Management

Test selection is performed at the discretion of the investigator in conjunction with consultation with the site pediatric cardiologist. Key testing that may assist in diagnosis and management of myocarditis includes but is not limited to:

- Laboratory testing: A variety of laboratory testing can be useful in the diagnosis and management of myocarditis, including but not limited to complete blood count and differential, complete metabolic panel, troponin I and/or troponin T, N-terminal pro b-type natriuretic peptide, sedimentation rate, C-reactive protein, infectious assessments (eg, nasopharyngeal and rectal swabs, viral titers), CK with isoenzymes to identify elevations in the myocardial isoenzyme (CK-MB), and interferon gamma (IFN-γ) enzyme-linked immunosorbent spot assay (IFN-γ ELISpot) for MTM1 peptide pool and AAV8 peptide pool. Anti-MTM1 antibodies are optional.

- ECG
- Transthoracic ECHO
- Cardiac MRI
- Cardiac catheterization with endomyocardial biopsy (in exceptional cases)

4.6.2 Monitoring for and Management of Hepatobiliary Toxicity

Hepatobiliary toxicity has been reported with AAV gene therapy treatment (Chan, 2020) and in this study (ATX-MTM-002). In Study ATX-MTM-002, AT132-related SAEs involving the hepatobiliary system, including intrahepatic cholestasis, have occurred in subjects with XLMTM treated at the 1.0×10^{14} vg/kg and 3.0×10^{14} vg/kg dose levels. Subjects will be administered glucocorticoid prophylaxis and ursodiol prophylaxis as outlined in Sections 4.4 and 4.5, respectively. Routine surveillance and evaluation of any signs or symptoms of hepatobiliary toxicity is an essential safety parameter. The primary monitoring strategy for identifying potential hepatobiliary toxicity will be through frequent, scheduled testing of the following LFTs: ALT, AST, gamma-glutamyltransferase (GGT), total bilirubin (TBL), and direct bilirubin. Additional monitoring will include scheduled liver ultrasound procedures and as of protocol v10 serum bile acid levels in order to better understand the long-term behavior of intrahepatic cholestasis in XLMTM subjects. The frequency of these assessments is outlined in the Schedule of Events (Sections 5.4, 5.5, and 5.6).

Site Investigators are responsible for prompt review of all laboratory or procedural results related to potential hepatobiliary toxicity. To enhance the monitoring and management of potential hepatotoxicity, sites will identify a site hepatologist (preferably with expertise in pediatric cholestatic disease). The site hepatologist will assist the study site and any managing physician responsible for patient care with interpretation of abnormal hepatobiliary test results and treatment. A high level of awareness and vigilance for potential hepatobiliary toxicity should be maintained in the setting of gene therapy clinical studies. Details related to the monitoring, diagnosis, and treatment of hepatobiliary toxicity are described herein. Any laboratory tests and/or procedures performed for these safety events should be reported in the eCRF.

4.6.2.1 Clinical Role of the Site Hepatologist

Given that AT132-related SAEs involving the hepatobiliary system, including severe intrahepatic cholestatic disease, have been observed in subjects with XLMTM administered AT132 at the 1.0×10^{14} vg/kg and 3.0×10^{14} vg/kg dose levels, the site hepatologist will serve as the:

- Expert hepatology resource for the study site and clinicians who manage ASPIRO subjects
- Primary specialist to assist in the diagnosis and management of hepatobiliary toxicity noted in subjects following treatment with AT132

As a member of the study site medical team, the site hepatologist will work together with the Investigator to assist with interpretation of abnormal surveillance results, and if indicated,

additional diagnostic workup and treatment. In the case where the site hepatologist does not have direct access to the subject and no local hepatologist is available, the site hepatologist will provide guidance to the treating clinician to ensure subject safety. In all cases, the clinician that is providing direct patient care is ultimately responsible and the decision maker for the management and treatment of the subject.

4.6.2.2 Monitoring for Hepatobiliary Toxicity Risk

A combination of hepatobiliary laboratory tests and procedures will be used to monitor for cases of potential toxicity after treatment with AT132. The following notification and consultative parameters are provided to assist Investigators, and/or other clinicians caring for ASPIRO subjects, to identify clinically significant abnormalities during routine hepatobiliary monitoring. All laboratory reference ranges are per the reporting of the local or central laboratory, as applicable.

Routine Hepatobiliary Surveillance

- Investigators will consult the site hepatologist for any of the following criteria:
- GGT, AST, or ALT: ≥ 5 × ULN (or ≥ 3 × the Baseline Visit value if it was > ULN)
- TBL or direct bilirubin: ≥ 3 × ULN (or ≥ 2 × the Baseline Visit value if it was > ULN)
- Any abnormal liver ultrasound finding
- Serum bile acid, total or fraction: ≥ 2 × ULN

Abnormal Hepatobiliary Laboratory or Procedural Results not Associated with Routine Surveillance per the Protocol Schedule of Events

- The approach for identifying potential hepatobiliary toxicity in this setting may be more complex (eg, the subject is hospitalized)
- The Investigator should consult with the site hepatologist and notify the Medical Monitor to determine an appropriate follow-up plan based on the clinical circumstance

Communication Guidelines

- Details and discussions around the monitoring, diagnostic, and treatment (Section 4.6.2.3) activities of a subject that include the site hepatologist are required to be documented

4.6.2.3 Risk Management of Hepatobiliary Toxicity

Management of subjects with hepatobiliary toxicity will depend on the interpretation of all available clinical data.

- The site hepatologist must be actively involved in any case where the LFTs are higher than the values listed in the routine surveillance guidelines (Section 4.6.2.2). Management in this setting may be highly variable and may require additional diagnostic testing and/or alteration of the existing immunosuppressive regimen.

- In cases where the LFTs are rising but are within the values listed in the routine surveillance guidelines (Section 4.6.2.2) and the subject is on prophylactic glucocorticoids, prednisolone adjustments may be made at the discretion of the Investigator based on the stage of prophylaxis (steady dose or tapering). For cases refractory to prednisolone adjustment, consult the Medical Monitor.
- In cases where the ALT or AST are rising but are within the values listed in the routine surveillance guidelines (Section 4.6.2.2) and the subject is not on prophylactic glucocorticoids, consultation with the Medical Monitor is recommended, and management may require additional diagnostic testing and/or treatment with immunosuppressive agents
- In cases of clinically significant elevated LFTs and/or elevated CK that in the Investigator's judgment may be related to T cell responses, repeat cytokine panel and IFN-γ ELISpot response testing is suggested
- Following the Week 4 complement panel collection, in cases of clinically significant elevations in LFTs (and/or thrombocytopenia, acute renal insufficiency, or anemia), repeat complement panel testing is suggested

4.6.3 Monitoring for and Management of Neuromuscular Abnormalities

4.6.3.1 Monitoring for Neuromuscular Abnormalities

Acute, subacute, and chronic CK elevations can occur after gene therapy administration. Muscle abnormalities vary and can include myositis, which can be asymptomatic or display muscle weakness. Neuromuscular abnormalities may manifest as bulbar, motor, and/or respiratory weakness.

Site Investigators are responsible for prompt review of all laboratory or procedural results. Due to the importance of accurate diagnosis and management of neuromuscular dysfunction for subject safety, consultation with a pediatric pulmonologist is required for potential decline in respiratory function. The site pediatric pulmonologist will assist the study site and any managing physician responsible for patient care with interpretation of abnormal pulmonary test results and treatment. Due to risks associated with worsening bulbar function, a pediatric ear, nose and throat (ENT) specialist and/or speech-language pathologists will be consulted if worsening bulbar dysfunction is suspected.

The primary monitoring strategy for identifying potential neuromuscular abnormalities will be by clinical assessment and scheduled (surveillance) of CK levels in blood. The frequency of these assessments is outlined in the Schedule of Events (Sections 5.4, 5.5, 5.6, and 5.7). Additional assessments may be performed at the discretion of the investigator and pediatric neuromuscular specialist as outlined in Section 4.6.3.2.

4.6.3.2 Diagnosis and Management of Neuromuscular Abnormalities

Clinical assessment, laboratory testing, and muscle imaging can be used to detect neuromuscular abnormalities associated with AT132 administration. Documentation is required

for data and discussions related to diagnosis and treatment. The Investigator for the study is responsible for all study-related medical decisions.

Routine neuromuscular surveillance

- Physical examination with detailed neurological evaluation and respiratory function assessment
- CK will be measured at scheduled visits. If the CK is above the ULN for the laboratory reference range, the investigator will decide whether additional diagnostic evaluation is needed.

Additional testing for diagnosis and management of neuromuscular abnormalities

Test selection is performed at the discretion of the investigator in conjunction with consultation with the neuromuscular specialist as needed. Additional consultations with a pediatric ENT specialist, speech-language pathologist, pediatric pulmonologist, and a pediatric neuromuscular specialist may be needed. Key testing that may assist in diagnosis and management of neuromuscular abnormalities includes but is not limited to:

- Laboratory testing: A variety of laboratory testing can be useful in the diagnosis and management of neuromuscular abnormalities, including but not limited to complete blood count and differential, complete metabolic panel, sedimentation rate, C-reactive protein, infectious assessments (eg, nasopharyngeal and rectal swabs, viral titers), CK , CK-isoenzymes for identification of elevations in the skeletal muscle isoenzyme (CK-MM) or CK-MB, and IFN-γ ELISpot for MTM1 peptide pool and AAV8 peptide pool. Anti-MTM1 antibodies are optional.
- Electrophysiological assessment (electromyography [EMG])
- Skeletal muscle MRI with short tau inversion recovery (STIR) sequence
- Diagnostic muscle biopsy

4.7 Prior and Concomitant Medications

All prescription and over-the-counter medications taken by subjects for 3 months prior to the Screening Visit through the end of the study will be recorded on the concomitant medications eCRF. Receipt of SARS-CoV-2 vaccine products at *any* time prior to Screening Visit through the end of the study will be recorded on the concomitant medications eCRF. The Investigator may prescribe additional medications during the study, as long as the prescribed medication is not prohibited by the protocol as described in Section 4.8. In the event of an emergency, any needed medications may be prescribed without prior approval, but the Medical Monitor must be notified of the use of any excluded medications immediately thereafter. Any concomitant medications administered or discontinued during the study should be recorded on the eCRF. For guidance regarding the administration of vaccines, including timing considerations, to subjects on prolonged glucocorticoid regimens, refer to local country guidelines (eg, the *Centers for Disease Control and Prevention [CDC] General Best Practice Guidelines for Immunization:*

Best Practices Guidance of the Advisory Committee on Immunization Practices [ACIP]; Altered Immunocompetence [CDC, 2020]).

4.8 Excluded Therapies

Investigational therapies, other than AT132, for XLMTM are not allowed during the first 24 months after the subject receives AT132. The allowance of investigational therapies that are NOT related to XLMTM (eg, experimental vaccines or treatments for infectious diseases) will be determined on a case-by-case basis. Subjects should not be administered such investigational therapies without first obtaining approval from the Medical Monitor.

The therapies listed below are excluded during the course of the study:

- Pyridostigmine
- Other than the prescribed oral prednisolone or the use of an immunosuppressive agent in response to a specific condition called out in the protocol, any immune-modifying medication is strongly discouraged during the course of the study (use of inhaled corticosteroids to manage chronic respiratory conditions is allowed)

4.9 Physical Therapy

Physical therapists will continue to provide a consistent standard of care therapy for subjects in ASPIRO through Week 48. Following Week 48, if a subject has achieved new motor milestones, a physical therapist should alter their care to ensure a subject maintains these achieved milestones.

5 STUDY ASSESSMENTS AND SCHEDULE OF STUDY ASSESSMENTS

5.1 Study Assessments

The Investigator is responsible for ensuring that all staff involved in the study are familiar and comply with the content of this section. The following describes the study procedures to be performed during the study. The Schedule of Events is provided in Section 5.2. For each subject, assessments should be conducted at approximately the same time of day at each visit, and in the same order, if possible. All study assessments will be recorded on the eCRF.

In the event of unforeseen circumstances (eg, a global pandemic), Investigators are encouraged to engage with their governing Institutional Review Board (IRB)/Ethics Committee (EC) as early as possible when urgent or emergent changes to the protocol or informed consent are anticipated. Attempts should be made to conduct safety monitoring and other efficacy assessments even if on-site visits are not possible. Sites shall discuss any expected modifications to the protocol visit schedule or assessments with the Sponsor to protect study subjects and manage study conduct.

The total volume of blood collected from each study subject is to be as small as possible, using pediatric tubes and the smallest volumes possible, while still allowing for validity of each test and protecting the safety of subjects. For a 1-year-old child, the 50th percentile average weight is 10.5 kg/23 lb, with a corresponding blood volume limit of 31.5 mL daily and 78.75 mL for an 8-week period (Howie, 2011). Blood sample volumes for this study are approximately 2 to 18 mL per visit, with a maximum of approximately 90 mL over an 8-week period. If the blood volumes at a visit exceed the maximum amount allowed, the study site should prioritize the assessments to be conducted and discuss with the Medical Monitor.

UNIQUE to France: For a 1-year-old child, the 50th percentile average weight is 10.5 kg/23 lb, with a corresponding blood volume limit of 20 mL daily and 40 mL for a 4-week period (Howie, 2011). Blood sample volumes for this study are 2.0 to 10 mL per visit, with a maximum of approximately 36.1 mL over a 4-week period. For visits where the amount of blood exceeds the limits stated above, the site should discuss with the Medical Monitor which samples should be prioritized.

5.1.1 Description of Study Assessments

5.1.1.1 Medical History

All conditions and procedures occurring before the ICF is signed will be recorded as medical history. The name of the disease state (ie, XLMTM) does not need to be recorded as medical history. From the time of obtaining informed consent, all SAEs should be reported as such, if the Investigator believes they may have been caused by a protocol-required procedure. All other untoward medical occurrences observed during the screening period through Day -1, including exacerbation or changes in medical history, are to be captured on the medical history eCRF. The medical history at baseline should be updated to capture changes since the Screening

Visit. The Sponsor retains the right to request additional information regarding a medical history condition, if judged necessary. See Section 6 for details.

5.1.1.2 Procedures

All procedures occurring after the ICF is signed but prior to dosing (Day -1) will be recorded as medical history. All procedures occurring after dosing will be recorded on the procedures eCRF.

5.1.1.3 Genetic Testing

At screening, *MTM1* genetic testing will be conducted to confirm a diagnosis of XLMTM for subjects without previous confirmation by a Sponsor-approved testing facility.

Additionally, whole genome analysis may be conducted on leftover samples to further understand the molecular basis of XLMTM disease and interindividual heterogenicity in treatment response (see Appendix 9 Genetics for information regarding genetic research).

5.1.1.4 Human Leukocyte Antigen (HLA Testing)

A laboratory test to identify the major HLA genes that the subject has inherited and their corresponding antigens that are present on the surface of their cells. The sample will only need to be collected 1 time during the study. The sample will be collected at the Week 12 visit, unless HLA type has been obtained in INCEPTUS. For subjects in Part 1 who have already passed the timepoint listed above, the HLA sample will be collected at the Week 48 visit or the Month 24 visit, whichever occurs first. HLA typing will be used to study association of potential AEs in the ongoing and future potential gene therapy studies with particular HLA alleles.

5.1.1.5 Physical Examination

A complete physical examination should include all body systems pertinent to the subject. Abbreviated physical examinations should focus on general appearance, cardiovascular, pulmonary, and abdominal examinations, as well as investigation of reported symptoms. Any clinically significant abnormalities observed should be recorded as medical history or AEs as described in Section 6.

5.1.1.6 Vital Signs

Vital signs for this study include blood pressure, heart rate, respiration rate (RR), and temperature. Record whether the subject was on or off of ventilatory support when vital signs are taken.

5.1.1.7 Concomitant Medications

All medications from the time of informed consent to when the subject withdraws or completes the study will be recorded on the concomitant medications eCRF. In this study, subjects will receive concomitant prophylactic glucocorticoid (see Section 4.4) and ursodiol (see Section 4.5).

5.1.1.8 Standard Safety Laboratory Tests (Chemistry, Hematology, Coagulation, Urinalysis)

Standard safety laboratory tests (chemistry, hematology, coagulation, urinalysis; as specified in Appendix 1) will be conducted using a combination of the central laboratory and local laboratories. It is recommended to use the central laboratory to analyze laboratory tests that are drawn prior to dosing, and after the Week 16 Visit and to use the local laboratory to analyze laboratory tests that are drawn at Baseline, Dosing, Days 1 and 2, and Weeks 1 through Week 16. However, the local laboratory can be used for other visits if needed (for example, the local laboratory can accommodate the analysis with a smaller amount of blood) or if the local laboratory does not accommodate the appropriate analysis, the central laboratory may be used. Abnormal laboratory test results will be reported as described in Section 6. Laboratory samples may be collected by a trained home health nurse for protocol-required study visits and unscheduled visits, as needed. Samples collected by a home health nurse will be sent to the central laboratory for processing.

In addition to standard safety laboratory tests, samples for troponin T and/or I, cytokine panels, and complement panels will be collected as per the Schedule of Events in Sections 5.3.1, 5.4, 5.5, 5.6, and 5.7. Troponin T and/or troponin I will be monitored to assess cardiac safety. See Section 4.6.1 for details related to the evaluation of cardiac enzymes; monitoring, diagnosis, and treatment of myocarditis; and the role of the site pediatric cardiologist. The cytokine profile of each subject will be established before dosing of study drug. Complement panel details are provided in Section 5.1.1.28. It is the Investigator's responsibility to perform clinical laboratory assessments more frequently, if clinically indicated.

Samples for laboratory tests will be collected as per Appendix 1 and the Schedule of Events in Sections 5.3.1, 5.4, 5.5, 5.6, and 5.7. Additional samples may be collected as clinically indicated per Section 6.5. Refer to study Laboratory Manual for additional sample handling details.

5.1.1.9 Liver Ultrasound

Hepatic peliosis, an abnormality of the hepatic vasculature, which has the potential to cause a dramatic hemorrhage, is a rare but recognized complication of XLMTM. Serial ultrasound assessments will be used to assess change in size, shape, and structure of the liver, and will be evaluated in conjunction with serum LFTs. Refer to Section 4.6.2 for details related to the monitoring, diagnosis, and treatment of hepatobiliary toxicity and the role of the site hepatologist.

5.1.1.10 Cardiac Structure and Function (ECG, ECHO and Cardiac MRI)

Unlike many neuromuscular diseases, individuals with XLMTM do not exhibit cardiac muscle disease. Mice treated with rAAV8-Des-MTM1 had asymptomatic cardiac lesions detected on necropsy (in the presence of gross over expression of myotubularin) (Section 1.2.1.3). It is unknown whether a risk exists for human subjects. Cardiac structure and function will be

routinely assessed using 12-lead ECG and ECHOs. Cardiac MRI may be performed at the discretion of the investigator in conjunction with consultation with the site pediatric cardiologist. If myocarditis is observed, cardiac catheterization and endomyocardial biopsy may be needed in exceptional cases.

If troponin I and/or troponin T is elevated above the ULN of the laboratory reference range, test selection for diagnosis and management of myocarditis or asymptomatic troponin elevation is performed at the discretion of the investigator in conjunction with consultation with the site pediatric cardiologist. Key testing is discussed below. Refer to Section 4.6.1 for management of ECG, ECHO, and Cardiac MRI results and the role of the site pediatric cardiologist.

- ECG: In patients with suspected myocarditis, the ECG can display a variety of non-specific abnormalities. Interpretation of ECG by the site pediatric cardiologist may be required due to the array of ECG changes that can occur with myocarditis.
- Transthoracic ECHO: ECHO is a safe, widely available, and clinically extremely useful cardiac imaging tool, particularly for the initial assessment of myocarditis. The American College of Cardiology (ACC), American Heart Association (AHA), and European Society of Cardiology (ESC) Working Group on Myocardial and Pericardial Diseases recommend that all patients with clinically suspected myocarditis should undergo an ECHO at initial presentation (Adeboye, 2022).
- Cardiac MRI: Cardiac MRI is considered the non-invasive reference standard for diagnosis of myocarditis in the absence of contraindications to MRI. Gadolinium may be used to assess for late gadolinium enhancement, a technique used for cardiac tissue characterization due to various types of myocardial injury and fibrosis. Cardiac MRI findings change depending on the phase of disease and timing of imaging. Myocardial edema is a characteristic early feature of acute myocarditis which typically improves over days to weeks and may not be detected if cardiac MRI is performed weeks to months after symptom onset. Diagnostic sensitivity is highest within 2 to 4 weeks of symptom onset; therefore, cardiac MRI should be performed quickly in patients with suspected myocarditis (Urzua Fresno, 2023).
- Cardiac catheterization with endomyocardial biopsy in exceptional cases: Although the gold standard for diagnosis of myocarditis is endomyocardial biopsy, this procedure is usually not required for diagnosis and management. Considering this procedure's invasiveness, low sensitivity when myocardial involvement is focal, and a complication rate ranging from 1% to 15%, the pediatric cardiologist may consider the clinical presentation to be of sufficient severity to necessitate the procedure, especially in uncommon clinical scenarios such as severe heart failure, cardiogenic shock, ventricular arrhythmias, and/or when there is significant diagnostic uncertainty, aiming to ensure the requirement of the procedure (Pilati, 2022).

5.1.1.11 Respiratory Function

5.1.1.11.1 Assessment of Ventilator Requirements with Polysomnogram

Daytime polysomnograms (ie, respiratory physiology studies) will be performed at Screening and at Week 24 to assess the number of hours of ventilator support required. Daytime polysomnograms will also be performed at Week 48 only if the subject is still on ventilator at the

time of the visit. For example, if the subject has discontinued the ventilator at Week 40, the Week 48 daytime polysomnogram is not required.

At Screening, ventilatory support will be provided for approximately 3 hours while observing the subject and subsequently the ventilatory support will be removed.

At Week 24 and Week 48, ventilatory support will be provided for approximately 1 hour while observing the subject and subsequently the ventilatory support will be removed. Additionally, if a subject is already off ventilator for 1 hour or more, the 1-hour observation period will not be required.

At Screening, Week 24, and Week 48 if any of the following criteria are met following the removal of ventilatory support, the polysomnogram will be stopped and the ventilator support will be reintroduced:

- Oxygen saturation (SpO_2) < 94% on room air for at least 20 seconds
- Increased end tidal carbon dioxide ($ETCO_2$) or transcutaneous carbon dioxide (TCO_2) of 10 mmHg or 1.33 kPA for at least 20 seconds
- $ETCO_2$ or TCO_2 > 50 mmHg for at least 20 seconds
- Increased respiratory rate by 25% over baseline value

At Screening, if the subject does not reach criteria for the reintroduction of ventilation, they will remain off of the ventilator for a maximum of 4 hours.

At Week 24 and Week 48 if the subject does not reach criteria for the reintroduction of ventilation, they will remain off of the ventilator for a minimum of 6 hours plus the amount of time off at Screening. For example, if at Screening, the subject was off the ventilator for 15 minutes, the daytime polysomnogram at Week 24 should be a minimum of 6 hours and 15 minutes off the ventilator.

5.1.1.11.2 Assessment of Time Off Ventilator

5.1.1.11.2.1 Daily Ventilator Dependence Diary

For subjects in Part 1 who have not completed the Week 24 visit and for subjects in Part 2 (including any subjects enrolled under protocol v5 and beyond), an electronic diary will be completed by the parent/LAR/caregiver to collect the type of ventilatory support and the amount of time the subject spent off of ventilatory support in a 24-hour period. The diary should be completed daily by the same parent/LAR/caregiver (whenever possible). Site staff should set up the electronic diary prior to distribution to the parent/LAR/caregiver and review the training with the parent/LAR/caregiver. The daily diary collection will begin after informed consent (or reconsent to protocol v5 or later for Part 1 subjects) and continue through Week 48. However, if after the Week 24 visit, ventilator support has been discontinued, the daily diary will no longer need to be completed. If ventilator support is resumed, the daily diary completion will resume through Week 48 or discontinuation of the ventilatory support, whichever occurs first. For

subjects in Part 1 who have already completed the Week 24 visit, a daily diary will not be completed.

5.1.1.11.2.2 Ventilator Dependence Questionnaire

A questionnaire will be completed by the parent/LAR/caregiver to collect the type of ventilatory support and the amount of time the subject spent off of ventilatory support in a 24-hour period at clinic and contact visits (Appendix 4).

For subjects in Part 1 who have already completed the Week 24 visit, the parent/LAR/caregiver will complete the ventilator dependence questionnaire at clinic and contact visits through Month 120.

For subjects in Part 1 who have not completed the Week 24 visit and for subjects in Part 2 (including any subjects enrolled under protocol v5 and beyond), the daily diary will be completed per Section 5.1.1.11.2.1. After the daily diary has been stopped or the subject has completed Week 48, the parent/LAR/caregiver will complete the ventilator dependence questionnaire at clinic and contact visits through Month 120.

5.1.1.11.3 Guidelines for Initiating Ventilator Weaning

Weaning is the process of decreasing the amount of support that the subject receives from the mechanical ventilator, so that the subject assumes a greater proportion of the ventilatory effort. The objective is to assess the probability that mechanical ventilation can be successfully discontinued.

Weaning from invasive or noninvasive ventilatory support will be conducted in collaboration with the Investigator, site pulmonologist, and the subject's local pulmonologist, if applicable. The site pulmonologist and the local pulmonologist, if applicable, will review the results from the visit, assess the subject, and determine if the subject meets the criteria outlined in Table 1 to begin reduction of hours on the ventilator. Once the subject meets and maintains the criteria outlined in Table 1, the site pulmonologist and the local pulmonologist, if applicable, may change the ventilator settings and reduce the prescribed number of hours at their discretion. Refer to the ASPIRO: ATX-MTM-002 Ventilator Weaning and Discontinuation Manual for recommendations on ventilator weaning. The actual number of hours of ventilatory support a subject receives daily will be recorded via the electronic ventilator dependence diary or the Ventilator Dependence Questionnaire (Section 5.1.1.11.2.1).

Table 1: Required Parameters for Initiation of Ventilator Weaning

Assessments	Parameters
Vital signs	Normal for age
Weight	Normal for age
Respiratory function tests	
Maximal inspiratory pressure (MIP)	> 50 cmH_2O
Maximal expiratory pressure (MEP)	> 40 cmH_2O
Positive end-expiratory pressure (PEEP)	≤ 5 cmH_2O
Oxygen saturation (SpO_2)	> 94%
Transcutaneous CO_2 (TCO_2)	35-45 mmHg
End tidal CO_2 ($ETCO_2$)	35-45 mmHg
Gas exchange markers	
Serum bicarbonate	22-27 mEq/L
Clinical judgement	Consider week-to-week clinical improvements in motor milestones (rolling over, head control, sitting unassisted); vocalization; coughing; secretions, and neuromuscular function tests (CHOP INTEND)

CHOP INTEND = Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders

The above parameters are to be assessed by site personnel during the normal routine site visits through Month 42. As of protocol v12, beginning at the Month 48 visit, some parameters (ie, MIP/MEP, $ETCO_2$, TCO_2) will no longer be collected per protocol and decision making related to initiation of ventilator weaning will occur by the site and local pulmonologists in collaboration with the Site Investigator, upon review of relevant clinical assessments and in consideration of standard practice in ventilator weaning in patients with neuromuscular disease.

5.1.1.11.4 Guidelines for Discontinuation of Ventilatory Support

Discontinuation of mechanical ventilation can be attempted after the Week 12 visit, if appropriate. The site pulmonologist and the subject's local pulmonologist, if applicable, will determine if the subject is ready to discontinue ventilatory support based on the parameters outlined in Table 1 and Table 2.

At the Week 12 visit, the site pulmonologist and local pulmonologist, if applicable, should review the results from the previous assessments and determine if the subject continues to meet the criteria outlined in Table 1. If the subject meets the criteria in Table 1, the assessments in Table 2 should be conducted to assess readiness to discontinue ventilatory support. If the subject meets the criteria outlined in Table 2 and the pulmonologist and Investigator determine the subject is ready, the ventilator can be discontinued. The assessments in Table 2 should be conducted before discontinuing nighttime ventilation and should be repeated 3 to 4 weeks after discontinuation of nighttime ventilation. If the subject does not meet the criteria in either Table 1

and Table 2, the pulmonologist should continue to assess the subject at subsequent visits to determine readiness to discontinue ventilatory support. Details on assessment for discontinuation of ventilatory support and for decannulation will be provided in a separate procedure manual.

It is advisable to wean tracheostomized subjects directly off ventilation to spontaneous respiration rather than to noninvasive ventilation (NIV) as an intermediate step because the masks required for NIV are not likely to be tolerated by infants and toddlers unaccustomed to masks.

Table 2: Required Ventilator Discontinuation Parameters

Assessments	Parameters
Gas exchange markers	
Sprinting (video record the assessment)	• No distress • SpO_2 > 94% • TCO_2 ≤ 45 mmHg • No intercostal retraction, tachypnea, respiratory paradox, or phase delay
Nocturnal Polysomnogram (PSG) (performed with trach open)	
Apnea-hypopnea index (AHI)	< 5 (lower threshold for NMD than for obstructive processes)
TCO_2	35-45 mmHg
$ETCO_2$	• Consider % of sleep time with $ETCO_2$ < 45 mmHg and <50 mmHg • Steady state capnometry in NREM and in REM sleep
Oxygen saturation (SpO_2)	> 94%
Respiration rate (RR)	Within the age adjusted norms
Sleep architecture	Normal for age (Berry, 2012). The following parameters will be assessed: • Sleep efficiency (%) • Sleep latency (min) • REM latency (min) • Arousal index (N/hr) • Stage N1 (%TST) • Stage N2 (%TST) • Stage N3 (%TST) • Stage R (%TST)

$ETCO_2$ = End tidal carbon dioxide; min = minute(s); NMD = neuromuscular disorders; NREM = non-rapid eye movement; REM = rapid eye movement; TCO_2 = transcutaneous carbon dioxide; TST = total sleep time

The above parameters are to be assessed by site personnel during the normal routine site visits through Month 42. As of protocol v12, beginning at the Month 48 visit, some parameters (ie, MIP/MEP, $ETCO_2$, TCO_2) will no longer be collected per protocol and decision making related to

discontinuation of ventilator support will occur by the site and local pulmonologists in collaboration with the Site Investigator, upon review of relevant clinical assessments and in consideration of standard practice in ventilator discontinuation in patients with neuromuscular disease.

5.1.1.11.5 Assessment of Ventilator Parameters

End tidal CO_2, transcutaneous CO_2, O_2 saturation, and serum bicarbonate (from the local or central laboratory) will provide information about the effectiveness of ventilation.

Assessment of ventilator parameters will include the baseline ventilator settings and changes in ventilator settings over time, end tidal CO_2, transcutaneous CO_2, respiratory rate, O_2 saturation, and serum bicarbonate during clinic visits. During the contact calls, the site will ask the LAR if the subject is currently using ventilator support.

Details on assessment of ventilator parameters will be provided in a separate procedure manual. The above parameters are to be assessed by site personnel during the normal routine site visits through Month 42. As of protocol v12, beginning at the Month 48 visit, the Assessment of Ventilator Parameters will no longer be collected per protocol and decision making related to collection and analysis of clinical ventilator and respiratory parameters will occur by the site and local pulmonologists in collaboration with the Site Investigator.

5.1.1.11.5.1 MIP, MEP, P0.1, and Tidal Volume

Assessment of respiratory strength and endurance will include MIP, MEP, inspiratory occlusion pressure at 0.1 seconds (P0.1), and tidal volume. MIP and MEP will be obtained using a cuffed tracheostomy tube. Details on assessment of respiratory muscle strength and endurance will be provided in a separate procedure manual.

MIP and MEP are noninvasively obtained estimates of respiratory muscle strength. Adult and pediatric reference values exist (Gaultier, 1983; Wilson, 1984) and this measure is becoming increasingly utilized in the neuromuscular disease setting (Smith, 2014).

Inspiratory occlusion pressure at 0.1 seconds provides a measurement that represents a weighted sum of the effort of all respiratory muscles active at a given time (Whitelaw, 1993). This measure is not a separate procedure but rather is a calculated value from data collected during the assessment of MIP.

If a subject has discontinued the use of the ventilator and has 2 assessments with the MIP at 80 cmH_2O, testing for MIP, MEP, and P0.1 will not be required for future visits.

Respiratory strength and endurance assessments are to be performed by site personnel during the normal routine site visits through Month 42. As of protocol v12, beginning at the Month 48 visit, respiratory strength and endurance assessments will no longer be collected per protocol and decision making related to collection and analysis of respiratory strength, endurance and

function assessments will occur by the site and local pulmonologists in collaboration with the Site Investigator.

5.1.1.11.6 Respiratory Infections and Annualized Respiratory Hospitalization Rate

The incidence, severity, and duration of respiratory infections will be determined from AE reports. The annualized respiratory hospitalization rate will be calculated for all subjects.

5.1.1.12 Assessments of Neuromuscular Function

Assessments of neuromuscular function will be video recorded and may be reviewed to confirm that assessment procedures are being conducted correctly, as well as for optional future review of subject performance. To fully assess disease manifestations, video recordings will not be deidentified (will show faces and limbs). The procedures for video recording assessments will be provided in a separate procedure manual. In all subjects, the assessments will include a set of key motor development milestones (Section 5.1.1.12.1), the CHOP INTEND (Section 5.1.1.12.3), and the motor domain of the Bayley-III (Section 5.1.1.12.2). The MFM-32 will be used to assess children ≥ 2 years of age (Section 5.1.1.12.4).

As of protocol v12, beginning at the Month 48 visit, only the Motor Developmental Milestones assessment will be performed. Decision making regarding collecting other assessments of neuromuscular function will occur by the Site Investigator, in collaboration with other neuromuscular specialists as indicated.

5.1.1.12.1 Motor Developmental Milestones

The motor developmental milestones listed in Table 3 will be assessed and video recorded (on site or remotely) in all subjects according to the Schedule of Events. The motor developmental milestone assessments will be conducted separately from the Bayley-III scales and details on assessment of motor developmental milestones will be provided in a separate procedure manual. If a subject is unable to complete the motor developmental milestones of “Pulls to Stand”, “Stands with Assistance”, “Walks with Assistance”, “Stands Alone”, or “Walks Alone”, these may be evaluated with supra-malleolar or ankle-foot orthoses (SMO or AFOs).

Table 3: Motor Developmental Milestones

Development Milestone	Reference	Summary Description of Performance Criteria[a]
Head Control	Bayley Gross Motor Subtest Item #9	Child holds head erect for at least 15 seconds without support
Rolls from Back to Sides	Bayley Gross Motor Subtest Item #20	Child turns from back to both right and left sides
Sits Without Support	*WHO Multicentre Growth Reference Study*	Child sits alone without support for at least 10 seconds
Sits Without Support	Bayley Gross Motor Subtest Item #26	Child sits alone without support for at least 30 seconds
Stands with Assistance	Bayley Gross Motor Subtest Item #33	Child supports own weight for at least 2 seconds
Crawls	Bayley Gross Motor Subtest Item #34	Child makes forward progress of at least 5 feet by crawling on hands and knees
Pulls to stand	Bayley Gross Motor Subtest Item #35	Child raises self to standing position using chair or other convenient object for support
Walks with Assistance	Bayley Gross Motor Subtest Item #37	Child walks by making coordinated, alternating stepping movements. He/she may hold on with 1 or 2 hands for support.
Stands Alone	Bayley Gross Motor Subtest Item #40	Child stands alone for at least 3 seconds after you release his or her hands.
Walks Alone	Bayley Gross Motor Subtest Item #42	Child takes at least 3 steps without support, even if gait is stiff-legged and wobbly

[a] See motor milestone manual for full description of performance criteria

5.1.1.12.2 Bayley-III Scales (Gross and Fine Motor Domains)

The motor domains of the Bayley-III are a standard series of measurements to assess the motor (fine and gross motor subscales) development of infants and toddlers. The motor domain of the Bayley-III will be conducted in all children. The Bayley-III is a tool that is used commonly in clinical practice to assess a child's developmental progression. It has been used in many pediatric clinical studies to ensure that the children are attaining milestones at the requisite ages; and it has been used specifically in children with spinal muscular atrophy (SMA) (Mendell, 2015) and infants with Duchenne muscular dystrophy (Connolly, 2013). Raw Bayley-III scores will be reported.

5.1.1.12.3 CHOP INTEND

The CHOP INTEND is an assessment scale containing 16 questions, each of which is scored on a scale of 0 to 4. It was originally designed to quantify motor abilities in infants aged 1.4 to 37.9 months, with spinal muscular atrophy type I (SMA-I) (Glanzman, 2010) and has been validated for XLMTM (Duong, 2021). It was designed such that the repertoire of motor skills and

movements associated with neuromuscular disorders could be quantified, without relying heavily on inherently challenging tasks such as prone positioning, long duration, or head control items. Face validity and inter-rater reliability has since been established in multiple neuromuscular diseases, including SMA-I, myotubular myopathy, infant botulism, nemaline myopathy, central core myopathy and XLMTM (Glanzman, 2011).

If a subject has 2 assessments with a total score of 56, the CHOP INTEND will not be required for future visits.

5.1.1.12.4 Motor Function Measure 32 (MFM-32)

The MFM-32 contains 32 tests of muscle strength, each measured on a scale of 0 to 3, for a total score of 96. The measures capture 3 dimensions of muscle tone: standing and transfers, axial and proximal motor function, and distal motor function. The MFM-32 has been validated in patients aged 6 to 62 years with Duchenne muscular dystrophy, Becker muscular dystrophy, limb-girdle muscular dystrophy, facio-scapulo-humeral dystrophy, myotonic dystrophy, congenital myopathy, congenital muscular dystrophy, SMA, and hereditary neuropathy (n = 303) (de Lattre, 2013). XLMTM shares key characteristics with these disorders including reduced fetal movements and polyhydramnios in the pregnant mother; and severe hypotonia, myopathy, respiratory distress and/or failure, and underdeveloped motor skills in the child (Sewry, 2012).

The MFM-32 will be used to assess children ≥ 2 years of age.

5.1.1.13 Swallowing Assessments

5.1.1.13.1 Parental Swallowing Questionnaire

The Parental Swallowing Questionnaire is a Sponsor-designed instrument designed to reflect a parents' assessment of the child's ability to swallow and will be used to document the child's ability to feed without a gastrostomy or G-tube. The Parental Swallowing Questionnaire will be completed by a parent/LAR/caregiver based on their day-to-day observation of the child's condition in the home environment.

A 2-item questionnaire (Appendix 7) will evaluate elements of swallowing including the child's ability to swallow liquids and foods.

5.1.1.14 Speech Assessments

As of protocol v12, beginning at the Month 48 visit, speech assessments will no longer be performed per protocol. Decision making regarding collecting other clinical speech assessments will occur by the Site Investigator, in collaboration with other neuromuscular specialists as indicated.

5.1.1.14.1 Parental Speech Development Questionnaire

The Parental Speech Development Questionnaire is a Sponsor-designed instrument designed to assess major speech developments. A series of probative questions will be asked to the

parent/LAR/caregiver (the same person every time, whenever possible) by the Investigator or delegate at study visits (Appendix 8).

5.1.1.14.2 Communicative Development Inventories

The MacArthur Communicative Development Inventories (CDI) and their language adaptations, are parent-reported instruments for assessment of communicative skills in infants and toddlers (Fenson, 2000). The short-form versions of the English and Spanish CDIs have been validated. Adaptations in other languages of the MacArthur CDI will be used to assess communicative skills of children in other countries.

Parents/LARs/caregivers (the same person every time, whenever possible) in ASPIRO will be asked to complete the inventory for subjects 8 months and older. Communicative Development Inventories will be used to assess change in baseline language development over time.

5.1.1.15 Secretions Management Assessments (PGIS-S and PGIS-I)

Management of secretions is an important component of protecting the airway and lowering the risk of lung infections caused by aspiration.

The PGIS-S and PGIS-I are 2 separate but related Sponsor-designed instruments designed to reflect parents' assessment of the burden of care associated with the need to manage the secretions of a child with XLMTM. The scales assess both the severity of this aspect of the disease (PGIS-S) and change over time (PGIS-I). Related instruments are widely used and well validated as supportive endpoints (Busner, 2009). The PGIS (PGIS-I and PGIS-S) will be completed by a parent/LAR/caregiver based on their day-to-day observation of the child's condition in the home environment.

Parents/LAR/caregiver will report on the amount of suctioning and secretions management required over the previous week, using a scale of 1 to 7 to report impression of change from baseline and impression of current severity (Appendix 3). The same parent/LAR/caregiver should provide assessments throughout the study (whenever possible). During months that do not have a clinic visit, the parent/LAR/caregiver will be contacted by the study site. A minimum of 2 contact attempts will be made at each time point.

5.1.1.16 Clinical Global Impression Assessments (CGI-S and CGI-I)

A global assessment by the Investigator or designee will be done to assess the severity of the subject's disease utilizing a 7-point scale (CGI-S; Appendix 5).

A global assessment by the Investigator or designee will be done to assess if there has been any improvement of the subject's disease utilizing a 7-point scale (CGI-I; Appendix 5). The purpose of this assessment is to capture change in a subject's clinical status compared with baseline severity.

For both assessments, the same person should assess a subject for the duration of the study (whenever possible). In addition to the numerical score, clinicians will be asked to draft a brief clinical narrative, to justify the score that is being given.

5.1.1.17 Burden of Disease

5.1.1.17.1 Assessment of Caregiver Experience with Neuromuscular Disease (ACEND)

The ACEND was developed to measure impact on the lives of parents/LARs/caregivers caring for children with severe neuromuscular disorders (Matsumoto, 2011). Although currently validated for children aged 4 years and older, the ACEND was developed specifically for children with significant developmental disabilities. Several domains of the ACEND (time, finance, and emotion) are relevant to assessing the caregiver burden of the parents of children with XLMTM.

5.1.1.17.2 Pediatric Quality of Life Inventory (PedsQL)

The PedsQL is a 23-item tool designed to measure health-related quality of life in healthy children and adolescents and those with acute and chronic health conditions. The PedsQL measures the core dimensions of health as delineated by the World Health Organization, as well as role (school) functioning. The PedsQL neuromuscular module was designed to measure health-related quality of life dimensions specific to children aged 2-18 years with neuromuscular disorders and has been validated in other neuromuscular disorders such as SMA (Iannaccone, 2009). It will also be used for children < 2 years of age in this study. Subjects will continue to use the same version (from Baseline) of the questionnaire for the duration of the study even as the subject ages out of PedsQL version.

5.1.1.18 In-Depth Interviews of Caregivers and Subjects

Parents/LARs/caregivers will be invited to participate in in-depth interviews to elicit the stories of families (parents/LARs/caregivers) related to the child's illness, and their participation in the ASPIRO study. Participation in the interviews is optional and is not required to participate in the ASPIRO study. These interviews will explore and analyze parents/LARs/caregivers' perspectives on, and experiences with, the impact of the treatment received (by the child) in the ASPIRO study. In in-depth interviews, a single respondent is probed by an experienced interviewer to uncover underlying motivations, beliefs, attitudes, and feelings on a topic (Harris, 1996). Due to the rarity of the disease and the paucity of published literature describing the impact and burden of XLMTM, it is considered that these interviews will provide valuable information to help understand the disease. In addition, it is considered that detailed information regarding the involvement of XLMTM subjects in an interventional clinical study will facilitate the optimization of future clinical studies both in XLMTM and in other rare pediatric neuromuscular diseases.

These interviews will follow a generic qualitative design, with a critical qualitative component analyzing the narratives of parents in relation to their experiences, values, and perspectives on the child's illness trajectory and participation in the ASPIRO study. An open-ended, narrative approach to data collection will be utilized. This approach is interpretive and involves storytelling. The interviews will take place in-person or over a video conference, and will be audio-recorded. The personnel conducting all the interviews will be scientists at St. Michael's Hospital; all of whom are trained and experienced qualitative researchers, which is essential to manage interview dynamics, minimize social desirability bias, and ensure data quality.

Parents/LARs/caregivers will be interviewed a maximum of 2 times in the study, ideally coinciding with the Baseline and Week 24 or Week 48 assessments. For subjects who have already passed the timepoints listed above, only 1 interview will be conducted at a subsequent visit. Subjects (aged 2 and older) may also be interviewed at the same timepoints to capture children's stories through visual methods, including drawn images such as comics (Darnhofer, 2018).

5.1.1.19 Anti-AAV8 Antibody Testing

The timing of clearance of maternally acquired anti-adeno-associated virus 8 (anti-AAV8) antibodies in infants differs from individual to individual and has been described, but is not fully understood. In a study (Calcedo, 2011), approximately 18% of infants 0 to 2 months old tested positive for AAV8 NAbs with titers > 1:20. The prevalence of NAbs declined rapidly after birth, reaching a nadir at 7 to 11 months. The rate at which children develop anti-AAV8 antibodies in response to natural exposure to AAV8 is also incompletely understood. Because the presence of preexisting antibodies to AAV8 could affect the potential efficacy of AAV8 gene transfer therapy, information about binding, total antibodies (TAbs), and NAbs will be collected at Screening and following administration of AT132 per the Schedule of Events in Sections 5.3.1, 5.4, 5.5, 5.6, and 5.7. An isotype-independent cell-based NAb assay will detect neutralizing factors specific to AAV8 (NAbs) (Calcedo, 2009). The TAbs to AAV8 will be measured using an enzyme-linked immunosorbent assay (ELISA)-based assay (Falese, 2017).

5.1.1.20 Anti-MTM1 Antibody Testing

Anti-MTM1 is a specialty assay which will be used to measure binding human antibodies immunoglobulin G (IgG) against MTM1 protein. Titers of anti-MTM1 antibodies will be evaluated at Screening and following administration of AT132 per the Schedule of Events in Sections 5.3.1, 5.4, and 5.5.

5.1.1.21 Interferon-γ (IFN-γ) ELISpot Assay

Interferon-γ (IFN-γ) production is commonly used as an indicator of T cell immune reactivity. An ELISpot assay will measure IFN-γ producing T cells in subjects' peripheral blood mononuclear cells (PBMCs) after challenge with a pool of AAV8 peptides or myotubularin peptides. Samples for testing will be collected per the Schedule of Events in Sections 5.4 and 5.5. Additional samples will be collected as clinically indicated, as described in Section 4.6.

5.1.1.21.1 Flow Cytometry Assay

A Flow Cytometry exploratory analysis will be performed to access the phenotype of T cells, B cells, Natural Killer cells, Monocytes, and Macrophages in the subject's cryopreserved PBMCs, leftover from the IFN-γ ELISpot assay. This assay will be performed to assess the immune cells for available samples pre- and post-gene therapy administration.

5.1.1.22 Platelet Monitoring (not applicable for France)

Samples for platelet monitoring will be collected at Baseline and following study drug administration at Day 2, Week 1, and Week 2. The samples will be analyzed for the following:

- Complete blood count with differential (ensuring report of platelet counts and mean platelet volumes)
- Peripheral blood smears (performed as part of the hematology panel)
- Flow cytometry analysis of:
  - Activation surface antigens, degranulation and functional markers including:
    - CD62P (P-selectin)
    - Activated GP IIb/IIIa [αIIbβ3] with PAC-1 antibody
    - Phosphatidylserine
  - Platelet circulating antibodies
  - Reticulated platelets (platelet mRNA)

5.1.1.23 Growth and Developmental Parameters

5.1.1.23.1 Growth Parameters

Growth will be assessed by measuring height, weight, and head circumference.

5.1.1.23.2 Bone Age (Wrist X-ray) (not applicable for Germany)

X-ray of the left wrist in infants and children to ascertain bone age is a recognized method of assessing maturity and development of the skeleton. This is of particular relevance in XLMTM, because advanced bone age and a variety of skeletal abnormalities (although not well characterized) have been documented in some XLMTM patients (Herman, 1999). The radiation dose for this procedure is low (0.001 mSv per X-ray). Subjects in Germany will not have a wrist X-ray.

As of protocol v12, beginning at the Month 48 visit, bone age will no longer be collected per protocol.

5.1.1.23.3 Tanner Stage

Tanner stage will be assessed to monitor for premature adrenarche, which has been documented in some XLMTM patients. Tanner staging criteria are provided in Appendix 2.

As of protocol v12, beginning at the Month 48 visit, Tanner stage will no longer be collected per protocol.

5.1.1.24 Hospitalization Rate and Length of Stay

The annualized hospitalization rate and length of stay per hospitalization will be determined for all subjects.

5.1.1.25 Survival

Children with XLMTM have a high mortality rate (Section 1.1). Accordingly, survival will be assessed. Survival status should be assessed at each visit until the subject withdraws consent or completes the study. If the subject misses a visit or withdraws for a reason other than withdrawal of consent or death, the site should contact the parent(s)/LAR(s) to ascertain if the subject is alive. For subjects who withdraw from the study, the subject should be contacted every 6 months for 5 years after administration and every year for an additional 5 years (after the 5-year follow-up through 10-years follow-up) to assess for survival. If not alive, the cause and date of death should be requested and recorded.

In the case of a subject's death during participation in the study, an autopsy may be requested.

5.1.1.26 Muscle Biopsy (Quantitative Assessment of Tissue Changes)

Muscle biopsies will be collected using a standard muscle open biopsy protocol. Preference of muscles to biopsy will be (1) left gastrocnemius; (2) right gastrocnemius; and (3) vastus lateralis (either side). A different muscle may be biopsied if imaging studies (eg, muscle MRI) suggest a higher probability of muscle tissue collection in another muscle group (eg, biceps brachii). Muscles that have been biopsied previously will not be re-biopsied during this study to avoid the potential for sampling of the old biopsy site. Biopsy sites will be taken from the target muscle at least 3 cm away from myotendinous insertion sites and sites of known prior muscle injections or electromyography needle sites.

Muscle biopsies will be used to assess the efficacy and safety of AT132 by analyzing MTM1 protein expression, vector copy number quantitation, RNA transcripts assessment, histological characterization and inflammatory markers.

If a muscle biopsy is performed at the discretion of the investigator for diagnostic purposes, including but not limited to CK elevations and declining neuromuscular function

(Section 4.6.3.2), testing should be performed in accordance with standards of care for diagnostic muscle biopsy assessment.

When possible after diagnostic muscle biopsy, muscle should be collected and sent to the central lab for the AT132-related assessments listed below:

- MTM1 protein expression
- Vector copy number quantitation
- RNA transcript assessment
- Histological characterization
- Inflammatory markers

Automatic muscle specimen segmentation techniques will be used to assess pathological features (eg, myofiber size, internally/centrally nucleated myofibers) using machine learning, computer vision techniques, and algorithms that have been developed to measure challenging skeletal muscle specimens (Liu, 2013; Liu, 2014; Liu, 2015) and further adapted for XLMTM tissue. Muscle specimens will be processed by the clinical study site and sent to a Sponsor designated central reader for review. Details will be provided in a separate procedure manual.

If a subject had a diagnostic biopsy prior to study enrollment, it will not be eligible for use as the pre-treatment biopsy due to concerns about variable tissue handling procedures and age-related changes in pathology.

5.1.1.27 Viral Shedding

Viral shedding will be assessed by quantitative polymerase chain reaction (PCR) on samples of saliva (swab), stool sample, and urine (diaper collection or urine bag). Collection of each sample type will begin on Day 1 and will continue until each sample type has 3 consecutive data points at or below the limit of detection (BLOD). For example, saliva collection will stop once 3 consecutive saliva sample results are BLOD, but stool and urine collection will continue beyond that timepoint if they have not reached 3 consecutive BLOD results in each of those individual sample types. Sponsor will notify the site when collection can cease.

5.1.1.28 Complement Panel

The complement system consists of a large number of plasma proteins, either soluble or membrane bound, that play an important role in the innate immune system. Activation of the complement system is tightly regulated to ensure a specific response against pathogen infection and to maintain homeostasis. Samples for complement panel testing will be collected per Appendix 1 and the Schedule of Events in Sections 5.3.1, 5.4, and 5.5. Additional samples will be collected as clinically indicated per Section 4.6.2.

5.1.1.29 Serum Bile Acid Assay

Serum bile acid levels are elevated in some cholestatic disorders. To better understand cholestatic tendency in XLMTM, serum bile acid levels will be measured. This assay will be performed once, any time prior to treatment with AT132, for subjects dosed in protocol v8 and beyond. As of protocol v10, the assay will also be performed following AT132 administration according to the Schedule of Events (see Sections 5.3.1, 5.4, 5.5, and 5.6). Serum bile acid levels will preferably be collected in the fasting state; however, the samples may be collected in the non-fasting state per the Investigator's discretion.

5.1.1.30 Transient Elastography of the Liver by Ultrasound

Transient elastography by ultrasound is a noninvasive, widely available technique to assess liver stiffness. Liver stiffness may be increased in cholestatic disorders, particularly if resulting fibrosis occurs. To better understand liver stiffness characteristics in baseline XLMTM, this exploratory procedure will be performed once, any time prior to treatment with AT132, for subjects dosed in protocol v8 and beyond (see Sections 5.3.1 and 5.4).

5.1.1.31 Muscle MRI with STIR Sequence

The MRI STIR sequence is a type of MRI protocol that involves a short inversion time to nullify signals from tissues with short T1 relaxation times, highlighting areas with longer T1 values. It is useful for identifying edema, fluid, and inflammation in muscle. It is recommended that this imaging protocol be obtained in response to elevated CK to better understand the potential inflammatory changes that may occur after treatment with AT132. STIR sequences may be used to support identification of the cause for the neuromuscular abnormality, identify a muscle site for biopsy if needed, and monitor treatment response. In some circumstances, muscle MRI with STIR sequences may be performed when CK is elevated as part of the diagnostic assessment. This imaging is performed at the discretion of the investigator.

5.1.1.32 Electrophysiological Assessments (EMG)

EMG may be performed to assess neuromuscular abnormalities. Nerve conduction studies can be performed if a neuropathic process is suspected.

5.2 Schedule of Study Events

The assessments to be conducted for each subject enrolled in the study are presented in tabular form in Sections 5.3.1, 5.4, 5.5, 5.6, and 5.7. Additional information is provided above, in the study appendices, and study procedure manuals, as noted.

For each subject and visit, assessments may be conducted over multiple days and should be performed at approximately the same time of day and in the same order, when possible, for each visit. Week 3, 5, 6, 7, 9, 10, 11, 13, 14, or 15 procedures can be performed at the study site clinic, or in the subject's home by a trained home health nurse. Home health nurses may collect laboratory samples, perform a limited physical examination, vital signs, and review

concomitant medications and AEs upon request of the Investigator, as needed at any protocol scheduled visit or unscheduled visit. All subjects will undergo all assessments at the Screening Visit. Following randomization to treated or control arm, subjects will follow either the Treated or Control Subject Schedule of Events. Control subjects will use the Control Subjects Schedule of Events until they are assigned to receive AT132.

Long-term follow-up visits and/or assessments after the 5-year (Month 60) visit can be done remotely per discretion of investigator in consultation with Medical Monitor.

For subjects who end the study early, information related to post-dose safety assessments may continue to be collected via public data or Electronic Medical Record request per consent form until 10 years after AT132 dosing. Additional details can be found in the CRF Completion Guidelines.

5.3 Schedule of Events for Screening

All subjects will undergo assessments at the Screening Visit. Eligibility of the control subjects will be confirmed before assignment to receive AT132. The assessments from a control visit may be used to assess eligibility. Following assignment to receive AT132, control subjects will follow the Treated Subject Schedule of Events, starting with the Biopsy Visit and Baseline Visit in Section 5.4.

Informed consent must be obtained from the subject's parent(s)/LAR(s) before initiation of any protocol-specified screening procedure.

Subjects will be screened within 70 days before dosing to determine eligibility for participation in the study. Procedures and assessments may be done on different days. Procedures or assessments that have already been completed within 70 days prior to planned Day 1 as part of an Astellas Gene Therapies, Inc.-sponsored study do not need to be repeated and may be used as a Screening assessment for this study. Screening assessments that are not repeated at Day -1 will be considered baseline values. All results needed to evaluate study eligibility must be reviewed by prior to randomization. Eligibility should be re-confirmed by the Investigator prior to dosing (Day 1) by reviewing the Screening and Baseline results. In addition, delayed-treatment control subjects must meet a subset of the inclusion/exclusion criteria before receiving AT132, as indicated by an asterisk (*) in Sections 3.3.1 and 3.3.2.

Subjects meeting all of the inclusion criteria and none of the exclusion criteria will be randomized to treatment or control and will return to the clinic on Day -2 or -1 for baseline assessments. Subjects must be randomized at least 6 days prior to planned Day 1 to allow adequate time for delivery of study drug to study site.

Subjects moving from the delayed-treatment control period to the active treatment period should have screening procedures as noted above completed in order to confirm that the subject is still eligible for dosing.

From the time of obtaining informed consent through dosing of IMP, record all SAEs related to protocol-mandated procedures on the AE eCRF. All other untoward medical occurrences observed during the screening period, including exacerbation or changes in medical history are to be captured on the medical history eCRF. See Section 6 for details.

5.3.1 Schedule of Events for Screening Visit

Study Phase/Visit	**Screening [a]**
Study Day	**-70 to Day -7**
Visit Window (days)	**NA**
Informed consent	X
Inclusion/exclusion criteria	X
Randomization	X
Medical history	X
Complete physical examination [b]	X
Vital signs [c]	X
12-Lead ECG [d]	X
Growth parameters	X
Samples for:	
Genetic testing (central lab) [e]	X
Safety labs (hematology) (central lab) [f,g]	X
Safety labs (chemistry) (central lab) [f g,h,i]	X
Cytokine panel (central lab) [f]	X
Complement panel (central lab) [f]	X
Troponin T and/or I (central lab) [i]	X
Coagulation parameters (central lab) [f,g]	X
Anti-AAV8 NAb and TAb (central lab)	X
Anti-MTM1 antibody (central lab)	X
Urinalysis (central lab) [f,g]	X
Serum bile acid assay (fasting preferred; central lab)	Once any time prior to dosing
Liver ultrasound [h]	X
Transient elastography ultrasound of liver	Once any time prior to dosing
Begin electronic diary of ventilator dependence [j]	X
Assessment of ventilator parameters	X
Daytime polysomnogram	X
MIP, MEP, P0.1, tidal volume	X
Motor developmental milestone assessments [k]	X
AEs/concomitant medications	X

AEs = adverse events; anti-AAV8 = anti-adeno-associated virus 8; ECG = electrocardiogram; MTM1 = gene encoding the myotubularin protein; MEP = maximal expiratory pressure; MIP = maximal inspiratory pressure; NA = not applicable; NAb = neutralizing antibody; P0.1 = inspiratory occlusion pressure at 0.1 seconds; TAb = total antibody.

[a] Procedures and assessments may be done on different days. Procedures or assessments that have already been completed within 70 days prior to planned Day 1 as part of an Astellas Gene Therapies, Inc.-sponsored study do not need to be repeated and may be used as a Screening assessment for this study. All results needed to evaluate study eligibility must be reviewed by prior to randomization and prior to dosing.

[b] Complete physical examination at screening.

[c] Record whether the subject was on or off of ventilatory support when vital signs are taken.

[d] Particular attention should be paid to ECG abnormalities specific to myocarditis. Refer to Section 4.6.1 for management of ECG results and the role of the site pediatric cardiologist.

[e] Genetic testing performed only for subjects without previous confirmation by a Sponsor-approved testing facility.

[f] See Appendix 1 for list of laboratory assessments.

[g] It is recommended to use the central laboratory to analyze laboratory tests that are drawn prior to dosing, and after the Week 16 Visit and to use the local laboratory to analyze laboratory tests that are drawn at Baseline, Dosing, Days 1, 2, and Weeks 1 through 16. However, the local laboratory can be used for other visits if needed (for example, the local laboratory can accommodate the analysis with a smaller amount of blood) or if the local laboratory does not accommodate the appropriate analysis, the central laboratory may be used.

[h] See Section 4.6.2 for details related to the monitoring, diagnosis, and treatment of hepatobiliary toxicity and the role of the site hepatologist.

[i] See Section 4.6.1 for details related to the evaluation of cardiac enzymes; monitoring, diagnosis, and treatment of myocarditis; and the role of the site pediatric cardiologist.

[j] Site staff should set up the electronic diary prior to distribution to the parent/LAR/caregiver and review the training with the parent/LAR/caregiver. The electronic diary should be given to the parent/LAR/caregiver following informed consent (or reconsent to protocol v5 or later for Part 1 subjects). The diary should be completed daily by the same parent/LAR/caregiver (when possible).

[k] All physical therapy assessments should be videoed and performed according to the site manual. This assessment will be conducted for the evaluation of eligibility.

5.4 Schedule of Events for Subjects Treated with AT132: Biopsy – Week 8

Study Phase/Visit	Daily	Biopsy	Baseline	Dosing	Day 2	Day 3	Week 1	Week 2	Week 3	Week 4	Weeks 5, 6, 7	Week 8
Study Day(s)	**NA**	**By -6**	**-2 to -1**	**1**	**2**	**3**	**7**	**14**	**21**	**28**	**35, 42, 49**	**56**
Visit Window (days)	**-1**	**NA**	**-1**	**NA**	**NA**	**NA**	**±1**	**±3**	**±3**	**±3**	**±3**	**±3**
Inclusion/exclusion criteria			X [a]									
Medical history			X [b]									
Physical examination [c]			X [b]	X	X	X	X	X		X		X
Vital signs [d]			X [b]	X [e]	X [f]	X [f]	X	X		X		X
12-Lead ECG [g]			X		X		X	X		X		X
ECHO [g]			X					X		X		X
Growth parameters			X							X		X
Tanner stage (puberty assessment)			X									
Samples for:												
Safety labs (hematology) (local lab) [h,i]			X		X		X	X		X		X
Safety labs (chemistry) (local lab) [h,i,j,k]			X		X		X	X	X [l]	X	X [l]	X
Cytokine panel (central lab) [h,m]			X			X	X	X	X	X	As clinically indicated	
Complement panel (central lab) [h,n]						X	X	X	X	X	As clinically indicated	
Troponin T and/or I (local lab) [i,j]			X		X		X	X	X	X	Wk 6 only	X
Coagulation parameters (local lab) [h,i]			X				X			X		X
Platelet Monitoring [h,o,†]			X		X		X	X				
Anti-AAV8 NAb and TAb (central lab)										X	Wk 6 only	X
Anti-MTM1 antibody (central lab)										X	Wk 6 only	X
IFN-γ ELISpot (PBMCs) (central lab)		X		As clinically indicated [p]								
Urinalysis (local lab) [h,i]			X		X		X	X		X		X
Serum bile acid assay (fasting preferred; central lab)		Once any time prior to dosing										
Liver ultrasound [k]			X							X		

Study Phase/Visit	Daily	Biopsy	Baseline	Dosing	Day 2	Day 3	Week 1	Week 2	Week 3	Week 4	Weeks 5, 6, 7	Week 8
Study Day(s)	**NA**	**By -6**	**-2 to -1**	**1**	**2**	**3**	**7**	**14**	**21**	**28**	**35, 42, 49**	**56**
Visit Window (days)	**-1**	**NA**	**-1**	**NA**	**NA**	**NA**	**±1**	**±3**	**±3**	**±3**	**±3**	**±3**
Transient elastography ultrasound of liver		Once any time prior to dosing										
Bone age (wrist X-ray) (not applicable for Germany) [r]			X									
Diary of ventilator dependence [s]	X											
Assessment of ventilator parameters			X					X		X		
MIP, MEP, P0.1, tidal volume [t]			X					X		X		
Ventilator Weaning and Discontinuation Assessment [u]			X					X		X		
Secretions management assessment [v]			X [w]							X		X
Motor development milestone assessments [x]			X							X		
Bayley-III, motor domain [x]			X							X		
CHOP INTEND [x,y]			X					X		X		X
MFM-32 [x]			X					X		X		X
ACEND			X				X	X		X		
PedsQL [z]			X				X	X		X		
Clinical global impression scales [aa]			X [aa]				X	X		X		X
In-depth interviews with caregiver and subject			X									
Swallowing questionnaire			X				X	X		X		X
Speech questionnaire			X				X	X		X		X
Communicative Development Inventories [bb]			X				X	X		X		X
Muscle biopsy		X										
Glucocorticoid administration [cc]	Starting Day -1											

Study Phase/Visit	Daily	Biopsy	Baseline	Dosing	Day 2	Day 3	Week 1	Week 2	Week 3	Week 4	Weeks 5, 6, 7	Week 8
Study Day(s)	**NA**	**By -6**	**-2 to -1**	**1**	**2**	**3**	**7**	**14**	**21**	**28**	**35, 42, 49**	**56**
Visit Window (days)	**-1**	**NA**	**-1**	**NA**	**NA**	**NA**	**±1**	**±3**	**±3**	**±3**	**±3**	**±3**
Ursodiol administration [dd]	Starting between Days -6 and -4											
IMP administration [ee]				X								
Overnight stay [ff]			X	X	DC							
Viral shedding (central lab) [gg]				X	X	X	X		X		Wks 5, 7 only	
AEs/concomitant medications			X	X	X	X	X	X	X	X	X	X

AEs = adverse events; ACEND = Assessment of Caregiver Experience with Neuromuscular Disease; anti-AAV8 = anti-adeno-associated virus 8; BID = twice daily; CGI-I =Clinical Global Impression of Improvement; CGI-S = Clinical Global Impression of Severity; CHOP INTEND = Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders; CK = creatine kinase; DC = discharge; ECG = electrocardiogram; ECHO = echocardiogram; ELISpot = enzyme-linked immunosorbent spot assay; IFN-γ = interferon-gamma; IMP = investigational medicinal product; LAR = legally authorized representative; LFTs = liver function tests; MTM1 = gene encoding the myotubularin protein; MEP = maximal expiratory pressure; MFM-32 = Motor Function Measure-32; MIP = maximal inspiratory pressure; NA = not applicable; NAb = neutralizing antibody; P0.1 = inspiratory occlusion pressure at 0.1 seconds; PBMCs = peripheral blood mononuclear cells; PedsQL = Pediatric Quality of Life Inventory; PGIS-I = Parental Global Impression of Secretion Improvement; PGIS-S = Parental Global Impression of Secretion Severity; TAb = total antibody; TID = three times a day; WK = week.

NOTE: Week 3, 5, 6, and 7 procedures can be performed at the study site clinic, or in the subject's home by a trained home health nurse. Home health nurses may collect laboratory samples, perform a limited physical examination, vital signs, and review concomitant medications and AEs upon request of the Investigator, as needed at any scheduled protocol visit or unscheduled visit. If the home health nurse is used, then the safety laboratory tests will be sent to the central laboratory for processing.

† Not applicable to France.

[a] All inclusion/exclusion criteria should be reviewed on Day -1 to confirm that the subject continues to meet eligibility criteria before dosing.

[b] Baseline measures of medical history, physical examination, vital signs, and assessment of ventilator use should be collected on Day -1 if possible. Medical history at Baseline should capture changes since the Screening visit.

[c] Complete physical examination at Baseline. At all other timepoints, conduct an abbreviated physical examination based on the subject's circumstances and in accordance with the Investigator's clinical judgement.

[d] Record whether the subject was on or off of ventilatory support when vital signs are taken.

[e] Vital signs to be predose and collected every 30 (±10) minutes for 2 hours post-dose and then hourly until 4 hours post-dose.

[f] Day 2 and Day 3 vital signs to be collected every 12 hours (±30 minutes), prior to discharge, and daily after discharge.

[g] ECHOs should include an assessment of pulmonary hypertension as well as structural and functional abnormalities. Particular attention should be paid to ECG and ECHO abnormalities specific to myocarditis. Refer to Section 4.6.1 for management of ECG and ECHO results and the role of the site pediatric cardiologist.

[h] See Appendix 1 for list of laboratory assessments.

[i] It is recommended to use the central laboratory to analyze laboratory tests that are drawn prior to dosing, and after the Week 16 Visit and to use the local laboratory to analyze laboratory tests that are drawn at Baseline, Dosing, Days 1, 2, and Weeks 1 through 16. However, the local laboratory can be used for other visits if needed (for example, the local laboratory can accommodate the analysis with a smaller amount of blood) or if the local laboratory does not accommodate the appropriate analysis, the central laboratory may be used.

[j] See Section 4.6.1 for details related to the evaluation of cardiac enzymes; monitoring, diagnosis, and treatment of myocarditis; and the role of the site pediatric cardiologist.

[k] See Section 4.6.2 for details related to the monitoring, diagnosis, and treatment of hepatobiliary toxicity and the role of the site hepatologist.

[l] LFTs, CK (including CK isoenzymes), C-reactive protein, and serum bicarbonate only.

[m] Cytokine panel: Following Week 4 cytokine panel collection, in cases of clinically important elevated bilirubin, elevated aminotransferases, elevated troponins, and/or elevated creatine phosphokinase that in the Investigator's judgment may be related to T-cell responses, repeat cytokine panel testing is suggested. Appropriate care for an individual subject will be determined by the Investigator based on an assessment of the subject's overall medical status.

[n] Complement panel: Following Week 4 complement panel collection, in cases of clinically important elevated bilirubin, elevated aminotransferases, ascites, thrombocytopenia, acute renal insufficiency, and/or anemia that in the Investigator's judgment may be related to complement activation, repeat complement panel testing is suggested. Appropriate care for an individual subject will be determined by the Investigator based on an assessment of the subject's overall medical status.

[o] Platelet monitoring: Peripheral blood smears should be performed as part of the hematology panel. Platelet monitoring consists of the following tests: Platelet Activation Assays (CD62P, Activated GP IIb/IIIa w/ PAC-1, and Phosphatidylserine), Reticulated Platelets, Platelet Circulating Antibodies. For EU sites ONLY: Reticulated platelets will be tested locally. Sample for platelet activation assays must be fixated locally prior to shipping.

[p] IFN-γ ELISpot: Following AT132 administration, in cases of clinically important elevated bilirubin, elevated aminotransferases, elevated troponins, and/or elevated creatine phosphokinase that in the Investigator's judgment may be related to T-cell responses, repeat IFN-γ ELISpot response testing is suggested. Appropriate care for an individual subject will be determined by the Investigator based on an assessment of the subject's overall medical status.

[q] Footnote was deleted.

[r] For control subjects whose wrist X-ray was within the past 6 months, this does not need to be repeated at their second baseline assessment before treatment with AT132. Subjects in Germany will not have a wrist X-ray.

[s] The diary should be completed daily by the same parent/LAR/caregiver (whenever possible); see Section 5.1.1.11.2.1.

[t] If a subject has discontinued the use of the ventilator and has 2 assessments with the MIP at 80 cmH_2O, testing for MIP, MEP, and P0.1 will not be required for future visits.

[u] The site pulmonologist and local pulmonologist, if applicable, will review the results from the visit, assess the subject, determine if the subject meets the criteria outlined in Table 1 to begin reduction of hours on the ventilator, and determine if the subject meets the criteria outlined in Table 2 to discontinue the ventilator.

[v] The severity of secretions and burden of secretions management will be assessed using the PGIS-S and PGIS-I (Appendix 3). The same parent/LAR/caregiver should report throughout the study (whenever possible).

[w] At baseline, the PGIS-S is conducted for all subjects. At baseline, the PGIS-I is conducted only for control subjects who are attending their second baseline assessments before treatment with AT132 (whenever possible).

[x] All physical therapy assessments should be videoed and performed according to the site manual.

[y] If a subject has a total score of 56 for 2 consecutive assessments, the CHOP INTEND will not be required for future visits.

[z] The PedsQL is to be performed for all subjects, regardless of age. Subjects < 2 years of age should be administered the version for 2- to 4-year-olds.

[aa] See Appendix 5. At baseline, the CGI-S is conducted for all subjects. At baseline, the CGI-I is conducted only for control subjects who are attending their second baseline before treatment with AT132.

[bb] Parents/LARs/caregivers (the same person every time, whenever possible) in ASPIRO will be asked to complete forms for subjects 8 months and older.

[cc] Subjects will be administered a course of glucocorticoid therapy commencing Day -1 and continuing for a period of 16 weeks as a preventative measure for immune-mediated hepatic injury. For subjects weighing < 60 kg, the dose will be 1 mg/kg prednisolone orally daily for 8 weeks, then tapered down for 8 weeks per Investigator discretion. For subjects weighing ≥ 60 kg, the dose will be 60 mg prednisolone orally daily for 8 weeks, then tapered down for 8 weeks per Investigator discretion.

[dd] Ursodiol to be dosed at 20 mg/kg/day (divided either BID or TID at the Investigator's discretion), beginning approximately 1 week (ie, between Days -6 and -4, at the Investigator's discretion) before AT132 administration and continuing to Week 12.

[ee] As of protocol v9: Subjects yet to be dosed will be administered 1.3×10^{14} vg/kg AT132 (see Section 1.4.2).

[ff] Subject may be admitted to the study clinic on Day –2 or –1 for baseline procedures, including confirmation of baseline ventilator settings. If medically appropriate, subject may be discharged from the study clinic after completion of Day 2 assessments.

[gg] Viral shedding will be assessed on samples of saliva (swab), stool, and urine (diaper collection or urine bag). Collection of each sample type will begin on Day 1 and will continue until each sample type has 3 consecutive data points at or below the limit of detection (BLOD) (see Section 5.1.1.27). Sponsor will notify site when collection can cease. Not conducted at Week 6.

5.5 Schedule of Events for Subjects Treated with AT132: Week 9 – Month 60

Study Phase/Visit	Daily	Weeks 9, 10, 11	Week 12	Weeks 13, 14, 15	Week 16	Weeks 20, 28, 32, 40, 44	Week 24	Week 36	Week 48 or Early Withdrawal	Month 15	Months 18, 24, 30, 36, 42	Months 48, 54, 60
Study Day(s)	**NA**	**63, 70, 77**	**84**	**91, 98, 105**	**112**	**140, 196, 224, 280, 308**	**168**	**252**	**336**	**456**	**548, 730, 913, 1095, 1278**	**1460, 1643, 1825**
Visit Window (days)	**-1**	**±3**	**±3**	**±3**	**+10**	**±3**	**±14**	**±14**	**±14**	**±14**	**±14**	**±14**
Diary of ventilator dependence [a,b]	X											
Ventilator Dependence Questionnaire [b]						X		X	X	X	X	X
Contact subject's caregiver [c]						X						
Physical examination [d]			X		X		X	X	X	X	X	X
Vital signs [e]			X		X		X	X	X	X	X	X
12-Lead ECG [f]			X		X		X	X	X		X	X
ECHO [f]			X				X		X		X [g]	X [g]
Growth parameters			X		X		X	X	X	X	X	X
Tanner stage (puberty assessment)							X		X		X [ff]	
Samples for:												
Safety labs (hematology) (central lab) [h,i]							X	X	X		X	X
Safety labs (hematology) (local lab) [h,i]			X		X							
Safety labs (chemistry) (central lab) [h,i,j,k,l]							X	X	X		X	X
Safety labs (chemistry) (local lab) [h,i,j,k]		X [m]	X	X [m]	X							
Cytokine panel (central lab) [h,n]		As clinically indicated										
Complement panel (central lab) [h,o]		As clinically indicated										
Troponin T and/or I (central lab) [h,j]							X	X	X		X	X
Troponin T and/or I (local lab) [h,j]		X	X	X	X							

Study Phase/Visit	Daily	Weeks 9, 10, 11	Week 12	Weeks 13, 14, 15	Week 16	Weeks 20, 28, 32, 40, 44	Week 24	Week 36	Week 48 or Early Withdrawal	Month 15	Months 18, 24, 30, 36, 42	Months 48, 54, 60
Study Day(s)	**NA**	**63, 70, 77**	**84**	**91, 98, 105**	**112**	**140, 196, 224, 280, 308**	**168**	**252**	**336**	**456**	**548, 730, 913, 1095, 1278**	**1460, 1643, 1825**
Visit Window (days)	**-1**	**±3**	**±3**	**±3**	**+10**	**±3**	**±14**	**±14**	**±14**	**±14**	**±14**	**±14**
Coagulation parameters (central lab) [h,i]							X	X	X		X	X
Anti-AAV8 NAb and TAb (central lab)			X		X		X	X	X		X	X
Anti-MTM1 antibody (central lab)			X		X		X	X	X		X	X
IFN-γ ELISpot (PBMCs) (central lab)		As clinically indicated [p]										
HLA typing (central lab) [q]			X [q]						X [q]		X [q]	
Urinalysis (central lab) [h,i]							X	X	X		X	X
Urinalysis (local lab) [h,i]		X	X		X							
Serum bile acid assay (fasting preferred; central lab)			X				X	X	X	X	X	X
Liver ultrasound [k]			X		X		X	X	X		X	X
Bone age (wrist X-ray) (not applicable for Germany)			X				X		X		X [ee]	
Assessment of ventilator parameters			X		X		X		X		X [ff]	
MIP, MEP, P0.1, tidal volume [r]			X		X		X		X		X [ff]	
Ventilator Weaning and Discontinuation Assessment			X [s]		X [s]		X [s]		X [s]		X [s, ff]	
Daytime polysomnogram							X		X [t]			
Secretions management assessment [u]			X		X	X	X	X	X		X	X
Motor milestone assessments [v]			X		X		X	X	X	X	X	X
Bayley-III, motor domain [v]			X		X		X	X	X	X	X [ff]	
CHOP INTEND [v,w]			X		X		X	X	X		X [ff]	
MFM-32 [v]			X		X		X	X	X		X [ff]	

Study Phase/Visit	Daily	Weeks 9, 10, 11	Week 12	Weeks 13, 14, 15	Week 16	Weeks 20, 28, 32, 40, 44	Week 24	Week 36	Week 48 or Early Withdrawal	Month 15	Months 18, 24, 30, 36, 42	Months 48, 54, 60
Study Day(s)	**NA**	**63, 70, 77**	**84**	**91, 98, 105**	**112**	**140, 196, 224, 280, 308**	**168**	**252**	**336**	**456**	**548, 730, 913, 1095, 1278**	**1460, 1643, 1825**
Visit Window (days)	**-1**	**±3**	**±3**	**±3**	**+10**	**±3**	**±14**	**±14**	**±14**	**±14**	**±14**	**±14**
ACEND			X		X		X	X	X		X	X
PedsQL [x]			X		X		X	X	X		X [ff]	
Clinical global impression scales [y]			X		X		X	X	X		X	X
In-depth interviews with caregiver and subject [z]							X		X			
Swallowing questionnaire			X		X		X	X	X		X	X
Speech questionnaire			X		X		X	X	X		X [ff]	
Communicative Development Inventories [aa]			X		X		X	X	X		X [ff]	
Muscle biopsy							X		X			
Glucocorticoid administration [bb]	to Wk 16											
Ursodiol administration [cc]	to Wk 12											
Viral shedding (central lab) [dd]		Wks 9, 11 only		Wks 13, 15 only			X	X	X		X	X
AEs/concomitant medications		X	X	X	X	X	X	X	X	X	X	X

AEs = adverse events; ACEND = Assessment of Caregiver Experience with Neuromuscular Disease; anti-AAV8 = anti-adeno-associated virus 8; BID = twice daily; CHOP INTEND = Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders; CK = creatine kinase; ECG = electrocardiogram; ECHO = echocardiogram; ELISpot = enzyme-linked immunosorbent spot assay; IFN-γ = interferon-gamma; IMP = investigational medicinal product; LFTs = liver function tests; MTM1 = gene encoding the myotubularin protein; MEP = maximal expiratory pressure; MFM-32 = Motor Function Measure-32; MIP = maximal inspiratory pressure; NA = not applicable; NAb = neutralizing antibody; ; P0.1 = inspiratory occlusion pressure at 0.1 seconds; PBMCs = peripheral blood mononuclear cells; PedsQL = Pediatric Quality of Life Inventory; PGIS-I = Parental Global Impression of Secretion Improvement; PGIS-S = Parental Global Impression of Secretion Severity; TAb = total antibody; TID = three times a day; WK = week.

NOTE: Week 9, 10, 11, 13, 14, or 15 procedures can be performed at the study site clinic, or in the subject's home by a trained home health nurse. If the home health nurse is utilized then the safety laboratory tests will be sent to the central laboratory for processing. Home health nurses may collect laboratory samples, perform a limited physical examination, vital signs, and review concomitant medications and AEs upon request of the Investigator, as needed at any protocol scheduled visit or unscheduled visit.

[a] The diary should be completed daily by the same parent/LAR/caregiver (whenever possible); see Section 5.1.1.11.2.1. The daily diary collection will continue through Week 48. However, if after the Week 24 visit, ventilator support has been discontinued, the daily diary will no longer need to be completed. If ventilator support is resumed, the daily diary completion will resume through Week 48 or discontinuation of the ventilatory support, whichever occurs first.

[b] For subjects in Part 1 who have already completed the Week 24 visit, the parent/LAR/caregiver will complete the ventilator dependence questionnaire at clinic and contact visits through Month 120. For subjects in Part 1 who have not completed the Week 24 visit and for subjects in Part 2, parent/LAR/caregivers will complete the daily diary (Section 5.1.1.11.2.1). After the daily diary has been stopped or after subject has completed Week 48, the parent/LAR/caregiver will complete the ventilator dependence questionnaire at clinic and contact visits through Month 120.

[c] PGI-S, PGI-I, assessment of subject's ventilator status, review of adverse events and concomitant medications should be assessed at each contact visit.

[d] Complete physical examination at Week 24, Week 48, and Month 60 or Early Withdrawal. At all other timepoints, conduct an abbreviated physical examination based on the subject's circumstances and in accordance with the Investigator's clinical judgement.

[e] Record whether the subject was on or off of ventilatory support when vital signs are taken.

[f] ECHOs should include an assessment of pulmonary hypertension as well as structural and functional abnormalities. Particular attention should be paid to ECG and ECHO abnormalities specific to myocarditis. Refer to Section 4.6.1 for management of ECG and ECHO results and the role of the site pediatric cardiologist.

[g] After the Week 48 visit, ECHO only needs to be performed annually thereafter (Month 24, Month 36, Month 48, Month 60).

[h] See Appendix 1 for list of laboratory assessments.

[i] It is recommended to use the central laboratory to analyze laboratory tests that are drawn prior to dosing, and after the Week 16 Visit and to use the local laboratory to analyze laboratory tests that are drawn at Baseline, Dosing, Days 1, 2, and Weeks 1 through 16. However, the local laboratory can be used for other visits if needed (for example, the local laboratory can accommodate the analysis with a smaller amount of blood) or if the local laboratory does not accommodate the appropriate analysis, the central laboratory may be used.

[j] See Section 4.6.1 for details related to the evaluation of cardiac enzymes; monitoring, diagnosis, and treatment of myocarditis; and the role of the site pediatric cardiologist.

[k] See Section 4.6.2 for details related to the monitoring, diagnosis, and treatment of hepatobiliary toxicity and the role of the site hepatologist.

[l] See Section 4.6.3 for details related to the monitoring, diagnosis, and treatment of neuromuscular abnormalities.

[m] LFTs, CK (including CK isoenzymes), C-reactive protein, and serum bicarbonate only.

[n] Cytokine panel (see Appendix 1): Following Week 4 cytokine panel collection, in cases of clinically important elevated bilirubin, elevated aminotransferases, elevated troponins, and/or elevated creatine phosphokinase that in the Investigator's judgment may be related to T cell responses, repeat cytokine panel testing is suggested. Appropriate care for an individual subject will be determined by the Investigator based on an assessment of the subject's overall medical status.

[o] Complement panel (see Appendix 1): Following Week 4 complement panel collection, in cases of clinically important elevated bilirubin, elevated aminotransferases, ascites, thrombocytopenia, acute renal insufficiency, and/or anemia that in the Investigator's judgment may be related to complement activation, repeat complement panel testing is suggested. Appropriate care for an individual subject will be determined by the Investigator based on an assessment of the subject's overall medical status.

[p] IFN-γ ELISpot: Following AT132 administration, in cases of clinically important elevated bilirubin, elevated aminotransferases, elevated troponins, and/or elevated creatine phosphokinase that in the Investigator's judgment may be related to T-cell responses, repeat IFN-γ ELISpot response testing is suggested. Appropriate care for an individual subject will be determined by the Investigator based on an assessment of the subject's overall medical status.

[q] The sample will only need to be collected 1 time during the study. The sample will be collected at the Week 12 visit, unless HLA type has been obtained in INCEPTUS. For subjects in Part 1 who have already passed the timepoint listed above, the HLA sample will be collected at the Week 48 visit or the Month 24 visit, whichever occurs first.

[r] If a subject has discontinued the use of the ventilator and has 2 assessments with the MIP at 80 cmH_2O, testing for MIP, MEP, and P0.1 can be stopped.

[s] The site pulmonologist and local pulmonologist, if applicable, will review the results from the visit, assess the subject, determine if the subject meets the criteria outlined in Table 1 to begin reduction of hours on the ventilator, and determine if the subject meets the criteria outlined in Table 2 to discontinue the ventilator.

[t] Daytime polysomnogram not required at Week 48 if subject has already been weaned off the ventilator.

[u] The severity of secretions and burden of secretions management will be assessed using the PGIS-S and PGIS-I (Appendix 3). The same parent/LAR/caregiver should report throughout the study (whenever possible).

[v] All physical therapy assessments should be videoed and performed according to the site manual.

[w] If a subject has a total score of 56 for 2 consecutive assessments, the CHOP INTEND will not be required for future visits.

[x] The PedsQL is to be performed for all subjects, regardless of age. Subjects < 2 years of age should be administered the version for 2- to 4-year-olds.

[y] See Appendix 5.

[z] In-depth interviews are optional and will be completed at either Week 24 or Week 48.

[aa] Parents/LARs/caregivers (the same person every time, whenever possible) in ASPIRO will be asked to complete forms for subjects 8 months and older.

[bb] Subjects will be administered a course of glucocorticoid therapy commencing Day -1 and continuing for a period of 16 weeks as a preventative measure for immune-mediated hepatic injury. For subjects weighing < 60 kg, the dose will be 1 mg/kg prednisolone orally daily for 8 weeks, then tapered down for 8 weeks per Investigator discretion. For subjects weighing ≥ 60 kg, the dose will be 60 mg prednisolone orally daily for 8 weeks, then tapered down for 8 weeks per Investigator discretion.

[cc] Ursodiol to be dosed at 20 mg/kg/day (divided either BID or TID at the Investigator's discretion) to Week 12 and at the Investigator's discretion thereafter.

[dd] Viral shedding will be assessed on samples of saliva (swab), stool, and urine (diaper collection or urine bag). Collection of each sample type will begin on Day 1 and will continue until each sample type has 3 consecutive data points at or below the limit of detection (BLOD). Sponsor will notify site when collection can cease. Not conducted at Week 10 or 14.

[ee] Subjects in Germany will not have a wrist X-ray. After the Week 48 visit, wrist X-ray only needs to be performed annually thereafter (Month 24, Month 36). As of protocol v12, the wrist X-ray assessment will no longer be collected at Month 48 visit onward.

[ff] As of protocol v12, these assessments will no longer be collected at Month 48 visit onward.

5.6 Schedule of Events for Subjects Treated with AT132: Month 72 (Year 6) – Month 120 (Year 10) (End of Study)

Study Phase/Visit	**Month 72**	**Month 84**	**Month 96**	**Month 108**	**Month 120**
Study Day	**2190**	**2555**	**2920**	**3285**	**3650**
Visit Window (days)	**±60**	**±60**	**±60**	**±60**	**±60**
Physical Examination [a]	X	X	X	X	X
Ventilator Dependence Questionnaire [b]	X	X	X	X	X
Samples for:	X	X	X	X	X
Safety labs (hematology) (central lab) [c,d]	X	X	X	X	X
Safety labs (chemistry) (central lab) [d,e,f,g] Comprehensive metabolic profile ALT, AST, Total and direct bilirubin, GGT Serum bile acids, total (fasting preferred) PTT and PT/INR Troponin I and T CK Alpha fetoprotein	X	X	X	X	X
Liver ultrasound (local) [f]	X	X	X	X	X
Motor milestone assessments [h]	X	X	X	X	X
ACEND	X	X	X	X	X
Clinical global impression scales [i]	X	X	X	X	X
Swallowing questionnaire	X	X	X	X	X
Secretions management assessment [j]	X	X	X	X	X
AEs/concomitant medications	X	X	X	X	X

NOTE: Long-term follow-up visits and/or assessments after the 5-year (Month 60) visit can be done remotely per discretion of investigator in consultation with Medical Monitor.

AEs = adverse events; ACEND = Assessment of Caregiver Experience with Neuromuscular Disease; ALT = alanine aminotransferase; AST = aspartate aminotransferase; CK = creatine kinase; GGT = gamma-glutamyltransferase; INR = international normalized ratio; LAR = legally authorized representative; PGIS-I = Parental Global Impression of Secretion Improvement; PGIS-S = Parental Global Impression of Secretion Severity; PT = prothrombin time; PTT = partial thromboplastin time.

[a] Complete physical examination with detailed neurological evaluation and respiratory function assessment will be conducted. It can be done remotely per discretion of investigator in consultation with Medical Monitor. When visit is done remotely, an abbreviated physical examination is acceptable.

[b] After the daily diary has been stopped or after subject has completed Week 48, the parent/LAR/caregiver will complete the ventilator dependence questionnaire at clinic and contact visits through Year 10 (Month 120).

[c] See Appendix 1 for list of laboratory assessments.

[d] The local laboratory can be used for visits, if needed (for example, the local laboratory can accommodate the analysis with a smaller amount of blood) or if the local laboratory does not accommodate the appropriate analysis, the central laboratory may be used.

[e] See Section 4.6.1 for details related to the evaluation of cardiac enzymes; monitoring, diagnosis, and treatment of myocarditis; and the role of the site pediatric cardiologist.

[f] See Section 4.6.2 for details related to the monitoring, diagnosis, and treatment of hepatobiliary toxicity and the role of the site hepatologist.

[g] See Section 4.6.3 for details related to the monitoring, diagnosis, and treatment of neuromuscular abnormalities.

[h] All physical therapy assessments should be videoed and performed according to the site manual.

[i] See Appendix 5.

[j] The severity of secretions and burden of secretions management will be assessed using the PGIS-S and PGIS-I (Appendix 3). The same parent/LAR/caregiver should report throughout the study (whenever possible).

5.7 Schedule of Events for Control Subjects: Control Baseline – Control Month 30 (End of Study)

Study Phase/Visit	Daily	Baseline	Week 4	Week 12	Week 16	Week 24	Week 36	Week 48, Months 15, 18, 21, 24, 30, or EW
Study Day(s)	**NA**	**-2 to -1**	**28**	**84**	**112**	**168**	**252**	**336, 420, 504, 588, 672, 840**
Visit Window (days)	**-1**	**-1**	**±3**	**±3**	**±3**	**±14**	**±3**	**±3**
Inclusion/exclusion criteria		X [a]						
Medical history		X [b]						
Physical examination [c]		X [b]	X	X	X	X	X	X
Vital signs [d]		X [b]	X	X	X	X	X	X
12-Lead ECG [e]		X	X	X	X	X	X	X
ECHO [e]		X	X	X	X	X	X	X
Growth parameters		X	X	X	X	X	X	X
Tanner stage (puberty assessment)		X						
Samples for:								
Safety labs (hematology) (central lab) [f,g]		X	X	X	X	X	X	X
Safety labs (chemistry) (central lab) [f,g,h,i]		X	X	X	X	X	X	X
Troponin T and/or I (central lab) [f,h]		X	X	X	X	X	X	X
Coagulation parameters (central lab) [f,g]		X	X	X	X	X	X	X
Anti-AAV8 antibody NAb and TAb (central lab)			X	X	X	X	X	X
Urinalysis (local lab) [f,g]		X	X	X	X	X	X	X
Liver ultrasound [i]		X	X	X	X	X	X	X
Bone age (wrist X-ray) (not applicable for Germany) [j]		X						
Diary of ventilator dependence [k]	X							
Ventilator Weaning and Discontinuation Assessment [l]		X	X	X	X	X		W48, Months 18, 24, 30 or EW
Assessment of ventilator parameters		X	X	X	X	X	X	X
MIP, MEP, P0.1, tidal volume		X	X	X	X	X	X	X

Study Phase/Visit	Daily	Baseline	Week 4	Week 12	Week 16	Week 24	Week 36	Week 48, Months 15, 18, 21, 24, 30, or EW
Study Day(s)	**NA**	**-2 to -1**	**28**	**84**	**112**	**168**	**252**	**336, 420, 504, 588, 672, 840**
Visit Window (days)	**-1**	**-1**	**±3**	**±3**	**±3**	**±14**	**±3**	**±3**
Secretions management assessment [m]		X [n]	X	X	X	X	X	X
Daytime polysomnogram						X		
Motor development milestone assessments [o]		X	X	X	X	X	X	X
Bayley-III, motor domain [o]		X	X	X	X	X	X	X
CHOP INTEND [o]		X	X	X	X	X	X	X
MFM-32 [o]		X	X	X	X	X	X	X
ACEND		X	X	X	X	X	X	X
PedsQL [p]		X	X	X	X	X	X	X
Clinical global impression scales [q]		X [q]	X	X	X	X	X	X
Swallowing questionnaire		X	X	X	X	X	X	X
Speech questionnaire		X	X	X	X	X	X	X
Communicative Development Inventories [r]		X	X	X	X	X	X	X
AEs/concomitant medications		X	X	X	X	X	X	X

AEs = adverse events; ACEND = Assessment of Caregiver Experience with Neuromuscular Disease; anti-AAV8 = anti-adeno-associated virus 8; CGI-S = Clinical Global Impression of Severity; CHOP INTEND = Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders; ECG = electrocardiogram; ECHO = echocardiogram; EW = early withdrawal; LAR = legally authorized representative; MEP = maximal expiratory pressure; MFM-32 = Motor Function Measure-32; MIP = maximal inspiratory pressure; NA = not applicable; P0.1 = inspiratory occlusion pressure at 0.1 seconds; PedsQL = Pediatric Quality of Life Inventory; PGIS-I = Parental Global Impression of Secretion Improvement; PGIS-S = Parental Global Impression of Secretion Severity.

[a] All inclusion/exclusion criteria should be reviewed on Day -1 to confirm that the subject continues to meet eligibility criteria before dosing.

[b] Baseline measures of medical history, physical examination, vital signs, and assessment of ventilator use should be collected on Day -1. Medical history at baseline should capture changes since the screening visit.

[c] Complete physical examination at Baseline. At all other timepoints, conduct an abbreviated physical examination based on the subject's circumstances and in accordance with the Investigator's clinical judgement.

[d] Record whether the subject was on or off of ventilatory support when vital signs are taken.

[e] ECHOs should include an assessment of pulmonary hypertension as well as structural and functional abnormalities. Particular attention should be paid to ECG and ECHO abnormalities specific to myocarditis. Refer to Section 4.6.1 for management of ECG and ECHO results and the role of the site pediatric_cardiologist.

[f] See Appendix 1 for list of laboratory assessments.

[g] It is recommended to use the central laboratory to analyze laboratory tests for delayed-treatment control visits. However, the local laboratory can be used if needed (for example, the local laboratory can accommodate the analysis with a smaller amount of blood) or if the local laboratory does not accommodate the appropriate analysis, the central laboratory may be used.

[h] See Section 4.6.1 for details related to the evaluation of cardiac enzymes; monitoring, diagnosis, and treatment of myocarditis; and the role of the site pediatric cardiologist.

[i] See Section 4.6.2 for details related to the monitoring, diagnosis, and treatment of hepatobiliary toxicity and the role of the site hepatologist.

[j] Subjects in Germany will not have a wrist X-ray.

[k] The diary should be completed daily by the same parent/LAR/caregiver (whenever possible); see Section 5.1.1.11.2.1.

[l] The site pulmonologist and local pulmonologist, if applicable, will review the results from the visit, assess the subject, determine if the subject meets the criteria outlined in Table 1 to begin reduction of hours on the ventilator, and determine if the subject meets the criteria outlined in Table 2 to discontinue the ventilator.

[m] The severity of secretions and burden of secretions management will be assessed using the PGIS-S and PGIS-I (Appendix 3). The same parent/LAR/caregiver should report throughout the study (whenever possible).

[n] At baseline, the PGIS-S is conducted for all subjects.

[o] All physical therapy assessments should be videoed and performed according to the site manual.

[p] The PedsQL is to be performed for all subjects, regardless of age. Subjects < 2 years of age should be administered the version for 2- to 4-year-olds.

[q] See Appendix 5. At baseline, only the CGI-S is conducted for all subjects.

[r] Parents/LARs/caregivers (the same person every time, whenever possible) in ASPIRO will be asked to complete forms for subjects 8 months and older.

5.8 Unscheduled Visits

Additional assessments and optional assessments conducted as part of the study should be recorded on the unscheduled visit eCRFs.

5.9 Assessments for Early Withdrawal from Study

If a subject chooses to withdraw from the study, every attempt should be made to perform the early withdrawal procedures as listed for Week 48 in Section 5.5 for treated subjects and for Control Week 48 in Section 5.7 for control subjects. Medical and public records and/or laboratory results should continue to be obtained once the subject has withdrawn consent for safety and scientific reporting. Additional details can be found in the CRF Completion Guidelines.

5.10 Assessments for Untreated Delayed-Treatment Control Subjects

If a delayed-treatment control subject is not eligible to be dosed, and the subject agrees to remain on study, the subject will be followed up to Month 30 (from enrollment) as per the Schedule of Events in Section 5.7.

5.11 Amount of Blood (UNIQUE to France)

Blood Samples for:	**Blood Volume per Visit**
Genetic testing (central lab)	3 mL
Safety labs (hematology) (central lab)	0.5 mL
Safety labs (hematology) (local lab)	0.5 mL
Safety labs (chemistry) (central lab), including cytokine panel and Troponin T and/or I	2.5 mL
Safety labs (chemistry) (local lab)	2.0 mL
Complement panel (central lab)	3.2 mL
Coagulation parameters (central lab)	1.8 mL
Anti-AAV8 NAb and TAb, and anti-MTM1 antibody (central lab)	2.5 mL
IFN-γ (PBMCs) (central lab)	4.0 mL
HLA typing (central lab)	2.0 mL
Serum bile acid assay (fasting preferred; central lab)	1.0 mL

Anti-AAV8 = anti-adeno-associated virus 8; anti-MTM1 = gene that codes for myotubularin (a mutation in the *MTM1* gene results in myotubular myopathy); HLA = human leukocyte antigen; IFN-γ = interferon-gamma; NAb = neutralizing antibody; PBMCs = peripheral blood mononuclear cells; TAb = total antibody.

6 ADVERSE EVENT MANAGEMENT

6.1 Adverse Events

6.1.1 Definition of Adverse Events

According to the ICH 2EA guideline Definitions and Standards for Expedited Reporting and 21 CFR 312.32, IND Safety Reporting, an AE is any untoward medical occurrence associated with the use of the IMP in a study subject whether or not considered drug-related. An AE can therefore be any unfavorable and/or unintended sign, symptom, or disease temporally associated with the use of an IMP or study procedure, whether or not considered related to the IMP. This definition includes the following:

- Any pre-existing condition that increases in severity or changes in nature during or as a consequence of the IMP treatment administration or study procedure.
- All AEs (regardless of relationship to IMP) should be recorded from the Baseline Visit (Day -2 to -1) through the end of the safety reporting period.
- AEs occurring as a result of product withdrawal, abuse, overdose
- A change in a laboratory test results (eg, hematology, clinical chemistry, or urinalysis) or other safety assessments (eg, ECG, radiological scans, vital signs measurements) if considered by the Investigator to be clinically significant or if it caused (or should have caused) the Investigator to initiate a non-protocol therapy or procedure (refer to Section 6.5 for further details regarding assessment of abnormal laboratories). This includes results that worsen from baseline.

An AE does not include the following:

- Elective surgery or procedures for pre-existing conditions that are stable and have not worsened (eg, hospitalization for elective surgery, social and/or convenience admissions).
- Pre-existing diseases, conditions, or laboratory abnormalities present or detected before the IMP administration that do not worsen; such events should be recorded in the medical history eCRF.
- Any medical condition or clinically significant laboratory abnormality with an onset date before the consent form is signed and not related to a protocol-associated procedure is not an AE. It is considered to be pre-existing and should be documented on the medical history eCRF.

The Investigator and any qualified designees are responsible for detecting, documenting and recording events that meet the definition of an AE or SAE and remain responsible for following up AEs that are serious, considered related to AT132 and other study treatments or procedures, or that caused the subject to discontinue the study.

6.1.2 Adverse Event Reporting and Collection Period

The Investigator is responsible for monitoring the safety of subjects who have entered this study and for providing appropriate medical care.

The Investigator is responsible for evaluating all AEs, obtaining supporting documents, and for final review and confirmation of accuracy and completeness of event information and documentation. All SAEs will be reported on the eCRF and SAE worksheet. All AEs will be reported on the eCRF.

If the National Cancer Institute Common Terminology Criteria for AEs (NCI-CTCAE) grade of an SAE/AE changes, the event should be relisted on the eCRF, and SAE worksheet for SAEs, with the new NCI-CTCAE grade and new onset date.

The exception is ongoing pre-dose events that continue post-dose and improve post-dose. Such events should not be re-listed.

If the NCI-CTCAE grade of an SAE reduces, the details of the AE should be provided on the SAE worksheet for the medical assessor to be able to assess the course of the event.

6.1.3 Start of Adverse Event Collection Period

If a subject experiences an AE after the informed consent document is signed, but before the Baseline/Control Baseline visit, the event will be considered medical history.

If a control subject experiences an AE after the Control Baseline visit but before the Baseline visit prior to dosing, the event will be considered a Control AE.

If the subject experiences an SAE after signing consent and before Baseline, it should only be reported as an SAE if the Investigator believes that the event may have been caused by a protocol-required procedure.

6.1.3.1 Ongoing Adverse Event Collection During the Study

Before the Baseline visit, site personnel will note the occurrence and severity of each medical condition for the subject. During the study, site personnel will note any change in the severity of Baseline medical condition(s) and the occurrence and nature of any AEs. Whenever possible, an integrated diagnosis should be given for AE signs and symptoms of a common pathology. When recording AEs/SAEs, describe the event using commonly accepted medical terminology, if possible.

The details of each event should be discussed with the subject. If the event is serious, it must be reported to the Sponsor or its designee ***within 24 hours*** of the Investigator/designee/site personnel learning of the event.

6.1.3.2 End of Adverse Event Collection Period

AEs will be collected and reported until the subject completes the study or withdraws from the study.

AEs and SAEs will be followed as clinically indicated until resolution or, if non-resolving, until considered stable or until the end of the AE collection period, whichever comes first.

The Sponsor retains the right to request additional information for any study subject with ongoing AE(s)/SAE(s) at the end of the study, if judged necessary.

Investigators are not obligated to actively seek AEs or SAEs after conclusion of the study participation. However, if the Investigator learns of any SAE, including a death, at any time after a subject has been discharged from the study, and the event is considered to be reasonably related to the study drug or study participation, the Investigator must promptly notify the Sponsor.

6.1.3.3 Recording and Assessment of Seriousness, Severity, and Causality

It is the responsibility of the Investigator to review all documentation (eg, hospital progress notes, laboratory reports, and diagnostics reports) related to the event. Assessment of AEs must include seriousness, severity, relationship to study drug, duration, and outcome. At the Screening Visit, site personnel will note the occurrence and severity of each medical condition for the subject and document in Medical History. During the study, site personnel will note any change in the severity of medical condition(s) and the occurrence and nature of any AEs. Use precise medical terminology when recording AEs or SAEs. Record only 1 diagnosis, sign, or symptom in the AE eCRF (eg, nausea and vomiting should not be recorded in the same entry, but as 2 separate entries).

Whenever possible, a diagnosis should be recorded as the AE term rather than a series of terms relating to a diagnosis. If the diagnosis is unknown, record sign(s) and/or symptom(s). If a diagnosis subsequently becomes available, the diagnosis should be recorded, replacing the original entry(s) where appropriate.

The details of each event should be discussed with the subject.

All SAEs will be recorded and reported to the Sponsor or designee immediately and under no circumstance should this exceed 24 hours. The Investigator will submit any updated SAE data to the Sponsor ***within 24 hours*** of it being available.

The Sponsor retains the right to request additional information for any study subject with ongoing AE(s)/SAE(s) at the end of the study, if judged necessary. The Investigator will evaluate all AEs considered ongoing at the end of the study and document whether event has resolved, resolved with sequelae, or stabilized, with update to source and eCRF as appropriate.

It is not acceptable for the Investigator to send photocopies of the subject’s medical records to the Sponsor in lieu of completion of the SAE worksheet. There may be instances when copies of medical records for certain cases are requested by the Sponsor. In this case, all subject identifiers, with the exception of the subject number, will be redacted on the copies of the medical records before submission to the Sponsor.

6.1.3.4 Eliciting Adverse Events

The site personnel will instruct the subject to report any changes in health or new symptoms they notice during the study. At each visit, AEs should be elicited with minimal connotations. Care will be taken not to introduce bias when detecting AEs and/or SAEs. For example: “Have you had any health problems since your last visit?”

6.2 Serious Adverse Events

6.2.1 Definition of Serious Adverse Events

An SAE is defined as any untoward medical occurrence at any dose that results in the following:

- Death
- Life-threatening (The term “life-threatening” in the definition of “serious” refers to an event that, in the view of either the Investigator or Sponsor, places the subject at risk of death at the time of the event; it does not refer to an AE that hypothetically might have caused death if it were more severe.)
- Requires inpatient hospitalization or prolongs existing hospitalization
- Elective surgery or procedures for preexisting conditions that are stable and have not worsened (eg, hospitalization for elective surgery, social and/or convenience admissions) are not considered SAEs; however, an event that prolongs this type of hospitalization would be considered an SAE
- Persistent or significant disability/incapacity (The term disability means a substantial disruption of a person’s ability to conduct normal life functions).
- A congenital anomaly/birth defect
- An important medical event that may not result in death, be life-threatening, or require hospitalization, but may be considered an SAE when, based upon appropriate medical judgment, it may jeopardize the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These important medical events should usually be considered medically significant, and therefore serious. Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias, or convulsions that do not result in hospitalization. Potential drug induced liver injury (DILI) should be considered a medically significant event.

6.2.2 Serious Adverse Event Reporting Requirements

Prompt notification by the Investigator to the Sponsor of a SAE is essential so that legal obligations and ethical responsibilities towards the safety of subjects and the safety of a study drug under clinical investigation are met. The Investigator must complete and submit an SAE worksheet containing all information that is required by local and/or regional regulations to the Sponsor by fax or email immediately (***within 24 hours*** of awareness).

The SAE worksheet must be signed by a medically qualified Investigator (as identified on delegation of authority log). Signature confirms accuracy and completeness of the SAE data, as

well as the Investigator causality assessment including the explanation for the causality assessment.

Regardless of whether the SAE is deemed related to use of the IMP or study procedure, the data for the SAE must be reported with the appropriate information to the Sponsor or its designee ***within 24 hours*** of Investigator/designee/site personnel learning of the event. The Sponsor's Medical Monitor or designee may be contacted at any time for immediate discussion regarding such an event.

Please refer to the Study Management Plan for additional guidance regarding SAE reporting to the Sponsor.

Sites must report all SAEs to their governing IRB/EC, as required by local regulations and guidelines.

The Investigator is encouraged to discuss with the Medical Monitor any AEs for which the issue of seriousness is unclear or questioned. Contact information for the Medical Monitor can be found in the study contact list.

All deaths, regardless of causality, occurring from the time the subject signs the ICF until the Month 60 Visit are to be reported as SAEs to the Sponsor or its designee ***within 24 hours*** of the Investigator, designee, and/or site personnel's awareness of the event. For fatal or life-threatening events, copies of hospital case reports, autopsy reports, and other documents are also to be submitted by email or fax when requested and applicable. Any supportive SAE documentation (eg, hospital records, autopsy reports, progress notes) provided to the Sponsor or its designee should have all personal subject identification fully redacted in accordance with governing privacy regulations.

Additional information may be requested by the Sponsor or its designee to ensure the timely completion of accurate safety reports.

Any medications necessary for treatment of the SAE must be recorded onto the medication section of the subject's eCRF and the event description of the SAE.

6.2.2.1 Follow-Up of Serious Adverse Events

After the initial SAE report, the Investigator is required to proactively follow each subject at subsequent visits/contacts. All SAEs will be followed until resolution, stabilization, the event is otherwise explained, or the subject is lost to follow-up. New follow-up data for SAEs must be reported ***within 24 hours*** of receipt. All SAEs will be followed as clinically indicated until resolution or, if non-resolving, until considered stable or until the end of the AE collection period (Section 6.1.2) whichever comes first.

6.2.2.2 Serious Adverse Event Onset and Resolution Dates

The onset date of the SAE is defined as the date the event meets the criteria of a serious event. The resolution date is the date the event no longer meets the criteria of a serious event, the

date the symptoms resolve, or the event is deemed chronic. In cases of hospitalizations, the hospital admission date is considered the onset date.

6.2.3 Suspected Unexpected Serious Adverse Reaction

6.2.3.1 Definition of Suspected Unexpected Serious Adverse Reaction

A suspected unexpected serious adverse reaction (SUSAR) is an SAE for which there is a reasonable possibility that the drug caused the SAE and is considered unexpected per the current Reference Safety Information (RSI). For the purposes of expedited safety reporting, “reasonable possibility” means there is evidence to suggest a causal relationship between the drug and the AE.

An AE is considered “unexpected” for regulatory reporting purposes if it is not listed in the current RSI within the Investigator’s Brochure or is not listed at the specificity or severity that has been observed.

6.2.3.2 Suspected Unexpected Serious Adverse Reaction Expedited Reporting Requirements

The Sponsor or its designee will submit SUSARs to appropriate Regulatory Authorities (including Competent Authorities in all Member States concerned), IRBs/ECs, and Investigators as per local laws and regulations. Fatal and life-threatening SUSARs will be submitted no later than 7 calendar days of first knowledge of the event and follow-up information submitted within an additional 8 days. All other SUSARs will be submitted within 15 calendar days of first knowledge of the event.

The Investigator will notify the IRBs/ECs of SUSARs in accordance with IRB/EC requirements and local laws and regulations. A copy of this notification must be provided to the Sponsor and/or its designee.

6.2.3.3 Urgent Safety Measures and Non-Suspected Unexpected Serious Adverse Reaction Reporting

An urgent safety measure (USM) is an intervention that is not defined by the protocol and can be put in place with immediate effect without needing to gain prior approval by the Sponsor, relevant competent authorities, IRB/EC, where applicable, in order to protect subjects from any immediate hazard to their health and/or safety. Either the Investigator or the Sponsor can initiate a USM. The cause of a USM can be safety-, product-, or procedure-related.

Investigators are required to report any USMs to the Sponsor or its designee ***within 24 hours***. The Sponsor or its designee will inform the Regulatory Authorities, IRBs/ECs, and other Investigators of any events that may occur during the clinical study that do not fall within the definition of a SUSAR, but may affect the safety of subjects participating in the clinical study, as required, in accordance with applicable laws and regulations. The reporting collection period for

urgent safety issues is the period from the signing of the ICF until the Year 10 Visit or the subject withdraws from the study.

The Investigator will notify the IRB/ECs of USMs in accordance with IRB/EC requirements and local laws and regulations. A copy of this notification must be provided to the Sponsor and/or its designee.

Non-SUSARs will be maintained by the Sponsor and provided in annual safety reports and/or other aggregate periodic summary reports to Regulatory Authorities and IRB/ECs as per local laws and regulations.

6.3 Adverse Event and Serious Adverse Event Severity Grades and Relationship to Investigational Medicinal Product

6.3.1 Assessment of Severity

The seriousness of an AE should not be confused with its severity. Severity is a measure of intensity (eg, Grades 1 through 5 or mild, moderate, and severe), whereas seriousness is based on subject/event outcome as defined by the criteria in Section 6.2. The Investigator is responsible for assessing the severity of an AE according to the NCI CTCAE version 4.03 (Appendix 6).

It is important to distinguish between seriousness (AE versus SAE) and severity (Grades 1 to 5) of AEs. Seriousness, not severity, serves as a guide for defining regulatory reporting obligations.

An AE of severe intensity need not necessarily be considered serious. For example, nausea that persists for several hours may be considered Grade 3 nausea, but not an SAE. On the other hand, a stroke that results in only a limited degree of disability may be considered a Grade 1 stroke, but would be a SAE.

Many common AEs are able to be graded according to NCI-CTCAE criteria. AEs that do not have a corresponding NCI-CTCAE term will be assessed according to the general guidelines for grading used in the NCI-CTCAE version 4.03 as shown in Table 4.

Table 4: Adverse Event Severity Grading Scale (NCI-CTCAE)

Grade	Description
1	Mild: asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
2	Moderate: minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL) [a]
3	Severe or medically significant but not immediately life-threatening: hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL [b]
4	Life threatening consequences; urgent intervention indicated
5	Death related to AE

ADL = activities of daily living; AE = adverse event; NCI-CTCAE = National Cancer Institute Common Terminology Criteria for AEs.

[a] Instrumental ADL refer to the following examples: preparing meals, shopping for groceries or clothes, using the telephone, managing money.

[b] Self-care ADL refer to the following examples: bathing, dressing and undressing, feeding oneself, using the toilet, taking medications, not bedridden.

Source: Appendix 6.

Any Grade 3 and 4 clinical laboratory result that represents an increase in severity from baseline will be reported as an AE if it is not associated with a diagnosis already reported on the eCRF. A Grade 1 or 2 clinical laboratory abnormality should be reported as an AE only if it is considered clinically significant by the Investigator.

6.3.2 Assessment of Relationship

The Investigator is responsible for assessing whether the event was related to IMP based on the guidelines in Table 5.

Table 5: Assessment of Relatedness

Not related to IMP	Another cause of the event is most plausible; and/or clinically plausible temporal sequence is inconsistent with the onset of the event and the IMP; and/or a causal relationship is considered biologically implausible.
Possibly related to IMP	An event that follows a reasonable temporal sequence with the IMP, but that could readily have been produced by a number of other factors.
Related to IMP	An event that follows a reasonable temporal sequence with the IMP, and the event could not be reasonably explained by the known characteristics of the subject's clinical state.

IMP = investigational medicinal product.

For the purposes of reporting to regulatory agencies, AEs deemed as possibly or related to IMP will be considered related and those deemed not related will be considered unrelated.

There may be situations in which an SAE has occurred and the Investigator has minimal information to include in the initial report to the Sponsor. While it is very important that the

Investigator always assesses causality for every event before the initial transmission of the SAE data to the Sponsor, the initial report should be submitted without delay (ie, ***within 24 hours*** of awareness). With limited or insufficient information about the event to make an informed medical judgment and in absence of any indication or evidence to establish a causal relationship, a causality assessment of “no” is to be considered. In such instance, the Investigator is expected to obtain additional information regarding the event as soon as possible and to re-evaluate the causality upon receipt of additional information. The medically qualified Investigator may revise the assessment of causality in light of new information regarding the SAE and shall send an SAE follow-up report in the eCRF and follow-up SAE worksheet with the new information and updated causality assessment.

6.4 Clinical Laboratory Abnormalities and Other Abnormal Assessments as Adverse Events or Serious Adverse Events

The Investigator must review the laboratory report, document this review, and record any clinically significant changes occurring during the study as an AE. The laboratory reports must be filed with the source documents.

Clinical significance of out-of-range laboratory findings is to be determined and documented by the Investigator or Sub-Investigator who is a qualified physician. Abnormal laboratory findings associated with the underlying disease are not considered clinically significant unless judged by the Investigator to be more severe than expected for the subject's condition.

Laboratory abnormalities that are considered to be clinically significant by the Investigator must be recorded as AEs. Laboratory abnormalities (eg, clinical chemistry, hematology, coagulation parameters, and urinalysis) that require medical or surgical intervention must be recorded as an AE, as well as an SAE if the abnormality meets SAE criteria (defined in Section 6.2.1). In addition, laboratory or other abnormal assessments (eg, ECG, X-rays, vital signs) that are associated with signs and/or symptoms must be recorded as an AE or SAE if they meet the definition of an AE or SAE as described in Sections 6.1.1 and 6.2.1, respectively. If the laboratory abnormality is part of a syndrome, record the syndrome or diagnosis (eg, anemia), not the laboratory result (eg, decreased hemoglobin).

The following laboratory abnormalities should be recorded as AEs regardless of whether considered to be clinically significant, unless the abnormality is considered to be part of an integrated diagnosis:

- Troponin I or Troponin T values above the ULN (or a non-zero value if no ULN is reported) for which additional diagnostic workup and/or treatment is indicated per consultation with the site pediatric cardiologist (see Section 4.6.1)
- Platelet values below the lower limit of normal, or below the subject’s baseline value if abnormal at baseline

Please contact the Medical Monitor if there are questions regarding how to capture laboratory abnormalities.

6.5 AEs and Laboratory Results of Special Interest

See current AT132 Investigator's Brochure for current RSI.

6.5.1 Cardiac Enzyme Elevations

Troponin elevations have been observed in the ASPIRO study. Elevation of cardiac enzymes (including troponin levels) should be evaluated as described in Section 4.6.1.

6.5.2 Management of Potential Immune Responses

Elevations of hepatic transaminases, CK, and troponin I, likely representing an immune response to the study treatment, have been reported in Study ATX-MTM-002 and are considered identified risks associated with AT132 administration. Refer to the Investigator's Brochure for details.

Guidelines for monitoring and management of such events are provided in Section 4.6. Appropriate care for an individual subject will be determined by the Investigator based on an assessment of the subject's overall medical status. Corticosteroid treatment typically resolves the elevation of liver transaminases. However, when adverse events related to the immune response are of significant concern (eg, rAAV vector dose 1×10^{14} vg/kg and/or a transgene-mediated immune response), immunomodulation may include medications such as rapamycin (sirolimus), mycophenolate mofetil, calcineurin inhibitors (eg, cyclosporine, tacrolimus), and rituximab (Prasad, 2022).

6.5.3 Potential Cases of Drug-Induced Liver Injury

Abnormal values in AST and/or ALT concurrent or with abnormal elevations in TBL that meet the criteria outlined below, in the absence of other causes of liver injury, are considered potential cases of drug-induced liver injury (eg, Hy's Law) and are always to be considered clinically significant medical events and reported as SAEs.

The 3 "requirements" for Hy's Law are:

1. Evidence that a drug can cause hepatocellular-type injury, generally shown by an increase in serum aminotransferases (AT) elevations > 3 × ULN ("2 × ULN elevations are too common in treated and untreated subjects to be discriminating").
2. Cases of increased TBL (at least 2 × ULN) with concurrent AT elevations at least 3 × ULN and no evidence of intra- or extra-hepatic bilirubin obstruction (elevated alkaline phosphatase) or Gilbert's syndrome (Temple, 2006).
3. No other reason can be found to explain the combination of increased AT and TBL, such as viral hepatitis A, B, or C; preexisting or acute liver disease; or another drug capable of causing the observed injury.

For studies for which a central laboratory is used, alerts will be generated by the central laboratory regarding moderate and severe liver abnormality to inform the Investigator and study team. Subjects should be further evaluated for additional symptoms suggestive of hepatobiliary dysfunction.

6.5.3.1 Definition of Liver Abnormalities

For laboratory abnormalities (including liver) reported as AEs, severity grading assessment should follow the NCI-CTCAE (v4.03) Investigation system organ class.

In addition, the subject should be considered to have **severe hepatic abnormalities for any of the following:**

- ALT or AST > 8 × ULN
- ALT or AST > 5 × ULN for more than 2 weeks
- ALT or AST > 3 × ULN and* TBL > 2 × ULN or international normalized ratio (INR) > 1.5 (If INR testing is applicable/evaluated)
- ALT or AST > 5 × ULN and* TBL > 2 × ULN
- ALT or AST > 3 × ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (> 5%)

* Samples taken simultaneously or within a maximum of 24 hours.

The Investigator may determine that abnormal liver function results, other than those described above, may qualify as moderate or severe abnormalities and require additional monitoring and follow-up.

6.5.3.2 Liver Abnormality Follow-Up Procedures

Confirmed moderate and severe abnormalities in hepatic functions should be thoroughly characterized by obtaining appropriate expert consultations, detailed pertinent history, physical examination, and clinical laboratory tests. Subjects with confirmed abnormal liver function testing should be followed as described below.

Confirmed moderately abnormal liver function tests should be repeated at least once per week, and then weekly or less if abnormalities stabilize and the subject is asymptomatic.

Severe hepatic liver function abnormalities as defined above will be considered an important medical event and should be reported as an SAE. The Sponsor should be contacted and informed of all subjects for whom severe hepatic liver function abnormalities possibly attributable to study drug are observed.

To further assess abnormal hepatic laboratory findings, the Investigator is expected to:

- Obtain a more detailed history of symptoms and prior or concurrent diseases. Symptoms and new-onset diseases are to be recorded as AEs. Illnesses and conditions such as hypotensive events and decompensated cardiac disease that may lead to secondary liver abnormalities should be noted. Nonalcoholic steatohepatitis is seen in obese hyperlipoproteinemic and/or diabetic subjects and may be associated with fluctuating AT levels. The Investigator should ensure that the medical history form captures any illness that predates study enrollment that may be relevant in assessing hepatic function.
- Obtain a history of concomitant drug use (including nonprescription medication, complementary and alternative medications), alcohol use, recreational drug use, and

special diets. Medications, including dose, are to be entered in the eCRF. Information on alcohol, other substance use, and diet should be entered on the eCRF or an appropriate document.

- Obtain a history of exposure to environmental chemical agents.
- Based on the subject's history, other testing may be appropriate including:
  - Acute viral hepatitis (A, B, C, D, E, or other infectious agents such as cytomegalovirus, Epstein-Barr virus)
  - Ultrasound or other imaging to assess biliary tract disease
  - Other relevant clinical laboratory tests including INR, total and direct bilirubin, albumin
- Consider gastroenterology or hepatology consultations.
- Submit results for any additional testing and possible etiology on the eCRF or an appropriate document.

6.5.3.3 Hyperbilirubinemia

Investigations into the nature of liver dysfunction in patients with XLMTM before and after AT132 administration suggest intrahepatic cholestasis as a likely central feature. Ursodiol is an enterally administered hydrophilic bile acid that can decrease the hydrophobic bile acid content within bile (Poupon, 2012). As hydrophilic bile acids are generally non-toxic to hepatocytes, hydrophobic bile acids can be toxic to these same cells in direct contact, ursodiol has been used to treat severe cholestatic syndromes, such as progressive intrafamilial intrahepatic cholestasis (Balistreri, 1997; Strauss, 2006; Suskind, 2006) and intra-hepatic and extra-hepatic cholestatic diseases in childhood (Kriegermeier, 2020). Ursodiol has also been used for prophylaxis of hepatic veno-occlusive disease (Cheuk, 2015). Subjects in this study will begin treatment with prophylactic ursodiol prior to AT132 administration, as described in Section 4.5.

7 STATISTICAL CONSIDERATIONS

The Statistical Analysis Plan (SAP) will provide a detailed description of the planned statistical analyses and data summaries. This section presents an overview of the planned analyses. Final analyses are not limited to the summaries described herein. If discrepancies exist between the text of the statistical analysis as planned in the protocol and the final SAP, the final SAP will define the planned analysis of record. Reasons for such discrepancies, will be described in the final study report.

Data listings and summaries will be presented for all subjects and by dose levels. All continuous study assessments will be summarized using descriptive statistics (ie, n, mean, standard deviation, median, Q1, Q3, minimum, and maximum). All categorical study assessments will be summarized by time point, as applicable, using frequency counts and percentages. Details for the efficacy analyses will be provided below and in the SAP.

As of protocol v9 and beyond: The 1.0×10^{14} vg/kg dose level based on a 1st generation titer method equates to 1.3×10^{14} vg/kg as determined by a 2nd generation vg titer assay (see Section 1.4.2); and the 3.0×10^{14} vg/kg dose level equates to 3.5×10^{14} vg/kg based on an analysis of the historical Study ATX-MTM-002 drug product lots using the 2nd generation vg titer assay.

The study is no longer able to address the stated objectives, hence the statistical analysis will provide descriptive summaries by treatment groups.

7.1 Sample Size

The following language was from protocol v9 that planned a formal analysis to compare 1.3×10^{14} vg/kg and delayed-treatment control, however given that the study will not be able to address the objectives, no such formal analysis will be conducted.

A power analysis for the primary analysis of the primary endpoint was conducted at 80% power and 0.05 level of alpha to estimate the required sample size based on Mean of 13.0 vs. 0.0 hours of reduction in ventilation need, with common SD of 6.0 by Week 24 using a t-test, resulting in at least N = 10 for a balanced 1:1 allocation between 1.0×10^{14} vg/kg AT132 (equates to 1.3×10^{14} vg/kg as determined by the 2nd generation vg titer assay) and delayed-treatment control. It is further assumed, that the same difference in means (SD), will provide at least 80% power using a Mixed Effect Model Repeat Measurement (MMRM) analysis proposed for the primary endpoint. However, it is possible that 1:1 allocation may not be feasible; 1 control subject serving as control for multiple treated subjects, or multiple control subjects being a match for a single treated subject. Therefore, all qualified control subjects will be used in the analyses to ensure adequate power is available to detect the intended difference between treated and control subjects.

7.2 Analysis Populations

The following analysis sets for subjects treated with AT132 will be used:

- Full Analysis Set (FAS) is defined as all randomized and/or enrolled subjects who received AT132 and had at least 1 postdose efficacy assessment. The FAS will be used for the primary analysis of the primary efficacy endpoint.
- Safety Analysis Set (SAF) is defined as all randomized and/or enrolled subjects who received AT132. The SAF will be used for the analysis of the safety endpoints.

In each analysis set, 3 treatment groups are defined by: (i) subjects treated at 1.3×10^{14} vg/kg (low dose), (ii) subjects treated at 3.5×10^{14} vg/kg (high dose), and (iii) all subjects treated with AT132 (overall; not applicable to efficacy endpoints).

7.3 Subject Disposition, Demographic Data, Baseline Characteristics, and Extent of Exposure

The number and percentage of study subjects who complete and discontinue the study as well as reasons for early discontinuation will be presented by low dose, high dose, and overall for the SAF.

Demographic and baseline measurements will be summarized by low dose, high dose, and overall on the FAS and SAF. Medical history will be summarized by low dose, high dose, and overall for the SAF.

Exposure will be confirmed based on the report of dosing recorded in the eCRF, and will be presented in a listing.

7.4 Statistical Analysis

7.4.1 Safety Analyses

The analysis of safety endpoints will be conducted by treatment group (low dose, high dose, and overall) for the SAF.

7.4.1.1 Adverse Events

AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) dictionary.

Events will be identified as treatment-emergent (TEAE) and non-treatment-emergent (non-TEAE) on the basis of the date of onset relative to the date of AT132 dosing.

Number and percentage of subjects with TEAEs classified by system organ class (SOC) and preferred term (PT); by severity, causality, seriousness, and action taken with regard to study drug will be summarized. Number (%) of subjects with TEAEs leading to discontinuation of study will also be summarized. Hospitalization will be summarized by frequency (%) and duration. TEAEs and/or hospitalizations may be annualized.

7.4.1.2 Laboratory and Other Safety Evaluations

For clinical safety laboratory test results, vital signs, and ECG parameters, the numeric values and corresponding changes from baseline will be summarized using descriptive statistics by parameter and time point.

Incidence of laboratory abnormalities that increase at least 1 toxicity grade from baseline at any time postbaseline will be summarized. If baseline data are missing, then any graded abnormality (ie, at least a Grade 1) will be considered.

ECHO findings, liver ultrasounds, antibody formation (anti-AAV8, anti-MTM1), and viral shedding will be summarized.

Change in special liver laboratory parameters of interest (eg, bilirubin, AST, ALT, and CK) will be summarized.

7.4.2 Efficacy Analysis

7.4.2.1 Analysis of Primary Efficacy Endpoint

The primary efficacy endpoint is change from baseline in hours of ventilation support at Week 24. The hours of ventilation support will be based on diary data from subjects for whom diary data was collected at baseline (those administered AT132 under protocol v5 and beyond, including all subjects in Part 2), and by assessment of time off ventilator questionnaire for all other subjects (those administered AT132 under protocol v4 and prior). Weekly scores will be the average of ventilation hours needed for at least 5 out of the 7 days. Missing values will be ignored if number of ventilation hours are available for at least 5 days, otherwise they will be imputed by the worst score of the week.

7.4.2.1.1 Primary Analysis

The primary efficacy endpoint will be analyzed using a MMRM with baseline, treatment (low dose or high dose), time (Weeks 1, 4, 12, 16, 24, 36, and 48), and treatment by time interaction as fixed effects and subject as random effect. The change from baseline in hours of ventilation support at Week 24 will be summarized by treatment group based on this model. The primary analysis will be conducted using the FAS.

7.4.2.1.2 Secondary Analysis

Based on the MMRM described in Section 7.4.2.1.1, the change from baseline in hours of ventilation support at Week 1, 4, 12, 16, 36, and 48 will be summarized by treatment group.

7.4.2.2 Analysis of Secondary Efficacy Endpoints

7.4.2.2.1 Key Secondary Efficacy Endpoints

The analysis of key secondary efficacy endpoints will be conducted using the FAS.

The number and percentage of subjects achieving functionally independent sitting for at least 30 seconds by Week 24 will be summarized by low dose and high dose.

7.4.2.2.2 Other Secondary Efficacy Endpoints

The analysis of other secondary efficacy endpoints will be conducted using the FAS.

Time to reduction in required daily ventilator support to ≤ 16 hours by Week 24, time to reduction of at least 6 hours in daily ventilator support by Week 24, and time to death will be summarized for low dose and high dose. Kaplan-Meier plots along with median (95% CI) time from the study start date to event will be provided.

The CHOP INTEND, MIP, ACEND, and PedsQL, as well as their changes from baseline, will be summarized descriptively over time, and the changes from baseline will be analyzed using the MMRM in a similar method to that used for the primary analysis of the primary efficacy endpoint.

The number and percentage of subjects attaining age-appropriate clinically relevant gross motor function milestones through Week 24, and subjects achieving full ventilator independence at Week 24, will be summarized for low dose and high dose.

7.4.2.3 Analysis of Exploratory Efficacy Endpoints

The analysis of exploratory efficacy endpoints will be conducted using the FAS.

Exploratory efficacy variables will be summarized descriptively over time for low dose and high dose.

Growth parameters will be evaluated graphically and compared to standard World Health Organization or National Institutes of Health normal curves, where possible.

7.5 Data Monitoring Committee

This study will utilize an independent DMC comprising at least 3 experts with relevant expertise who will monitor subject safety and efficacy and provide recommendations to the Sponsor regarding expansion of dose cohorts and dose escalation or decreases. The DMC may recommend accelerated dosing of control subjects if compelling efficacy results support such action.

DMC review will occur, at minimum, at the following events:

- A sentinel subject has been dosed and observed for at least 4 weeks in each of the cohorts (DMC chair review)
- Consideration of a dose escalation after at least 3 subjects at a dose level have been dosed and observed for at least 4 weeks following AT132 administration (full DMC review)
- Dose escalation has been stopped and participating subjects have 24 weeks of post-dose follow up, at which point it is anticipated that an optimal dose can be selected (full DMC review; applicable to Part 1 of study)

- Consideration of expanding enrollment at a specific dose level (full DMC review)
- Review of important medical events

In addition, the DMC will perform an ad hoc review, if requested by the Sponsor, or if stopping criteria listed in Section 7.6 are met.

The DMC's specific activities will be defined by a mutually agreed ATX-MTM-002 charter, which will define the DMC's membership, conduct, and meeting schedule.

While the DMC will be asked to advise the Sponsor regarding future conduct of the study, including possible early study termination due to treatment-related AEs, the Sponsor retains final decision-making authority on all aspects of the study.

7.6 Study Safety Stopping Rules

Dosing of additional subjects will be paused and the DMC will conduct a review if any of the following stopping criteria are reached:

- One Grade ≥ 4 AE or the occurrence of the same treatment-related Grade ≥ 3 AE on 2 or more occasions
- Any Grade ≥ 3 neuromuscular AE in a treated subject considered possibly related or related to AT132, as assessed by the Investigators
- Any Grade ≥ 3 cardiac AE in a treated subject considered possibly related or related to AT132, as assessed by the Investigators
- Any Grade ≥ 3 hepatobiliary AE in a treated subject considered possibly related or related to AT132, as assessed by the Investigators

The DMC may advise that the study should be terminated or that clinical protocol should be revised to mitigate the risk to subjects. Such revisions may include changes to the enrollment criteria, changes in the monitoring plan, and/or changes to the informed consent form.

If dosing of additional subjects is paused, all remaining protocol specified study visits, safety assessments, and end-of-study procedures should be completed in subjects who have already been dosed. If any Study Safety Stopping Rules are met while the study is already paused or on hold, these will be reported to the DMC since such criteria may change management of a previously treated subject.

7.7 Data Review Committees

In addition to the DMC described in Section 7.5, the following independent expert committee will adjudicate endpoints and review data:

- Biopsy Review Committee: Responsible for the blinded adjudication of histopathology endpoints in muscle biopsies.

8 RESPONSIBILITIES AND ADMINISTRATIVE PROCEDURES

The Sponsor aims to conduct its studies according to the highest scientific standards. The following sections articulate standards to which sites are held accountable, as well as matters of compliance to document adherence to such standards.

8.1 Ethical Conduct of the Study

It is expected that Investigators understand and comply with the letter and spirit of this protocol. This includes and is not limited to establishing and meeting enrollment commitments, including only eligible subjects in the study, adhering to diagnostic and other procedures as specified in the study, and assuring appropriate compliance with study medication.

This study will be conducted in accordance with the following:

- United States (US) CFR sections that address clinical research studies
- European Union (EU) Directive 2001/20/EC and EU Clinical Trial Regulation 536/2014
- Other National and Local regulations, as applicable
- International Council for Harmonisation (ICH) E6
- The ethical principles established by the Declaration of Helsinki
- All human clinical research and data privacy regulations in the countries where the study is to be conducted

8.2 Investigator Responsibilities

The Investigator is responsible for ensuring the study is conducted in accordance with the procedures and evaluations described in this protocol and that all persons involved in the conduct of the study are informed about the protocol, study procedures and any study related duties. The Investigator is responsible for assuring that study site staff are properly trained and credentialed to perform any delegated tasks.

Prior to beginning the study, the Investigator at each site must provide to the Sponsor or designee a fully executed Form FDA 1572 (or equivalent form) and Financial Disclosure Form. Financial Disclosure Forms must be completed for all Sub-Investigators listed on the Form FDA 1572 (or equivalent form) who will be directly involved in the evaluation of research subjects in this study.

8.3 Retention of Records

The Investigator must retain all study records required by the Sponsor and by the applicable regulations in a secure and safe facility. The Investigator must consult a Sponsor representative before disposal of any study records, and must notify the Sponsor of any change in the location, disposition, or custody of study files. The Investigator and/or institution must take measures to prevent accidental or premature destruction of essential documents, that is, documents that individually and collectively permit evaluation of the conduct of a study and the quality of data

produced, including paper copies of study records (eg, subject charts) as well as any original source documents that are electronic, as required by applicable regulatory requirements.

All study records must be retained for at least 2 years after the last approval of a marketing application in the US or an ICH region and until (1) there are no pending or contemplated marketing applications in the US or an ICH region or (2) at least 2 years have elapsed since the formal discontinuation of clinical development of the IMP. The Investigator and/or institution should retain subject identifiers for at least 25 years after completion or discontinuation of the study. Subject files and other source data must be kept for the maximum period of time permitted by the hospital, institution, or private practice but not less than 25 years. These documents should be retained per the applicable regulatory requirements in the country/local region in which the study is being conducted or by a Sponsor agreement. The Sponsor must be notified and will assist with retention should an Investigator and/or institution be unable to continue maintenance of subject files for the full 25 years. It is the responsibility of the Sponsor to inform the Investigator and/or institution when these documents no longer need to be retained.

8.4 Institutional Review Board (IRB)/Ethics Committee (EC) Review and Approval

Prior to initiating the study, the Investigator will obtain written confirmation that the IRB/EC is properly constituted and compliant with ICH Guidelines and GCP requirements, applicable laws and local regulations. A copy of the confirmation from the IRB/EC will be provided to the Sponsor, or designee. The Investigator (or Sponsor as appropriate according to local regulations) will submit this protocol, ICF (including compensation procedures), and any accompanying material to be provided to the subject (such as subject information sheets or descriptions of the study used to obtain informed consent) to an IRB/EC. The Investigator will not begin any study subject activities until approval from the IRB/EC has been documented and provided as a letter to the Investigator.

Before implementation, the Investigator will submit to and receive documented unconditional approval from the IRB/EC for any modifications made to the protocol or any accompanying material to be provided to the subject after initial IRB/EC approval, with the exception of those necessary to reduce immediate risk to study subjects. The approval document should refer to the study by protocol title, Sponsor, and study number (if possible), identify the documents reviewed, and include the date of the review and approval.

Other Investigator responsibilities include the following:

- Submit to the appropriate committees and to the Sponsor or its designee for review any advertisements that will be used to recruit subjects
- During the conduct of the study, submit progress reports to the appropriate committees, if required, and request re-review of the study at least once a year
- Report, in writing, to the appropriate committees any SAEs that occur during the study or SAEs reported in other studies using study treatment, per local regulations

- Inform the appropriate committees of any changes in the protocol and obtain documented approval of the changes
- Provide the appropriate committees with any other information it requests before or during the conduct of the study
- Maintain a file of study-related information, including all correspondence with the committees
- Provide the appropriate committees with a final report on the study within a time period consistent with local requirements

8.4.1 Informed Consent

A properly written and executed ICF, in compliance with ICH E6 and 21 CFR 50 (Subpart B [50.20-50.27], and 50.55, if applicable) and other applicable local regulations, will be obtained by the Investigator or a person delegated by the Investigator. Due to the pediatric nature of the disorder, the study will be in compliance with 21 CFR 50 Subpart D. The site will prepare the ICF and provide the documents to the Sponsor or designee for approval prior to submission to the IRB/EC. Both the Sponsor and the IRB/EC must approve the documents before they are implemented. A copy of the approved ICF and, if applicable, a copy of the approved subject information sheet must be received by the Sponsor prior to enrollment of subjects.

The Investigator or designee is responsible for obtaining written informed consent from each subject or parent/LAR after adequate explanation of the aims, methods, objectives, and potential hazards of the study and before undertaking any study-related procedures. The site must use the most current IRB-/EC-approved ICF for documenting written informed consent. Each ICF will be appropriately signed and dated by the subject or parent/LAR and the person conducting the consent discussion, and also by an impartial witness if required by IRB/EC or local requirements.

A subject younger than 18 years (or defined as a minor, depending on the region) will provide assent if required by IRB/EC or local requirements.

8.4.2 Subject Information and Confidentiality

The Investigator must assure that subjects' anonymity will be strictly maintained and that their identities are protected from unauthorized parties. Only a unique identifier (as allowed by local law), and an identification code will be recorded on any form or biological sample submitted to the Sponsor, IRB/EC, or laboratory. Laboratory specimens must be labeled in such a way as to protect subject identity while allowing the results to be recorded to the proper subject.
NOTE: The Investigator must keep a screening log showing codes, names, and addresses for all subjects screened and for all subjects enrolled in the study. Subject data will be processed in accordance with all applicable regulations.

The Investigator agrees that all information received from the Sponsor, including but not limited to this protocol, eCRF, and any other study information, remain the sole and exclusive property

of the Sponsor during the conduct of the study and thereafter. This information is not to be disclosed to any third party (except employees or agents directly involved in the conduct of the study or as required by law) without prior written consent from the Sponsor. The Investigator further agrees to take all reasonable precautions to prevent the disclosure by any employee or agent of the study site to any third party or otherwise into the public domain.

8.5 Sponsor Responsibilities

8.5.1 Protocol Modifications

Protocol modifications, except those intended to reduce immediate risk to study subjects, may be made only by the Sponsor. If there are any substantial changes to the study protocol, these changes will be documented in a study protocol amendment and, where required, in a new version of the study protocol. The amendment should be approved by the IRB/EC and the appropriate regulatory authority(ies) before implementation, as appropriate. Local requirements should be followed for amended protocols. If a protocol amendment requires a change to the ICF, the IRB/EC must approve the revised ICF before the revised form is used.

8.5.2 Study Discontinuation

The Sponsor reserves the right to terminate the study at any time. Should this be necessary, the Sponsor and the Investigators will arrange discontinuation procedures and notify the appropriate IRBs/ECs. In terminating the study, the Sponsor and the Investigators will assure that adequate consideration is given to the protection of the subjects' interests.

8.5.3 Study Reports

A clinical study report (CSR) will be prepared and provided to the regulatory agency(ies) by the Sponsor, as appropriate. The Sponsor will ensure that the report meets the standards set out in the ICH Guideline for Structure and Content of Clinical Study Reports (ICH E3). Note that an abbreviated report may be prepared in certain cases.

8.5.4 Study Monitoring and Auditing

Qualified individuals designated by the Sponsor will monitor all aspects of the study according to GCP and standard operating procedures for compliance with applicable government regulations. The Investigator agrees to allow these monitors direct access to the clinical supplies and storage areas and to the clinical files, including original medical records of the study subjects, and, if requested, agrees to assist the monitors. The Investigator and staff are responsible for being present or available for consultation during routinely scheduled site visits conducted by the Sponsor or its designees.

Members of the Sponsor or designee may conduct an audit of a clinical site, IRB, or EC at any time before, during, or after completion of the study. The Investigator will be informed if an audit is to take place and advised as to the scope of the audit. Representatives of the FDA or other regulatory agencies may also conduct an audit of the study. If informed of such an inspection,

the Investigator should notify the Sponsor immediately. The Investigator will ensure that auditors have access to clinical supplies, study site facilities, original source documentation, and all study files.

8.5.5 Data Monitoring Committee

A DMC consisting of at least 3 independent medical experts will review data for subject safety and provide recommendations for study conduct to the Sponsor (Section 7.5).

8.6 Joint Investigator/Sponsor Responsibilities

8.6.1 Case Report Forms

eCRFs will be provided by the Sponsor to the Investigator for each subject. eCRFs must be completed using a validated web-based application. Study site personnel, central reviewer, or designee will be trained to enter the clinical data onto the eCRFs from source documentation using the web-based application. Unless explicitly allowed in the eCRF instructions, blank data fields are not acceptable.

The Investigator must review and electronically sign the eCRF casebook completed by the site to verify its accuracy.

In the event of an entry error, or if new information becomes available, the value will be corrected by deselecting the initial response and then selecting or entering the updated response. In compliance with US 21 CFR Part 11, the web-based eCRF system will require the personnel making the correction to enter a reason for changing the value. The documented audit trail will include the reason for the change, the original value, the new value, the time of the correction, and the identity of the operator.

Study data entered into the eCRFs must be verifiable to source data, which necessitates access to all original recordings, laboratory reports, and subject records. In addition, all source data should be attributable (signed and dated). The Investigator must, therefore, agree to allow direct access to all source data. Subjects (or legally authorized representative) must also allow access to their medical records. Subjects will be informed of the necessity for such access and will confirm their agreement with this when providing informed consent. If an Investigator or institution refuses to allow access to subject records because of confidentiality, arrangements must be made to allow an “interview” style of data verification.

A Sponsor clinical research associate (CRA) (or designee) will compare eCRFs with original source documents at the study site and evaluate eCRFs for completeness and accuracy before designating them as “source data verified.” If an error is discovered at any time or a clarification is needed, the CRA (or designee) will create an electronic query on the associated field. Study site personnel will then answer the query by either correcting the data or responding to the query. The CRA will then review the response and determine either to close the query or re-query the site if the response does not fully address the question. This process is repeated until all open queries have been answered and closed.

8.6.2 Payment Reporting

The Investigator and Sub-Investigators are required to disclose any financial arrangement during the study and for 1 year after study completion, whereby the outcome of the study could be influenced by the value of the compensation for conducting the study, or other payments the Investigator received from the Sponsor. The following information is collected: any significant payments from the Sponsor or subsidiaries such as a grant to fund ongoing research, compensation in the form of equipment, retainer for ongoing consultation or honoraria; any proprietary interest in investigational product; any significant equity interest in the Sponsor or subsidiaries (Investigator, spouse, or dependent children) as defined in 21 CFR 54 2(b).

8.6.3 Financing and Insurance

Financing and insurance for this clinical study will be addressed in appropriate study documents, in the Clinical Trial Agreement between the Sponsor and the Investigator and/or institution, or in the clinical study insurance policy(ies).

9 PUBLICATION AND USE OF INFORMATION

The Sponsor recognizes the importance of communicating medical study data and, therefore, encourages publication of these data in reputable scientific journals and at seminars or conferences. The details of the processes of producing and reviewing reports, manuscripts, and presentations based on data from this study will be described in the Clinical Trial Agreement between the Sponsor and the Investigator and/or institution. Consideration for authorship of all publications will be based on compliance with the Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals (“ICMJE Recommendations”) of the International Committee of Medical Journal Editors (http://www.icmje.org/recommendations/), Good Publication Practices (GPP), and the Sponsor’s internal publication and authorship policies.

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11 SIGNATURE PAGE – Sponsor

Accepted for the Sponsor:

Required sponsor signatures as required by ICH GCP 4.5.1 are located in the first attachment.

Attachment 1	Electronic Sponsor Signatures

12 SIGNATURE PAGE – Principal Investigator

Accepted for the Principal Investigator:

I have read the protocol and agree to conduct this study as described herein. I confirm that I will comply with all obligations as detailed in all applicable regulations and guidelines. I will ensure that the subject(s) and parent(s)/legal guardian(s) are informed of important information that becomes available during the conduct of this study.

Principal Investigator signature	Date
Printed name	

13 APPENDICES

Appendix 1: Laboratory Assessments

The following laboratory parameters will be measured by the central or local laboratory per the timing specified in the Schedule of Events (Sections 5.3.1, 5.4, 5.5, 5.6, and 5.7):

Hematology	Serum Chemistry	Urinalysis
White blood cells	Liver Function Tests	Color
Red blood cells	• Albumin, serum	Appearance
Hemoglobin (HGB)	• Alkaline phosphatase, serum	Urine pH
Hematocrit	• Alanine transaminase, serum	Urine specific gravity
Platelet count	• Aspartate transaminase	Leukocyte esterase urine
Absolute neutrophil	• Bilirubin direct	Nitrite urine
Absolute lymphocytes	• Bilirubin, total	Urine protein semiquant random
Absolute monocytes	• Gamma-glutamyl transferase	Urine glucose
Absolute eosinophils	Bicarbonate	Urine ketones (acetone) random
Absolute basophils	Calcium, serum	Urine urobilinogen random
Neutrophils	Chloride, serum	Urine blood
Lymphocytes	Creatine kinase	Urine bile
Monocytes	- CK-MM	
Eosinophils	- CK-BB	
Basophils	- CK-MB	**Coagulation Parameters**
Mean corpuscular volume	C reactive protein	Prothrombin time (PT)
Mean corpuscular HGB	Creatinine, serum	Activated partial thromboplastin time (PTT)
Mean corpuscular HGB concentration	Glucose, serum	International normalized ratio (INR)
Red cell distribution width	Lactate dehydrogenase	
Mean platelet volume	Phosphorus, serum	
Additional Hematology for Platelet Monitoring*	Potassium, serum	
Platelet Activation Assays	Protein, total serum	
- CD62P	Sodium, serum	
- Activated GP IIb/IIIa w/PAC-1	Urate (Uric Acid), serum	
- Phosphatidylserine	Urea nitrogen (BUN), serum	
Reticulated Platelets	Alpha fetoprotein	
Platelet Circulating Antibodies	**Additional Serum Chemistry**	
	Troponin T and/or troponin I	
	Complement Panel	
	- Complement C3	
	- Complement C4	
	- Complement CH50	
	- Soluble Complement C5b-9	
	Cytokine Panel	
	- IL-1b, IL-2, IL-6, IL-8, IL-10, IL-15, IFN-y, and TNF-α	
	Serum Bile Acids	

*Not applicable for France.

Appendix 2: Assessment of Tanner Stage for Boys

Stage	Genital Development	Pubic Hair Growth
1	Prepubertal; no change in size or proportion of testes, scrotum, and penis from early childhood	Prepubertal; no pubic hair
2	Enlargement of scrotum and testes; reddening and change in texture in skin of scrotum; little or no penis enlargement	Sparse growth of hair at base of penis
3	Increase first in length then width of penis; growth of testes and scrotum	Darkening, coarsening and curling, increase in amount
4	Enlargement of penis with growth in breadth and development of glands; further growth of testes and scrotum, darkening of scrotal skin	Hair resembles adult type, but not spread to medial thighs
5	Adult size and shape genitalia	Adult type and quantity, spread to medial thighs

Source: Tanner, 1962

Appendix 3: Secretions Management Assessment: PGIS-S and PGIS-I

Parental Global Impression of Secretion Severity (PGIS-S)

Taking into consideration the experience and understanding you have of your child; in terms of secretion management (ie, volume and thickness of secretions, and how often you need to suction your child) – how affected has your child been over the past 7 days?

1 – not affected

2 – borderline affected

3 – mildly affected

4 – moderately affected

5 – markedly affected

6 – severely affected

7 – among the worst he has ever been

Parental Global Impression of Secretion Improvement (PGIS-I)

In comparison to the baseline assessment (ie, the start of the study), and specifically with regard to management of secretions (ie, volume, thickness, frequency of suctioning), how has he changed?

1 – very much improved

2 – much improved

3 – minimally improved

4 – no change

5 – minimally worse

6 – much worse

7 – very much worse

Name of parent/LAR/caregiver providing information: _____

Date of completion: _____

Appendix 4: Assessment of Time Off of Ventilator

Site No. Subject ID Subject No: 1 ___ ___ - 9 ___ ___ ___	Date of Assessment (DD/Mon/YYYY): ___ ___/___ ___ ___/20 ___ ___
Ventilator Dependence Questionnaire	

1. Over the past 24 hours, what type of ventilation has your child used?	☐ BiPAP/CPAP ☐ Invasive ☐ None (do not complete question 2)
2. Over the past 24 hours, total number of minutes or hours the child has spent off of ventilator support?	___ ___ # of hours OR ___ ___ ___ ___ # of minutes

Name of parent/LAR/caregiver providing information: _____

Date of completion: _____

Appendix 5: Clinical Global Impression Scales: CGI-S and CGI-I

The Clinical Global Impression – Severity scale (CGI-S)

Rate the severity of the patient's illness at the time of assessment, relative to the clinician's past experience with patients who have the same diagnosis. Considering total clinical experience, a patient is assessed on severity of illness at the time of rating.

□ Not assessed

□ 1 = Normal, shows no signs of illness

□ 2 = Borderline ill

□ 3 = Slightly ill

□ 4 = Moderately ill

□ 5 = Markedly ill

□ 6 = Severely ill

□ 7 = Among the most extremely ill of patients

Please provide rationale for score provided.

The Clinical Global Impression – Improvement scale (CGI-I)

Assess how much the patient's illness has improved or worsened relative to baseline (Study Day 1)

□ Not assessed

□ 1 = Very much improved

□ 2 = Much improved

□ 3 = Minimally improved

□ 4 = No change

□ 5 = Minimally worse

□ 6 = Much worse

□ 7 = Very much worse

Please provide rationale for score provided.

Appendix 6: National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE)

Adverse events will be graded according to CTCAE v4.03.

The NCI CTCAE criteria are available at the following web location (accessed 22 February 2017): http://evs.nci.nih.gov/ftp1/CTCAE/About.html

Appendix 7: Parental Swallowing Questionnaire

1. Since the last visit how often do you use the following with your child (check one box for each line)

a. Tube Feeding
- ☐ Never
- ☐ Almost Never
- ☐ Sometimes
- ☐ Often
- ☐ Almost Always
- ☐ Always

b. Bottle Feeding
- ☐ Never
- ☐ Almost Never
- ☐ Sometimes
- ☐ Often
- ☐ Almost Always
- ☐ Always

c. Spoon from caregiver
- ☐ Never
- ☐ Almost Never
- ☐ Sometimes
- ☐ Often
- ☐ Almost Always
- ☐ Always

d. Fingers (self)
- ☐ Never
- ☐ Almost Never
- ☐ Sometimes
- ☐ Often
- ☐ Almost Always
- ☐ Always

e. Utensils (self)
- ☐ Never
- ☐ Almost Never
- ☐ Sometimes
- ☐ Often
- ☐ Almost Always
- ☐ Always

f. No spill cup
- ☐ Never
- ☐ Almost Never
- ☐ Sometimes
- ☐ Often
- ☐ Almost Always
- ☐ Always

g. Straw
- ☐ Never
- ☐ Almost Never
- ☐ Sometimes
- ☐ Often
- ☐ Almost Always
- ☐ Always

h. Open cup
- ☐ Never
- ☐ Almost Never
- ☐ Sometimes
- ☐ Often
- ☐ Almost Always
- ☐ Always

2. Since the last visit, how often does your child consume drinks or food of the following consistency?

a. **Thin**

Description

- Flows like water
- Fast flow
- Can drink through any type of teat/nipple, cup or straw as appropriate

- ☐ Never
- ☐ Almost Never
- ☐ Sometimes
- ☐ Often
- ☐ Almost Always
- ☐ Always

b. **Slightly Thick**

Description

- Thicker than water
- Requires a little more effort to drink than thin liquids
- Flows through a straw, syringe, teat/nipple
- Similar to the thickness of commercially available ‘Anti-regurgitation’ (AR) infant formula

- ☐ Never
- ☐ Almost Never
- ☐ Sometimes
- ☐ Often
- ☐ Almost Always
- ☐ Always

c. **Mildly Thick**

Description

- Flows off a spoon
- Sippable, pours quickly from a spoon, but slower than thin drinks
- Effort is required to drink this thickness through standard straw

- ☐ Never
- ☐ Almost Never
- ☐ Sometimes
- ☐ Often
- ☐ Almost Always
- ☐ Always

d. **Moderately Thick/Liquidized**

Description

- Will not hold its shape on a spoon
- Sippable, pours slowly off a spoon
- Difficult to suck through a standard or wide straw
- Cannot be piped, layered or moulded
- Cannot be eaten with a fork because it drops through the prongs

☐ Never
☐ Almost Never
☐ Sometimes
☐ Often
☐ Almost Always
☐ Always

e. **Extremely Thick/Pureed**

Description

- Holds shape on spoon
- Flows very slowly under gravity
- Does not require chewing
- Could be piped, layered or moulded
- No lumps
- Falls off spoon in a single spoonful when tilted and continues to hold a shape on a plate
- Cannot be sucked through a straw
- Not sticky
- Liquid does not separate from solid

☐ Never
☐ Almost Never
☐ Sometimes
☐ Often
☐ Almost Always
☐ Always

f. **Minced and Moist**

Description

- Very small pieces of moist food
- Holds shape on spoon
- Falls off spoon if the spoon is tilted or turned sideways or shaken slightly
- Not sticky

☐ Never
☐ Almost Never
☐ Sometimes
☐ Often
☐ Almost Always
☐ Always

g. **Soft**

Description

- Food can be mashed with fork

☐ Never
☐ Almost Never
☐ Sometimes
☐ Often
☐ Almost Always
☐ Always

h. **Regular**

Description

- Food too hard to be mashed with fork
- Can be eaten with a fork

☐ Never
☐ Almost Never
☐ Sometimes
☐ Often
☐ Almost Always
☐ Always

Name of parent/LAR/caregiver providing information: _____

Date of completion: _____

Appendix 8: Parental Speech Development Questionnaire

1.	Does your child speak?	☐ Yes, off of ventilator ☐ Yes, only with a speaking valve ☐ No
2.	Does your child cry audibly?	☐ Yes ☐ No
3.	Does your child giggle or laugh audibly?	☐ Yes ☐ No
4.	Does your child produce coos or gurgles?	☐ Yes ☐ No
5.	Does your child produce the following sounds?	
a.	da	☐ Yes ☐ No
b.	ba	☐ Yes ☐ No
c.	ga	☐ Yes ☐ No
d.	ma	☐ Yes ☐ No
e.	pa	☐ Yes ☐ No
6.	Does your child produce the following sounds in a sequence?	
a.	dada	☐ Yes ☐ No
b.	baba	☐ Yes ☐ No
c.	gaga	☐ Yes ☐ No
d.	mama	☐ Yes ☐ No

e.	papa	☐ Yes ☐ No
7.	Does your child jabber loudly?	☐ Yes ☐ No
8.	Does your child try to imitate your speech?	☐ Yes ☐ No
9.	Does your child produce animal sounds from the following animals?	
a.	cat	☐ Yes ☐ No
b.	dog	☐ Yes ☐ No
c.	bird	☐ Yes ☐ No
d.	pig	☐ Yes ☐ No
e.	horse	☐ Yes ☐ No
f.	duck	☐ Yes ☐ No
10.	Does your child use mama or dada to call for that specific parent?	☐ Yes ☐ No
11.	When your child sees an animal (i.e. dog, cat, bird) do they say the animal name consistently?	☐ Yes ☐ No
12.	Does your child use specific words to label everyday objects (i.e. juice, cookie, blanket, etc.)?	☐ Yes ☐ No
13.	Can your child produce their own name?	☐ Yes ☐ No
14.	Can the following people understand what your child is saying?	
a.	mother	☐ Yes ☐ No
b.	father	☐ Yes ☐ No

c.	sibling	☐ Yes ☐ No
d.	grandparent	☐ Yes ☐ No
e.	stranger	☐ Yes ☐ No
15.	Can your child alert you in another room?	☐ Yes ☐ No

Name of parent/LAR/caregiver providing information: _____

Date of completion: _____

Appendix 9: Genetics Testing

Use/Analysis of DNA

- Genetic variation may impact a subject's response to study intervention, susceptibility to, and severity and progression of disease. Variable response to study intervention may be due to genetic determinants that impact drug absorption, distribution, metabolism, and excretion; mechanism of action of the drug; disease etiology; and/or molecular subtype of the disease being treated. Therefore, where local regulations and IRB/IEC allow, a blood sample will be collected for DNA analysis from consenting subjects.
- DNA samples will be used for research related to gene therapy or XLMTM and related diseases. They may also be used to develop tests/assays, including diagnostic tests related to gene therapy and XLMTM disease. Genetic research may consist of the analysis of one or more candidate genes or the analysis of the entire genome as appropriate.
- The samples may be analyzed as part of a multistudy assessment of genetic factors involved in the response to the gene therapy or study interventions of this class to understand the study disease or related conditions.
- The results of genetic analyses may be reported in the clinical study report (CSR) or in a separate study summary or may be published in medical or scientific journals and conferences.
- The Sponsor will store the DNA samples in a secure storage space with adequate measures to protect confidentiality.
- The samples will be retained while research on AT132 is ongoing but no longer than 15 years after the final study results have been reported and then destroyed, or other period as per local requirements.

PROTOCOL AMENDMENT
SUMMARY OF CHANGES FOR PROTOCOL VERSION 12.0

Study Title:	ASPIRO: A Phase 1/2/3, Randomized, Open-Label, Ascending-Dose, Delayed-Treatment Concurrent Control Clinical Study to Evaluate the Safety and Efficacy of AT132, an AAV8-Delivered Gene Therapy in X-Linked Myotubular Myopathy (XLMTM) Patients	
Protocol Number:	ATX-MTM-002	
Protocol Versions and Dates:	1.0 (Global)	22 February 2017
	2.0 (Global)	14 June 2017
	3.0 (Global)	25 January 2018
	4.0 (Global)	21 November 2018
	5.0 (Global)	01 May 2019
	6.0 (Global)	08 October 2019
	7.0 (Global)	07 April 2020
	8.0 (Global)	16 November 2020
	9.0 (Global)	15 January 2021
	10.0 (Global)	03 February 2022
	11.0 (Global)	16 May 2023
	12.0 (Global)	17 May 2024

Amendment 12 [Substantial]

This amendment is considered to be substantial based on the criteria set forth in Article 10(a) of Directive 2001/20/EC of the European Parliament and the Council of the European Union and EU Clinical Trial Regulation.

Overall Rationale for Amendment

The main purpose of this protocol amendment was to 1) update the overall risks and benefits based on the latest risk evaluations and available information, 2) update and reorganize the monitoring and management plan for safety events as well as potential immune responses, 3) update study assessments for safety events and 4) streamline efficacy assessments in the long-term follow-up period.

Summary of Changes

Substantial Changes

Deleted text is denoted with strikethrough font and added text is underlined. When needed, "…" indicates a skipped sentence or paragraph.

Location(s)	Description of Change	Rationale for Change	Justification for Substantial Designation
Section 1.5	**1.5 Overall Risks and Benefits** ~~Key risks of the study include the following:~~ • ~~Subjects participating in this study will have study procedure-related risks, including invasive assessments and muscle biopsies.~~ • ~~Subjects participating in this study will receive prophylactic prednisolone and ursodiol. Information regarding the risks associated with these medications can be found in the respective USPIs (Prednisolone USPI, 2013; Ursodiol USPI, 2013).~~ • ~~The important identified risks associated with AT132 that have been observed during the clinical development program include:~~ – ~~Hepatobiliary events (hyperbilirubinemia, cholestatic liver dysfunction, transaminases increase and elevated serum bile acids levels): In Study ATX-MTM-002, SAEs representing significant hepatobiliary dysfunction have been observed in 4 subjects (1 at the 1.0 × 10^{14} vg/kg dose; 3 at the 3.0 × 10^{14} vg/kg dose), and led to fatal outcomes. Each of the 4 subjects had a history of hyperbilirubinemia prior to treatment.~~ – ~~Myocardial events (myocarditis and troponin increased): In Study ATX-MTM-002, 2 subjects (1 at the 1.0 × 10^{14} vg/kg dose; 1 at the 3.0 × 10^{14} vg/kg dose) presented with SAEs of myocarditis. In both cases, neither subject was hemodynamically unstable, and echocardiograms and magnetic resonance imaging (MRIs) showed normal biventricular systolic function, with no wall motion abnormalities. Both events of myocarditis responded to administration of IV steroids and both events were considered recovered without sequelae.~~ • ~~Subclinical inflammatory hepatitis, manifest by elevations in hepatic transaminases have occurred in the setting of AAV gene therapies in clinical development; for example, in Hemophilia B (factor IX deficiency), Hemophilia A (factor VIII deficiency), and spinal muscular atrophy (SMA). This inflammatory hepatitis has been responsive to treatment with prednisolone (Mendell, 2015; Nathwani, 2014; Nathwani, 2011; Pasi, 2016; Spark Therapeutics, 2016).~~ • ~~As with all biological agents, severe inflammatory responses at the systemic or organ level may result from unpredicted reactivity to AT132 or to cells transduced by AT132.~~ • ~~Subjects may develop thrombocytopenia after receiving AT132, due to platelet and/or complement activation. Events of thrombocytopenia in Study ATX-MTM-002 have been consistently mild to moderate in severity, and self-limiting or responsive to additional steroid treatment.~~	To reflect the latest risk evaluation and available information	New safety findings which are likely to impact the risk/benefit assessment

Location(s)	Description of Change	Rationale for Change	Justification for Substantial Designation
	• ~~Subjects may develop an antibody response to the AAV capsid and/or the myotubularin protein after administration of AT132.~~ • ~~An effective dose for AT132 is not known for humans. AT132, if only partly effective, may expose existing comorbidities, worsen existing comorbidities (eg, uneven muscle response causing a worsening of scoliosis or ophthalmoplegia), or result in new comorbidities (eg, bone fractures resulting from increased capacity for mobility, or as yet unknown disease manifestations occurring in children who live longer).~~ **Key study risks** are listed below: • Subjects will have study procedure-related risks: - **Blood collection:** The risks of venous blood draws include discomfort at the site of puncture, possible bruising and swelling around the puncture site, rarely an infection; and, uncommonly, fainting, nausea, vomiting, and light-headedness from the procedure. - **Muscle biopsy:** The muscle biopsy has potential complications from the procedure that include infection at the biopsy site, bleeding and/or pain at the site of the biopsy, and psychological trauma from the scar at the site of the incision; in some cases, keloid formation can increase scarring. - **Cardiac or muscle magnetic resonance imaging (MRI):** The MRI scan is not associated with risks unless a subject has certain types of metal implants such as pacemakers. - **Procedural sedation risk**: Sedation risks in children with weak respiratory muscles varies with the medication used. Sedation risks will be explained to the family. Sedation for the imaging must be carefully administered in patients with XLMTM, since most anesthetic agents act directly on the muscle. Bulbar dysfunction, respiratory muscle weakness, gastroesophageal reflux, and scoliosis increase the risk of pneumonia. Children with respiratory and bulbar muscle weakness who are not ventilator dependent may require invasive or noninvasive ventilation after the procedure. The duration of increased ventilatory requirements would vary depending on the degree of respiratory muscle weakness. The potential side effects of specific medications administered for sedation (eg, vomiting, respiratory depression, laryngospasm, emergence reaction), and the likely duration of sedation will be discussed during informed consent for the procedure. - **Prophylaxis (prednisolone, ursodiol):** Subjects receive prophylactic prednisolone and ursodiol as part of the clinical study. Information regarding the risks associated with these medications can be found in the respective local prescribing information. • Overall risks associated with rAAV gene therapy administration include hepatotoxicity, cardiac events such as myocarditis, myositis, thrombocytopenia, thrombotic microangiopathy (TMA), and sensory ganglionopathy (Arjomandnejad, 2023; FDA, 2021; Lek, 2023; Shen, 2022; Whiteley, 2023).		

Location(s)	Description of Change	Rationale for Change	Justification for Substantial Designation
	- Key identified risks associated with AT132 administration observed in Study ATX-MTM-002 are listed below. Of the overall risks associated with rAAV gene therapy, thrombotic microangiopathy and sensory ganglionopathy have not been observed in ATX-MTM-002 study subjects. Long-term follow-up is important in understanding whether these AEs have long-term implications. Refer to the Investigator's Brochure for further details. - **Hepatobiliary events:** Many XLMTM patients have liver dysfunction and cholestasis, which places them at increased risk for hepatobiliary dysfunction or even hepatic failure with rAAV administration. A prospective natural history study (Study ATX-MTM-009) reported that overall, 91% of subjects had histories of hepatic disease and/or prospectively experienced related AEs or laboratory or imaging abnormalities (Dowling, 2022). ◦ In Study ATX-MTM-002, SAEs due to hepatobiliary dysfunction occurred after AT132 administration and were characterized by hyperbilirubinemia, cholestatic liver dysfunction, transaminases elevations, ascites, and serum bile acid elevations. Fatal outcomes were observed in 4 subjects due to multifactorial causes including hepatobiliary failure: 1 subject who received the 1.0×10^{14} vg/kg dose and 3 subjects who received the 3.0×10^{14} vg/kg dose. All 4 subjects showed increases in direct and total bilirubin values (> upper limit of normal [ULN]) beginning 1 to 4 weeks after AT132 administration (Shieh, 2023). Liver findings included intrahepatocellular and canalicular cholestasis, periportal and bile ductular reaction, secondary fibrosis, and notable lack of prominent liver parenchymal inflammatory cellular infiltrates (Shieh, 2020). - **Myocardial events:** ◦ *Acute cardiac risks:* Acute myocardial events manifested as myocarditis and increases in troponins. In Study ATX-MTM-002, variable increases in troponin levels after AT132 administration were commonly observed. Two subjects experienced SAEs of myocarditis: 1 subject who received the 1.0×10^{14} vg/kg dose and 1 subject who received the 3.0×10^{14} vg/kg dose. Prolonged prednisolone administration plus addition of other immunosuppressive medications were used for treatment of myocarditis. ◦ *Chronic cardiac risks:* Following prolonged immunosuppression for over 3 years, 1 subject experienced an increase in cardiac troponin levels after withdrawal of immunosuppression. Abnormalities in cardiac function have not been observed in this subject. However, long term assessment is required to understand whether there could be more significant impact on cardiac function. - **Muscle abnormalities:** A skeletal muscle immune response to the rAAV capsid and to the transgene can occur after rAAV gene therapy administration. ◦ *Acute and subacute creatine kinase (CK) elevations*: In Study ATX-MTM-002, variable increases in CK levels after AT132 administration were commonly observed, occurring soon		

Location(s)	Description of Change	Rationale for Change	Justification for Substantial Designation
	after dosing. These CK levels generally returned to normal within the first year after AT132 administration. ◦ *Chronic CK elevations and inflammatory infiltrates in skeletal muscles*: Assessment of muscle biopsies demonstrated treatment-associated inflammatory changes in muscle biopsy specimens of some subjects. No SAEs were reported in association to these findings. Improvements in neuromuscular function (respiratory and motor) have been maintained. Although the CK levels generally returned to normal within the first year after AT132 administration, chronic CK elevation over several years can occur. - **Thrombocytopenia:** Subjects may develop transient thrombocytopenia or decreases in platelet levels from baseline after AT132 administration. In Study ATX-MTM-002, transient thrombocytopenia related to AT132 occurred within the first 2 weeks of dosing (Shieh, 2023). **Key potential study benefits** include the following: • Independent of efficacy, study subjects may receive better-coordinated and more comprehensive overall clinical care at centers of excellence. • AT132, even if only partially effective, could cause clinically important improvements in respiratory function and mobility and a lowered risk of disease -related comorbidities, leading to greater independence for the subject and caregivers. • Other patients may benefit from knowledge gained about XLMTM. ~~Subjects randomized to the control delayed treatment arm are not considered to have additional risk from receiving treatment at a later date, because AT132 has not yet been shown to be an effective treatment for XLMTM.~~		
Section 4.6 and Section 4.6.1	**4.6. Monitoring for and Management of Cardiac, ~~and~~ Liver, and Neuromuscular Related Safety Events** **4.6.1 Monitoring for and Management of Myocarditis** **4.6.1.1 Monitoring for Myocarditis** ~~Troponin elevations have been observed in the ASPIRO study. Troponin elevations can be an early signal of myocarditis, a serious cardiac condition that is critical to promptly diagnose and treat. The clinical presentation of myocarditis is variable. Affected patients can present with a broad clinical spectrum of signs and symptoms ranging from subclinical disease to cardiogenic shock, arrhythmias, and sudden death. A high level of awareness and vigilance for potential myocarditis should be maintained in the setting of gene therapy clinical studies.~~ ~~The primary monitoring strategy for identifying potential myocarditis will be through frequent, scheduled (surveillance) testing of high-sensitivity Troponin T and/or I parameters. Additional monitoring that may identify potential myocarditis will include scheduled CK (with isoenzymes) laboratory testing as well as scheduled procedures such as electrocardiogram (ECG) and echocardiogram (ECHO). The frequency of these assessments is outlined in the Schedule of Events (Sections 5.4, 5.5, 5.6, and 5.7).~~	To update the monitoring and management plan for safety events based on the latest safety information	Change of medical monitoring procedure which is likely to have a significant impact on the safety

Location(s)	Description of Change	Rationale for Change	Justification for Substantial Designation
	~~Site Investigators are responsible for prompt review of all laboratory or procedural results related to cardiac monitoring. Interpretation of abnormal test results is complex as it relates to identifying signals for potential myocarditis. Given that this interpretation is critical to subject safety, sites will identify and establish a site pediatric cardiologist preferably with expertise/experience in the diagnosis and treatment of myocarditis. The site pediatric cardiologist will assist the study site, as well as other clinicians who manage study subjects (eg, if the subject lives remote from the site), with interpretation of abnormal cardiac test results indicative of potential myocarditis. Details related to the monitoring, diagnosis, and treatment of myocarditis are described herein.~~ Site Investigators are responsible for prompt review of all laboratory or procedural results. Due to the importance of accurate diagnosis and management of myocarditis for subject safety, sites must identify and establish a site pediatric cardiologist experienced in the treatment of myocarditis. The site pediatric cardiologist will assist the study site and any managing physician responsible for patient care with interpretation of abnormal cardiac test results and treatment. A high level of awareness and vigilance for potential myocarditis should be maintained in the setting of gene therapy clinical studies. The primary monitoring strategy for identifying potential myocarditis will be by clinical assessment and scheduled (surveillance) high-sensitivity Troponin T and/or Troponin I testing. Troponin elevations can be an early signal of myocarditis, a serious cardiac condition that is critical to promptly diagnose and treat. The frequency of assessments is outlined in the Schedule of Events (Sections 5.4, 5.5, 5.6, and 5.7). Additional assessments may be performed at the discretion of the investigator and pediatric cardiologist as outlined in Section 4.6.1.3.		
Section 4.6.1.1	**4.6.1.~~1~~2 Clinical Role of the Site Pediatric Cardiologist** The site pediatric cardiologist will serve as ~~the~~: … As a member of the study site medical team, the site pediatric cardiologist will work together with the Investigator to assist with diagnosis, ~~of abnormal surveillance results, and if indicated, additional diagnostic workup~~ interpretation of cardiac testing, and treatment. In the case where the site pediatric cardiologist does not have direct access to the subject and no local cardiologist is available, the site pediatric cardiologist will provide guidance to the managing physician ~~treating clinician to ensure subject safety. In all cases, the clinician that is providing direct patient care is ultimately responsible and the decision maker for the management and treatment of the subject~~.	To update the monitoring and management plan for safety events based on the latest safety information	Change of medical monitoring procedure which is likely to have a significant impact on the safety
Section 4.6.1.2	~~**4.6.1.2 Monitoring for Potential Myocarditis**~~ ~~A combination of cardiac laboratory tests and cardiac procedures will be used to monitor for cases of potential myocarditis after treatment with AT132. The following notification and consultative parameters are provided to assist Investigators, and/or other clinicians caring for ASPIRO subjects, to identify clinically significant abnormalities during routine cardiac monitoring. All laboratory reference ranges are per the reporting of the local or central laboratory, as applicable.~~ ~~Routine Troponin Surveillance~~	To update the monitoring and management plan for safety events based on the latest safety information	Change of medical monitoring procedure which is likely to have a significant impact on the safety

Location(s)	Description of Change	Rationale for Change	Justification for Substantial Designation
	• ~~Investigators will consult the site pediatric cardiologist for any Troponin T or I value that meets either of the following criteria:~~ ~~– Above the ULN if a reference range is reported~~ ~~– Any non-zero value if no ULN is reported or if the result indicates that the upper limit reflects myocardial ischemia (thus not informative for myocarditis monitoring); if this is not clear, the Investigator will contact the site pediatric cardiologist to discuss the case~~ ~~Other Routine Cardiac Surveillance~~ • ~~Investigators will consult the site pediatric cardiologist in the following scenarios:~~ ~~– For any clinically significant abnormal ECHO or ECG~~ ~~– For any elevated routine CK-MB that is considered clinically significant by the Investigator~~ ~~Abnormal Cardiac Laboratory or Procedural Results not Associated with Routine Cardiac Surveillance per the Protocol Schedule of Events~~ • ~~The approach for identifying potential myocarditis in this setting may be more complex (eg, the subject is hospitalized)~~ • ~~The Investigator should consult with the site pediatric cardiologist and notify the Medical Monitor to determine an appropriate follow-up plan based on the clinical circumstance~~ ~~Communication Guidelines~~ • ~~Details and discussions around the monitoring, diagnostic (Section 4.6.1.3), and treatment activities (Section 4.6.1.4) of a subject that include the site pediatric cardiologist are required to be documented~~ **4.6.1.3 Diagnosis and Management of Myocarditis or Asymptomatic Troponin Elevations** Clinical assessment, laboratory testing, and cardiac imaging can be used to detect myocarditis associated with AT132 administration. Documentation is required for data and discussions related to diagnosis and treatment. The Investigator for the study is responsible for all study-related medical decisions. Routine Myocarditis Surveillance • Troponin I and/or Troponin T will be assessed at scheduled visits. Investigators will consult the site pediatric cardiologist when Troponin T and/or Troponin I are above the ULN for the laboratory reference range. All laboratory reference ranges are per the reporting of the local or central laboratory, as applicable. Additional Testing for Myocarditis Diagnosis and Management Test selection is performed at the discretion of the investigator in conjunction with consultation with the site pediatric cardiologist. Key testing that may assist in diagnosis and management of myocarditis includes but is not limited to: • Laboratory testing: A variety of laboratory testing can be useful in the diagnosis and management of myocarditis, including but not limited to complete blood count and differential, complete metabolic		

Location(s)	Description of Change	Rationale for Change	Justification for Substantial Designation
	panel, troponin I and/or troponin T, N-terminal pro b-type natriuretic peptide, sedimentation rate, C-reactive protein, infectious assessments (eg, nasopharyngeal and rectal swabs, viral titers), CK with isoenzymes to identify elevations in the myocardial isoenzyme (CK-MB), and interferon gamma (IFN-γ) enzyme-linked immunosorbent spot assay (IFN-γ ELISpot) for MTM1 peptide pool and AAV8 peptide pool. Anti-MTM1 antibodies are optional. • ECG • Transthoracic ECHO • Cardiac MRI • Cardiac catheterization with endomyocardial biopsy (in exceptional cases)		
Section 4.6.1.4	~~**4.6.1.4 Classification and Management of Suspected Myocarditis**~~ ~~Classification and management of subjects in the setting of confirmed myocarditis, or where a high probability of myocarditis is suspected, will depend on the interpretation of all available clinical data by the treating physician in consultation with the site pediatric cardiologist. Table 1 outlines high level DMC treatment recommendations on the basis of severity. Initiation or alteration of immunosuppressive treatments (including prophylactic prednisolone) as a result of myocarditis will be implemented by the treating clinician in consultation with the site pediatric cardiologist (and the Investigator and the Medical Monitor, when appropriate).~~ ~~In the unlikely event a subject presents with or develops severe myocarditis (as described in Table 1), transfer to a tertiary referral hospital is warranted for institutions without advanced heart failure surgical and medical management capabilities, including expertise in myocarditis management.~~	To update the monitoring and management plan for safety events based on the latest safety information	Change of medical monitoring procedure which is likely to have a significant impact on the safety

<table>
<tr><th>Location(s)</th><th>Description of Change</th><th>Rationale for Change</th><th>Justification for Substantial Designation</th></tr>
<tr><td></td><td>~~Table 1: DMC Recommendations for Management of Myocarditis Events~~
<table>
<tr><th>~~Severity~~</th><th>~~Criteria~~</th><th>~~Management~~</th></tr>
<tr><td>~~**Mild**~~</td><td>~~Symptoms of chest pain; elevation of heart rate above expected by 20 bpm or less; ST segment changes on surface ECG; elevations of troponin; atrial or ventricular ectopy including single beats or couplets; or any combination of these findings, in the absence of any findings suggesting more serious forms of myocarditis. Clinical impression must support diagnosis of myocarditis as likely etiology of findings.~~</td><td>~~Methylprednisolone IV 10 mg/kg/day for 5 days.~~
~~Continue with prednisolone PO 1-2 mg/kg until resolution of myocarditis symptoms/signs.~~</td></tr>
<tr><td>~~**Moderate**~~</td><td>~~Elevation of heart rate by more than 20 bpm above expected; evidence of hypotension requiring support with either volume infusion or a single inotropic agent; atrial or ventricular tachycardia that is hemodynamically tolerated and self-terminating; mildly or moderately reduced ejection fraction of left ventricle as measured by any imaging technique; new onset pericardial effusion other than trivial in size; or any combination of these findings, in the absence of any findings suggesting more serious forms of myocarditis. Clinical impression must support diagnosis of myocarditis as likely etiology of findings.~~</td><td>~~Methylprednisolone IV 10 mg/kg/day for 5 days, plus tacrolimus at an initial starting dose of 0.1 mg/kg/day PO, divided BID. Target trough level: 10-12 ng/mL until resolution of myocarditis symptoms/signs.~~</td></tr>
<tr><td>~~**Severe**~~</td><td>~~Hemodynamic instability requiring treatment with multiple infusions of volume or more than a single inotropic agent; severely reduced ejection fraction of left ventricle as measured by any imaging technique; atrial or ventricular tachycardia requiring either pharmacologic management or cardioversion; end organ injury due to circulatory insufficiency or any combination of these findings. Clinical impression must support diagnosis of myocarditis as likely etiology of findings.~~</td><td>~~Methylprednisolone IV 10 mg/kg/day and rATG (eg, thymoglobulin) 1.5 mg/kg/dose, for 3 doses as needed to a maximum of 5 doses as deemed clinically appropriate.~~
~~Monitor the absolute CD3 count during this process and aim to reduce the count to < 20 cells/μL.~~</td></tr>
</table>
~~BID = twice daily; bpm = beats per minute; DMC = Data Monitoring Committee; ECG = electrocardiogram; IV = intravenous; PO = *per os* (by mouth).~~</td><td></td><td></td></tr>
<tr><td>Section 4.6.2</td><td>4.6.2 Monitoring for and Management of Hepatobiliary Toxicity
Hepatobiliary toxicity has been reported with AAV gene therapy treatment (Chan, 2020) and in this study (~~ASPIRO~~ ATX-MTM-002). In ~~ASPIRO~~ Study ATX-MTM-002, AT132-related SAEs involving the hepatobiliary system, including intrahepatic cholestasis, have occurred in subjects with XLMTM treated at the 1.0 × 1014 vg/kg and 3.0 × 1014 vg/kg dose levels. Subjects will be administered glucocorticoid</td><td>To update the monitoring and management plan for safety events</td><td>Change of medical monitoring procedure which is likely to have a significant impact on the safety</td></tr>
</table>

Location(s)	Description of Change	Rationale for Change	Justification for Substantial Designation
	prophylaxis and ursodiol prophylaxis as outlined in Sections 4.4 and 4.5, respectively. Routine surveillance and evaluation of any signs or symptoms of hepatobiliary toxicity is an essential safety parameter. The primary monitoring strategy for identifying potential hepatobiliary toxicity will be through frequent, scheduled testing of the following LFTs: ALT, AST, gamma-glutamyltransferase (GGT), total bilirubin (TBL), and direct bilirubin. Additional monitoring will include scheduled liver ultrasound procedures and as of protocol v10 serum bile acid levels in order to better understand the long-term behavior of intrahepatic cholestasis in XLMTM subjects. The frequency of these assessments is outlined in the Schedule of Events (Sections 5.4, 5.5, and 5.6). Site Investigators are responsible for prompt review of all laboratory or procedural results related to potential hepatobiliary toxicity. To enhance the monitoring and management of potential hepatotoxicity, sites will identify a site hepatologist (preferably with expertise in pediatric cholestatic disease). The site hepatologist will assist the study site and any managing physician responsible for patient care with interpretation of abnormal hepatobiliary test results and treatment. A high level of awareness and vigilance for potential hepatobiliary toxicity should be maintained in the setting of gene therapy clinical studies. Details related to the monitoring, diagnosis, and treatment of hepatobiliary toxicity are described herein. Any laboratory tests and/or procedures performed for these safety events should be reported in the eCRF.	based on the latest safety information	
Section 4.6.2.3	**4.6.2.3 Risk management of Hepatobiliary Toxicity** **…** • ~~Nasobiliary drainage (NBD) will be initiated in any subject who demonstrates total or direct bilirubin levels ≥ 5 × ULN (or ≥ 3 × the Baseline value if it was > ULN) within the first 16 weeks following AT132 administration, unless clinical contraindications are present. Beyond Week 16, strong consideration to NBD placement should be given to any subject who develops these laboratory abnormalities. If well tolerated, consider maintaining NBD through normalization of total and direct bilirubin levels (or return to Baseline Visit values), though duration will be determined on a case by case basis. Investigators and site hepatologists should consult the Medical Monitor when deciding on NBD placement and duration. Associated clinical and laboratory monitoring (including for pancreatitis which has been reported with NBD) will be determined on a case by case basis, but should include periodic monitoring of biochemical markers of pancreatitis (such as serum lipase and amylase levels) as well as hepatic laboratory parameters to assess response to NBD. See Section 5.1.1.30 for additional details regarding NBD.~~	To update the monitoring and management plan for safety events based on the latest safety information	Change of medical monitoring procedure which is likely to have a significant impact on the safety
Section 4.6.3.1	**4.6.3 Monitoring for and Management of Neuromuscular Abnormalities** **4.6.3.1 Monitoring for Neuromuscular Abnormalities** Acute, subacute, and chronic CK elevations can occur after gene therapy administration. Muscle abnormalities vary and can include myositis, which can be asymptomatic or display muscle weakness. Neuromuscular abnormalities may manifest as bulbar, motor, and/or respiratory weakness.	To update the monitoring and management plan for safety events based on the latest safety information	Change of medical monitoring procedure which is likely to have a significant impact on the safety

Location(s)	Description of Change	Rationale for Change	Justification for Substantial Designation
	Site Investigators are responsible for prompt review of all laboratory or procedural results. Due to the importance of accurate diagnosis and management of neuromuscular dysfunction for subject safety, consultation with a pediatric pulmonologist is required for potential decline in respiratory function. The site pediatric pulmonologist will assist the study site and any managing physician responsible for patient care with interpretation of abnormal pulmonary test results and treatment. Due to risks associated with worsening bulbar function, a pediatric ear, nose and throat (ENT) specialist and/or speech-language pathologists will be consulted if worsening bulbar dysfunction is suspected. The primary monitoring strategy for Identifying potential neuromuscular abnormalities will be by clinical assessment and scheduled (surveillance) of CK levels in blood. The frequency of these assessments is outlined in the Schedule of Events (Sections 5.4, 5.5, 5.6, and 5.7). Additional assessments may be performed at the discretion of the investigator and pediatric neuromuscular specialist as outlined in Section 4.6.3.2.		
Section 4.6.3.2	**4.6.3.2 Diagnosis and Management of Neuromuscular Abnormalities** Clinical assessment, laboratory testing, and muscle imaging can be used to detect neuromuscular abnormalities associated with AT132 administration. Documentation is required for data and discussions related to diagnosis and treatment. The Investigator for the study is responsible for all study-related medical decisions. Routine neuromuscular surveillance • Physical examination with detailed neurological evaluation and respiratory function assessment • CK will be measured at scheduled visits. If the CK is above the ULN for the laboratory reference range, the investigator will decide whether additional diagnostic evaluation is needed. Additional testing for diagnosis and management of neuromuscular abnormalities Test selection is performed at the discretion of the investigator in conjunction with consultation with the neuromuscular specialist as needed. Additional consultations with a pediatric ENT specialist, speech-language pathologist, pediatric pulmonologist, and a pediatric neuromuscular specialist may be needed. Key testing that may assist in diagnosis and management of neuromuscular abnormalities includes but is not limited to: • Laboratory testing: A variety of laboratory testing can be useful in the diagnosis and management of neuromuscular abnormalities, including but not limited to complete blood count and differential, complete metabolic panel, sedimentation rate, C-reactive protein, infectious assessments (eg, nasopharyngeal and rectal swabs, viral titers), CK , CK-isoenzymes for identification of elevations in the skeletal muscle isoenzyme (CK-MM) or CK-MB, and IFN-γ ELISpot for MTM1 peptide pool and AAV8 peptide pool. Anti-MTM1 antibodies are optional. • Electrophysiological assessment (electromyography [EMG]) • Skeletal muscle MRI with short tau inversion recovery (STIR) sequence • Diagnostic muscle biopsy	To update the monitoring and management plan for safety events based on the latest safety information	Change of medical monitoring procedure which is likely to have a significant impact on the safety

Location(s)	Description of Change	Rationale for Change	Justification for Substantial Designation
Section 5.1.1.10	**5.1.1.10 Cardiac Structure and Function (ECG, ~~and~~ ECHO and Cardiac MRI)** Unlike ~~most~~many neuromuscular diseases, individuals with XLMTM ~~subjects have no evidence of~~ do not exhibit cardiac muscle disease. Mice treated with rAAV8-Des-MTM1 had asymptomatic cardiac lesions detected on necropsy (in the presence of gross over expression of myotubularin) (Section 1.2.1.3). It is unknown whether a risk exists for human subjects. Cardiac structure and function will be routinely assessed using 12 lead ECG and ECHOs. ~~The ECHOs should include an assessment of pulmonary hypertension as well as structural and functional abnormalities. Particular attention should be paid to ECG and ECHO abnormalities specific to myocarditis. Refer to Section 4.6.1 for management of ECG and ECHO results and the role of the site pediatric cardiologist~~. Cardiac MRI may be performed at the discretion of the investigator in conjunction with consultation with the site pediatric cardiologist. If myocarditis is observed, cardiac catheterization and endomyocardial biopsy may be needed in exceptional cases. If troponin I and/or troponin T is elevated above the ULN of the laboratory reference range, test selection for diagnosis and management of myocarditis or asymptomatic troponin elevation is performed at the discretion of the investigator in conjunction with consultation with the site pediatric cardiologist. Key testing is discussed below. Refer to Section 4.6.1 for management of ECG, ECHO, and Cardiac MRI results and the role of the site pediatric cardiologist. • ECG: In patients with suspected myocarditis, the ECG can display a variety of non-specific abnormalities. Interpretation of ECG by the site pediatric cardiologist may be required due to the array of ECG changes that can occur with myocarditis. • Transthoracic ECHO: ECHO is a safe, widely available, and clinically extremely useful cardiac imaging tool, particularly for the initial assessment of myocarditis. The American College of Cardiology (ACC), American Heart Association (AHA), and European Society of Cardiology (ESC) Working Group on Myocardial and Pericardial Diseases recommend that all patients with clinically suspected myocarditis should undergo an ECHO at initial presentation (Adeboye, 2022). • Cardiac MRI: Cardiac MRI is considered the non-invasive reference standard for diagnosis of myocarditis in the absence of contraindications to MRI. Gadolinium may be used to assess for late gadolinium enhancement, a technique used for cardiac tissue characterization due to various types of myocardial injury and fibrosis. Cardiac MRI findings change depending on the phase of disease and timing of imaging. Myocardial edema is a characteristic early feature of acute myocarditis which typically improves over days to weeks and may not be detected if cardiac MRI is performed weeks to months after symptom onset. Diagnostic sensitivity is highest within 2 to 4 weeks of symptom onset; therefore, cardiac MRI should be performed quickly in patients with suspected myocarditis (Urzua Fresno, 2023). • Cardiac catheterization with endomyocardial biopsy in exceptional cases: Although the gold standard for diagnosis of myocarditis is endomyocardial biopsy, this procedure is usually not required for diagnosis and management. Considering this procedure's invasiveness, low sensitivity	To update the monitoring and management plan for safety events based on the latest safety information	Change of medical monitoring procedure which is likely to have a significant impact on the safety

Location(s)	Description of Change	Rationale for Change	Justification for Substantial Designation
	when myocardial involvement is focal, and a complication rate ranging from 1% to 15%, the pediatric cardiologist may consider that the clinical presentation to be of sufficient severity to necessitate the procedure, especially in uncommon clinical scenarios such as severe heart failure, cardiogenic shock, ventricular arrhythmias, and/or when there is significant diagnostic uncertainty, aiming to ensure the requirement of the procedure (Pilati, 2022).		
Section 5.1.1.11.3	… The actual number of hours of ventilatory support a subject receives daily will be recorded via the electronic ventilator dependence diary or the Ventilator Dependence Questionnaire (Section 5.1.1.11.2.1). … **Table ~~2~~1: Required Parameters for Initiation of Ventilator Weaning** … The above parameters are to be assessed by site personnel during the normal routine site visits~~.~~ through Month 42. As of protocol v12, beginning at the Month 48 visit, some parameters (ie, MIP/MEP, $ETCO_2$, TCO_2) will no longer be collected per protocol and decision making related to initiation of ventilator weaning will occur by the site and local pulmonologists in collaboration with the Site Investigator, upon review of relevant clinical assessments and in consideration of standard practice in ventilator weaning in patients with neuromuscular disease.	To streamline the efficacy assessment in the long-term follow-up period	Change in study design which reduces required study assessments
Section 5.1.1.11.4	**Table ~~3~~2: Required Ventilator Discontinuation Parameters** … The above parameters are to be assessed by site personnel during the normal routine site visits through Month 42. As of protocol v12, beginning at the Month 48 visit, some parameters (ie, MIP/MEP, $ETCO_2$, TCO_2) will no longer be collected per protocol and decision making related to discontinuation of ventilator support will occur by the site and local pulmonologists in collaboration with the Site Investigator, upon review of relevant clinical assessments and in consideration of standard practice in ventilator discontinuation in patients with neuromuscular disease.	To streamline the efficacy assessment in the long-term follow-up period	Change in study design which reduces required study assessments
Section 5.1.1.11.5	… Details on assessment of ventilator parameters will be provided in a separate procedure manual. The above parameters are to be assessed by site personnel during the normal routine site visits through Month 42. As of protocol v12, beginning at the Month 48 visit, the Assessment of Ventilator Parameters will no longer be collected per protocol and decision making related to collection and analysis of clinical ventilator and respiratory parameters will occur by the site and local pulmonologists in collaboration with the Site Investigator.	To streamline the efficacy assessment in the long-term follow-up period	Change in study design which reduces required study assessments
Section 5.1.1.11.5.1	If a subject has discontinued the use of the ventilator and has 2 assessments with the MIP at 80 cmH_2O, testing for MIP, MEP, and P0.1 will not be required for future visits. Respiratory strength and endurance assessments are to be performed by site personnel during the normal routine site visits through Month 42. As of protocol v12, beginning at the Month 48 visit, respiratory strength and endurance assessments will no longer be collected per protocol and decision	To streamline the efficacy assessment in the long-term follow-up period	Change in study design which reduces required study assessments

Location(s)	Description of Change	Rationale for Change	Justification for Substantial Designation
	making related to collection and analysis of respiratory strength, endurance and function assessments will occur by the site and local pulmonologists in collaboration with the Site Investigator.		
Section 5.1.1.12	… The MFM-32 will be used to assess children ≥ 2 years of age (Section 5.1.1.12.4). As of protocol v12, beginning at the Month 48 visit, only the Motor Developmental Milestones assessment will be performed. Decision making regarding collecting other assessments of neuromuscular function will occur by the Site Investigator, in collaboration with other neuromuscular specialists as indicated.	To streamline the efficacy assessment in the long-term follow-up period	Change in study design which reduces required study assessments
Section 5.1.1.14	**5.1.1.14 Speech Assessments** As of protocol v12, beginning at the Month 48 visit, speech assessments will no longer be performed per protocol. Decision making regarding collecting other clinical speech assessments will occur by the Site Investigator, in collaboration with other neuromuscular specialists as indicated.	To streamline the efficacy assessment in the long-term follow-up period	Change in study design which reduces required study assessments
Section 5.1.1.23.2	**5.1.1.23.2 Bone Age (Wrist X-ray) (not applicable for Germany)** X-ray of the left wrist in infants and children to ascertain bone age is a recognized method of assessing maturity and development of the skeleton. This is of particular relevance in XLMTM, because advanced bone age and a variety of skeletal abnormalities (although not well characterized) have been documented in some XLMTM patients (Herman, 1999). The radiation dose for this procedure is low (0.001 mSv per X-ray). Subjects in Germany will not have a wrist X-ray. As of protocol v12, beginning at the Month 48 visit, bone age will no longer be collected per protocol.	To update the monitoring and management plan for safety events based on the latest safety information	Change in study design which reduces required study assessments
Section 5.1.1. 23.3	**5.1.1.23.3 Tanner Stage** Tanner stage will be assessed to monitor for premature adrenarche, which has been documented in some XLMTM patients. Tanner staging criteria are provided in Appendix 2. As of protocol v12, beginning at the Month 48 visit, Tanner stage will no longer be collected per protocol.	To update the monitoring and management plan for safety events based on the latest safety information	Change in study design which reduces required study assessments
Section 5.1.1.26	Muscle biopsies will be used to assess the efficacy and safety of AT132 by analyzing MTM1 protein expression, vector copy number quantitation, RNA transcripts assessment, histological characterization and ~~histology data. Muscle biopsies may also be used to assess safety measures such as the presence of~~ inflammatory markers. If a muscle biopsy is performed at the discretion of the investigator for diagnostic purposes, including but not limited to CK elevations and declining neuromuscular function (Section 4.6.3.2), testing should be performed in accordance with standards of care for diagnostic muscle biopsy assessment. When possible after diagnostic muscle biopsy, muscle should be collected and sent to the central lab for the AT132-related assessments listed below: • MTM1 protein expression • Vector copy number quantitation • RNA transcript assessment	To add a recommended assessment/testing for safety events based on the latest safety information	Change of medical monitoring procedure which is likely to have a significant impact on the safety

<table>
<tr><th>Location(s)</th><th>Description of Change</th><th>Rationale for Change</th><th>Justification for Substantial Designation</th></tr>
<tr><td></td><td>• Histological characterization
• Inflammatory markers</td><td></td><td></td></tr>
<tr><td>Section 5.1.1.30</td><td>~~5.1.1.30 Nasobiliary Drainage~~
~~Nasobiliary drainage (NBD), including long-term placement and maintenance, has shown benefit in various intrahepatic cholestatic syndromes, as it allows for definitive disruption of the enterohepatic circulation of bile components. Improvements in clinical cholestasis, including laboratory hyperbilirubinemia, have been described, and the procedure is generally well- tolerated (Appleby, 2015; Hegade, 2016; Jannone, 2020; Stapelbroek, 2006; Sun, 2018; Zollos, 2012) See Section 4.6.2.3 for a description of subjects in whom NBD is required, and in whom it should be strongly considered, following AT132 administration. Endoscopic placement of NBD may be performed at study sites or at facilities local to the study subject. Investigators and site hepatologists should consult the Medical Monitor when deciding on NBD placement and duration. Refer to Section 4.6.2 for details related to the monitoring, diagnosis, and treatment of hepatobiliary toxicity and the role of the site hepatologist.~~</td><td>To update the monitoring and management plan for safety events based on the latest safety information</td><td>Change of medical monitoring procedure which is likely to have a significant impact on the safety</td></tr>
<tr><td>Section 5.1.1.31</td><td>5.1.1.31 Muscle MRI with STIR Sequence
The MRI STIR sequence is a type of MRI protocol that involves a short inversion time to nullify signals from tissues with short T1 relaxation times, highlighting areas with longer T1 values. It is useful for identifying edema, fluid, and inflammation in muscle. It is recommended that this imaging protocol be obtained in response to elevated CK to better understand the potential inflammatory changes that may occur after treatment with AT132. STIR sequences may be used to support identification of the cause for the neuromuscular abnormality, identify a muscle site for biopsy if needed, and monitor treatment response. In some circumstances, muscle MRI with STIR sequences may be performed when CK is elevated as part of the diagnostic assessment. This imaging is performed at the discretion of the investigator.</td><td>To add a recommended assessment/testing for safety events based on the latest safety information</td><td>Change of medical monitoring procedure which is likely to have a significant impact on the safety</td></tr>
<tr><td>Section 5.1.1.32</td><td>5.1.1.32 Electrophysiological Assessments (EMG)
EMG may be performed to assess neuromuscular abnormalities. Nerve conduction studies can be performed if a neuropathic process is suspected.</td><td>To add a recommended assessment/testing for safety events based on the latest safety information</td><td>Change of medical monitoring procedure which is likely to have a significant impact on the safety</td></tr>
<tr><td>Section 5.4</td><td>Nasobiliary drainage (NBD) was deleted from the Schedule of Events table.
5.4 Schedule of Events for Subjects Treated with AT132: Biopsy – Week 8
<table>
<tr><th>Study Phase/Visit</th><th>Daily</th><th>Biopsy</th><th>Baseline</th><th>…</th><th>Week 8</th></tr>
<tr><td>Study Day(s)</td><td>…</td><td>…</td><td>…</td><td>…</td><td>…</td></tr>
<tr><td>~~Nasobiliary drainage[q]~~</td><td></td><td></td><td></td><td colspan="2">~~As clinically indicated~~</td></tr>
</table>
…CGI-I =Clinical Global Impression of Improvement;…; ELI~~s~~Spot = enzyme-linked immunosorbent spot assay; …; ~~NBD = nasobiliary drainage~~; …</td><td>To update the study assessments accordingly with changes to the management plan for safety events</td><td>Change of medical monitoring procedure which is likely to have a significant impact on the safety</td></tr>
</table>

<table>
<tr><th>Location(s)</th><th>Description of Change</th><th>Rationale for Change</th><th>Justification for Substantial Designation</th></tr>
<tr><td></td><td>[q] ~~NBD (see Section 5.1.1.30) will be initiated in any subject who meets the criteria listed in Section 4.6.2.3 within the first 16 weeks following AT132 administration, unless clinical contraindications are present.~~ Footnote was deleted.</td><td></td><td></td></tr>
<tr><td>Section 5.5</td><td>Nasobiliary drainage (NBD) was deleted from the Schedule of Events table, and Month 48 visit and onward are listed separately from Month 18 through 42 visits.
5.5 Schedule of Events for Subjects Treated with AT132: Week 9 – Month 60
<table>
<tr><th>Study Phase/Visit</th><th>…</th><th>Weeks 9, 10, 11</th><th>Week 12</th><th>Weeks 13, 14, 15</th><th>…</th><th>Week 48 or Early Withdrawal</th><th>Months 18, 24, 30, 36, ~~42, 48, 54, 60~~</th><th>Months 48, 54, 60</th></tr>
<tr><td>Study Day(s)</td><td>…</td><td>…</td><td>…</td><td>…</td><td>…</td><td>…</td><td>548, 730, 913, 1095, 1278~~, 1460, 1643, 1825~~</td><td>1460, 1643, 1825</td></tr>
<tr><td>Visit Window (days)</td><td>…</td><td>…</td><td>…</td><td>…</td><td>…</td><td>…</td><td>…</td><td>±14</td></tr>
<tr><td>Diary of ventilator dependence [a,b]</td><td>…</td><td></td><td></td><td></td><td>…</td><td></td><td></td><td></td></tr>
<tr><td>Ventilator Dependence Questionnaire [b]</td><td>…</td><td>…</td><td>…</td><td>…</td><td>…</td><td>…</td><td>X</td><td>X</td></tr>
<tr><td>Contact subject's caregiver [c]</td><td>…</td><td>…</td><td>…</td><td>…</td><td>…</td><td>…</td><td></td><td></td></tr>
<tr><td>Physical examination [d]</td><td>…</td><td>…</td><td>…</td><td>…</td><td>…</td><td>…</td><td>X</td><td>X</td></tr>
<tr><td>Vital signs [e]</td><td>…</td><td>…</td><td>…</td><td>…</td><td>…</td><td>…</td><td>X</td><td>X</td></tr>
<tr><td>12-Lead ECG [f]</td><td>…</td><td>…</td><td>…</td><td>…</td><td>…</td><td>…</td><td>X</td><td>X</td></tr>
<tr><td>ECHO [f]</td><td>…</td><td>…</td><td>…</td><td>…</td><td>…</td><td>…</td><td>X[g]</td><td>X[g]</td></tr>
<tr><td>Growth parameters</td><td>…</td><td>…</td><td>…</td><td>…</td><td>…</td><td>…</td><td>X</td><td>X</td></tr>
<tr><td>Tanner stage (puberty assessment)</td><td>…</td><td>…</td><td>…</td><td>…</td><td>…</td><td>…</td><td>X[ff]</td><td></td></tr>
<tr><td>Samples for:</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Safety labs (hematology) (central lab) [h,i]</td><td>…</td><td>…</td><td>…</td><td>…</td><td>…</td><td>…</td><td>X</td><td>X</td></tr>
<tr><td>Safety labs (hematology) (local lab) [h,i]</td><td>…</td><td>…</td><td>…</td><td>…</td><td>…</td><td>…</td><td></td><td></td></tr>
<tr><td>Safety labs (chemistry) (central lab) [h,i,j,k,l]</td><td>…</td><td>…</td><td>…</td><td>…</td><td>…</td><td>…</td><td>X</td><td>X</td></tr>
<tr><td>Safety labs (chemistry) (local lab) [h,i,j,k]</td><td>…</td><td>X [m]</td><td>…</td><td>X [m]</td><td>…</td><td>…</td><td></td><td></td></tr>
</table></td><td>To update the study assessments accordingly with changes to the management plan for safety events

To streamline the efficacy assessment in the long-term follow-up period (between Months 48 and 60)</td><td>Change of medical monitoring procedure which is likely to have a significant impact on the safety

Change in study design which reduces required study assessments</td></tr>
</table>

Location(s)	Description of Change								Rationale for Change	Justification for Substantial Designation
	Cytokine panel (central lab) h,~~m~~q		As clinically indicated							
	Complement panel (central lab) h,~~m~~q		As clinically indicated							
	Troponin T and/or I (central lab) h,j	…	…	…		…	…	X	X	
	Troponin T and/or I (local lab) h,j	…	…	…		…	…			
	Coagulation parameters (central lab) h,i	…	…	…		…	…	X	X	
	Anti-AAV8 NAb and TAb (central lab)	…	…	…		…	…	X	X	
	Anti-MTM1 antibody (central lab)	…	…	…		…	…	X	X	
	IFN-γ ELISpot (PBMCs) (central lab)			As clinically indicated ~~eq~~						
	HLA typing (central lab) ~~pq~~			X ~~pq~~			X ~~pq~~	X ~~pq~~		
	Urinalysis (central lab) h,i	…	…	…		…	…	X	X	
	Urinalysis (local) h,i	…	…	…		…	…			
	Serum bile acid assay (fasting preferred; central lab)	…	…	…		…	…	X	X	
	~~Nasobiliary drainage q~~		~~As clinically indicated~~			~~As needed per Section 4.6.2.3~~				
	Liver ultrasound k	…	…	…		…	…	X	X	
	Bone age (wrist X-ray) (not applicable for Germany)	…	…	…		…	…	X ~~eee~~		
	Assessment of ventilator parameters	…	…	…		…	…	X ff		
	MIP, MEP, P0.1, tidal volume r	…	…	…		…	…	X ff		
	Ventilator Weaning and Discontinuation Assessment	…	…	…		…	…	X s, ff		
	Daytime polysomnogram	…	…	…		…	…			
	Secretions management assessment u	…	…	…		…	…	X	X	

Location(s)	Description of Change	Rationale for Change	Justification for Substantial Designation

Motor milestone assessments [v]	…	…	…		…	…	X	X
Bayley-III, motor domain [v]	…	…	…		…	…	X [ff]	
CHOP INTEND [v,w]	…	…	…		…	…	X [ff]	
MFM-32 [v]	…	…	…		…	…	X [ff]	
ACEND	…	…	…		…	…	X	X
PedsQL [x]	…	…	…		…	…	X [ff]	
Clinical global impression scales [y]	…	…	…		…	…	X	X
In-depth interviews with caregiver and subject [z]	…	…	…		…	…		
Swallowing questionnaire	…	…	…		…	…	X	X
Speech questionnaire	…	…	…		…	…	X [ff]	
Communicative Development Inventories [aa]	…	…	…		…	…	X [ff]	
…	…	…	…		…	…	…	…
Viral shedding (central lab) [dd]	…	…	…		…	…	X	X
AEs/concomitant medications	…	…	…		…	…	X	X

…; ~~NBD = nasobiliary drainage;~~ …

[b] For subjects in Part 1 who have already completed the Week 24 visit, the parent/LAR/caregiver will complete the ventilator dependence questionnaire at clinic and contact visits through Month ~~60~~120. For subjects in Part 1 who have not completed the Week 24 visit and for subjects in Part 2, parent/LAR/caregivers will complete the daily diary (Section 5.1.1.11.2.1). After the daily diary has been stopped or after subject has completed Week 48, the parent/LAR/caregiver will complete the ventilator dependence questionnaire at clinic and contact visits through Month ~~60~~120.

[g] After the Week 48 visit, ECHO ~~and wrist X ray~~ only needs to be performed annually thereafter (Month 24, Month 36, Month 48, Month 60). ~~Subjects in Germany will not have a wrist X ray.~~

[l] See Section 4.6.3 for details related to the monitoring, diagnosis, and treatment of neuromuscular abnormalities.

~~[n] NBD (see Section 5.1.1.30) will be initiated in any subject who meets the criteria listed in Section 4.6.2.3 within the first 16 weeks following AT132 administration, unless clinical contraindications are present. Postdosing, NBD will be conducted if clinically indicated per Section 4.6.2.3.~~

[u] The severity of secretions and burden of secretions management will be assessed using the PGIS-S and PGIS-I (Appendix 3). ~~When possible, t~~The same parent/LAR/caregiver should report throughout the study (whenever possible).

<table>
<tr><th>Location(s)</th><th>Description of Change</th><th>Rationale for Change</th><th>Justification for Substantial Designation</th></tr>
<tr><td></td><td>[ee] Subjects in Germany will not have a wrist X-ray. After the Week 48 visit, wrist X-ray only needs to be performed annually thereafter (Month 24, Month 36). As of protocol v12, the wrist X-ray assessment will no longer be collected at Month 48 visit onward.
[ff] As of protocol v12, these assessments will no longer be collected at Month 48 visit onward.</td><td></td><td></td></tr>
<tr><td>Section 5.6</td><td>5.6 Schedule of Events for Subjects Treated with AT132: Month 72 (Year 6) – Month 120 (Year 10) (End of Study)
<table>
<tr><th>Study Phase/Visit</th><th>Month 72</th><th>Month 84</th><th>Month 96</th><th>Month 108</th><th>Month 120</th></tr>
<tr><td>…</td><td>…</td><td>…</td><td>…</td><td>…</td><td>…</td></tr>
<tr><td>Physical Examination [a]</td><td>X</td><td>X</td><td>X</td><td>X</td><td>X</td></tr>
<tr><td>Ventilator Dependence Questionnaire [ab]</td><td>…</td><td>…</td><td>…</td><td>…</td><td>…</td></tr>
<tr><td>Samples for:</td><td>…</td><td>…</td><td>…</td><td>…</td><td>…</td></tr>
<tr><td>Safety labs (hematology) (central lab) [b,c,d]</td><td>…</td><td>…</td><td>…</td><td>…</td><td>…</td></tr>
<tr><td>Safety labs (chemistry) (central lab) [b,c,d,e,f,g]
Comprehensive metabolic profile
ALT, AST, Total and direct bilirubin, GGT
Serum bile acids, total (fasting preferred)
PTT and PT/INR
Troponin I and T
CK
Alpha fetoprotein</td><td>…
…</td><td>…
…</td><td>…
…</td><td>…
…</td><td>…
…</td></tr>
<tr><td>Liver ultrasound (local) [ef]</td><td>X</td><td>X</td><td>X</td><td>X</td><td>X</td></tr>
<tr><td>Motor milestone assessments [fh]</td><td>X</td><td>X</td><td>X</td><td>X</td><td>X</td></tr>
<tr><td>ACEND</td><td>X</td><td>X</td><td>X</td><td>X</td><td>X</td></tr>
<tr><td>Clinical global impression scales [i]</td><td>X</td><td>X</td><td>X</td><td>X</td><td>X</td></tr>
<tr><td>Swallowing questionnaire</td><td>X</td><td>X</td><td>X</td><td>X</td><td>X</td></tr>
<tr><td>Secretions management assessment [j]</td><td>X</td><td>X</td><td>X</td><td>X</td><td>X</td></tr>
</table>
…; CK = creatine kinase; …
[a] Complete physical examination with detailed neurological evaluation and respiratory function assessment will be conducted. It can be done remotely per discretion of investigator in consultation with Medical Monitor. When visit is done remotely, an abbreviated physical examination is acceptable.
[g] See Section 4.6.3 for details related to the monitoring, diagnosis, and treatment of neuromuscular abnormalities.
[i] See Appendix 5.
[j] The severity of secretions and burden of secretions management will be assessed using the PGIS-S and PGIS-I (Appendix 3). The same parent/LAR/caregiver should report throughout the study (whenever possible).</td><td>To update the study assessments accordingly with changes to the management plan for safety events

To capture necessary efficacy data in the long-term follow-up period (Years 6 and 10)</td><td>Change of medical monitoring procedure which is likely to have a significant impact on the safety

Change in study design which reduces required study assessments</td></tr>
</table>

Location(s)	Description of Change	Rationale for Change	Justification for Substantial Designation
Section 6.5.2	**6.5.2 Management of Potential Immune Responses** ~~There are potential immune responses that may be considered when administering a gene transfer agent. The 2 broad categories are a cell-mediated (T cell) response and a humoral (B cell) response. B cell responses for a gene therapy vector, such as AT132, might include the development of antibodies to the AAV capsid and/or to the resulting protein (myotubularin). Samples will be taken during the course of the study to monitor for both neutralizing and binding antibodies to AAV8 capsid protein and myotubularin protein.~~ ~~If a humoral response to capsid occurs, it would likely not impact the subject unless a poststudy situation develops whereby consideration is being given to re-dose the subject with AT132 (perhaps due to declining efficacy over time, after an initial positive response to treatment). It may be difficult to re-dose a subject in the context of high levels of circulating AAV8 antibodies.~~ ~~Transient, asymptomatic elevations in hepatic transaminases, likely due to T cell mediated inflammatory responses, were reported in a study of subjects with hemophilia B (factor IX deficiency) infused with a single dose of a gene transfer product using the AAV8 vector (Nathwani, 2014; Nathwani, 2011). An asymptomatic, transient increase in the mean ALT level to 86 IU/L (range, 36-202) occurred between Weeks 7 and Week 10 in 4 of the 6 subjects treated at the highest dose but resolved over a median of 5 days (range, 2-35) after a short course of glucocorticoid therapy (oral prednisolone). Such transient, asymptomatic, increases in liver transaminases have been noted in AAV hemophilia A (factor VIII deficiency) studies (Pasi, 2016) and in the AAV9 studies of SMA (Mendell, 2017). Use of prophylactic glucocorticoids is intended to prevent any immune-mediated hepatic injury, which has been observed in subjects who have received systemic AAV gene therapy (Section 4.4).~~ Elevations of hepatic transaminases, CK, and troponin I, likely representing an immune response to the study treatment, have been reported in ~~the ASPIRO study~~ Study ATX-MTM-002 and are considered identified risks associated with AT132 administration. Refer to the Investigator's Brochure for details. Guidelines for monitoring and management of such events are provided in Section 4.6. Appropriate care for an individual subject will be determined by the Investigator based on an assessment of the subject's overall medical status. Corticosteroid treatment typically resolves the elevation of liver transaminases. However, when adverse events related to the immune response are of significant concern (eg, rAAV vector dose 1 x 10^{14} vg/kg and/or a transgene-mediated immune response), immunomodulation may include medications such as rapamycin (sirolimus), mycophenolate mofetil, calcineurin inhibitors (eg, cyclosporine, tacrolimus), and rituximab (Prasad, 2022).	To update the monitoring and management plan for safety events based on the latest safety information	Change of medical monitoring procedure which is likely to have a significant impact on the safety

Nonsubstantial Changes

In addition to the changes detailed in this list below, other non-substantive changes, including change of the Sponsor name and logo, change of “Audentes” to “Sponsor”, correction of typographical errors, grammar, abbreviations, and formatting were made, but are not delineated here. Deleted text is denoted with strikethrough font and added text is underlined. When needed, “…” indicates a skipped sentence or paragraph.

Location(s)	Description of Change	Brief Rationale
COVER PAGE	**Sponsor:** ~~Audentes Therapeutics, Inc., an Astellas Company~~ ~~San Francisco, CA 94108~~ Astellas Gene Therapies, Inc. South San Francisco, CA 94080 **~~Eudra~~U CT Number:** ~~2017 000876 27~~ 2024-512637-32	Administrative change
COVER PAGE	**Investigational Product:** AT132 (resamirigene bilparvovec)	Administrative change
PROTOCOL SYNOPSIS	**NAME OF TEST PRODUCT:** AT132 (resamirigene bilparvovec)	Administrative change
PROTOCOL SYNOPSIS	**STUDY RATIONALE:** …. Following a single systemic therapeutic administration. ~~In the absence of progressive pathology in XLMTM,~~ Gene therapy is …	Administrative change for accuracy
PROTOCOL SYNOPSIS and Section 2 and Section 7.4.2.2.2	**ENDPOINTS, SAFETY ASSESSMENTS, AND ESTIMANDS:** ~~**Safety Endpoints:**~~ • ~~Adverse events (AEs), serious AEs (SAEs), and findings from safety laboratory tests, 12-lead electrocardiogram (ECG), echocardiograms (ECHOs), vital signs, growth parameters, physical examinations, liver ultrasounds, antibody formation (anti-AAV8, anti-MTM1), viral shedding, annualized hospitalization rate, annualized respiratory and non-respiratory SAE rate, and length of stay per hospitalization~~ **Key Secondary Efficacy Endpoint:** **…** **Other Secondary Efficacy Endpoints:** **…** **Safety Endpoints:** • Adverse events (AEs), serious AEs (SAEs), and findings from safety laboratory tests, 12-lead electrocardiogram (ECG), echocardiograms (ECHOs), vital signs, growth parameters, physical examinations, liver ultrasounds, antibody formation (anti-AAV8, anti-MTM1), viral shedding, annualized hospitalization rate, annualized respiratory and non-respiratory SAE rate, and length of stay per hospitalization	Administrative change for clarity

Location(s)	Description of Change	Brief Rationale
PROTOCOL SYNOPSIS and Section 2 and Section 7.4.2.2.2	**ENDPOINTS, SAFETY ASSESSMENTS, AND ESTIMANDS:** … **Other Secondary Efficacy Endpoints:** … • Percentage of subjects achieving full ventilator independence ~~in the absence of acute illness and perioperatively~~ at Week 24 • Survival … **Exploratory Endpoints:** … ~~In depth interviews to assess the experiences and perspectives of caregivers and children with XLMTM~~	To update the endpoints based on nature and limitations of the collected data
PROTOCOL SYNOPSIS	**STUDY DESIGN:** … The study consists of 2 parts. Part 1 would establish the optimal dose of AT132. Part 2 would confirm the safety and efficacy of AT132 at the optimal dose level. The following describes how each part was designed and the changes as of protocol v8 and beyond.	Administrative change for consistency
PROTOCOL SYNOPSIS	See Figure 1 for the individual study participation timeline (Parts 1 & 2) through Week 48. ~~**Summary of Individual Study Participation Timeline (Parts 1 & 2)**~~ ***Part 2 (described herein as planned prior to protocol v8; partially dosed as of protocol v8):*** …	Administrative change to remove an extra heading

<table>
<tr><th>Location(s)</th><th>Description of Change</th><th>Brief Rationale</th></tr>
<tr><td>LIST OF ABBREVI-ATIONS/ DEFINITION OF TERMS</td><td>
<table>
<tr><td>~~ACTIVE~~</td><td>~~abilities captured through interactive video evaluation~~</td></tr>
<tr><td>~~AD~~</td><td>~~Analytical Development~~</td></tr>
<tr><td>ADL</td><td>activities of daily living</td></tr>
<tr><td>~~CMH~~</td><td>~~Cochran-Mantel-Haenszel~~</td></tr>
<tr><td>ELI~~s~~Spot</td><td>enzyme-linked immunosorbent spot assay</td></tr>
<tr><td>EMG</td><td>electrophysiological assessments</td></tr>
<tr><td>ENT</td><td>ear, nose and throat</td></tr>
<tr><td>~~EU~~</td><td>~~European Union~~</td></tr>
<tr><td>FAS</td><td>full analysis set</td></tr>
<tr><td>~~FDA~~</td><td>~~Food and Drug Administration~~</td></tr>
<tr><td>~~NBD~~</td><td>~~nasobiliary drainage~~</td></tr>
<tr><td>SAF</td><td>safety analysis set</td></tr>
<tr><td>~~SD~~</td><td>~~summarized by N, Mean~~</td></tr>
<tr><td>STIR</td><td>short tau inversion recovery</td></tr>
<tr><td>TMA</td><td>thrombotic microangiopathy</td></tr>
<tr><td>~~US~~</td><td>~~United States~~</td></tr>
</table>
</td><td>Administrative change</td></tr>
<tr><td>Section 1.1</td><td>1.1 Background
…
Additionally, the recently published literature (Dowling, 2022) based on the data from Study ATX-MTM-009 (INCEPTUS, a prospective natural history study conducted by the Sponsor) suggested that hepatobiliary disease was identified as an under-recognized comorbidity, where 91% of subjects had histories of hepatic disease and/or prospectively experienced related adverse events (AEs) or laboratory or imaging abnormalities.
…
The more recent case series and reports (Molera, 2022; D'Amico, 2022~~1~~; Neese, 2021) offered additional insight into key clinical characteristics of intrahepatic cholestasis and liver dysfunction in XLMTM patients.</td><td>Administrative change</td></tr>
<tr><td>Section 1.3</td><td>1.2 Study Rationale
…
~~In the absence of progressive pathology in XLMTM, g~~Gene therapy is expected to provide persistent protein expression, corresponding long-term clinical benefit, and the potential for substantial recovery of muscle function</td><td>Administrative change for accuracy</td></tr>
</table>

Location(s)	Description of Change	Brief Rationale
Section 3.7	**3.7 Start and End of Study Definitions** *First act of recruitment* The study start date is the date on which the clinical study will be open for recruitment of participants. The first act of recruitment is the date the first participant signs the informed consent form (ICF) and will be the study start date. *End of study* The end of the study is defined as the last visit or assessment shown in schedule of assessments (Section 5.6) for the last participant in the study. A participant is considered to have completed the study if the participant has completed all periods of the study including the last assessment shown in the schedule of assessments.	To clarify the definition of start and end dates (no change to the definition itself)
Section 4.4	**4.4 Prophylaxis with Glucocorticoids** … Therefore, providing a modest dose [consistent with that used for a number of conditions, see ~~USPI (Prednisolone USPI, 2013)~~ the respective local prescribing information] of a short course of oral prednisolone is considered appropriate and an acceptable risk.	Administrative change
Section 4.5	**4.4 Prophylaxis with Ursodiol** … Ursodiol is generally well-tolerated and minimal side effects, most gastrointestinal in nature, have been described (~~Ursodiol USPI, 2013~~ see the respective local prescribing information).	Administrative change
Section 5.1.1.11.2.2	**5.1.1.11.2.2 Ventilator Dependence Questionnaire** … For subjects in Part 1 who have already completed the Week 24 visit, the parent/LAR/caregiver will complete the ventilator dependence questionnaire at clinic and contact visits through Month ~~60~~120. For subjects in Part 1 who have not completed the Week 24 visit and for subjects in Part 2 (including any subjects enrolled under protocol v5 and beyond), the daily diary will be completed per Section 5.1.1.11.2.1. After the daily diary has been stopped or the subject has completed Week 48, the parent/LAR/caregiver will complete the ventilator dependence questionnaire at clinic and contact visits through Month ~~60~~120.	Administrative changes for correction
Section 5.1.1.21	**5.1.1.21 Interferon-γ (IFN-γ) ELISpot Assay** Interferon-γ (IFN-γ) production is commonly used as an indicator of T cell immune reactivity. An ELISpot assay will ~~assess the~~ measure IFN-γ ~~response of~~ producing T cells in subjects' peripheral blood mononuclear cells (PBMCs) ~~when challenged~~ after challenge with a pool of AAV8 peptides ~~and~~ or myotubularin peptides. Samples for testing will be collected per the Schedule of Events in Sections 5.4 and 5.5. Additional samples will be collected as clinically indicated, as described in Section 4.6~~.2.3~~.	Administrative change for clarity
Section 5.8	**5.8 Unscheduled Visits** Additional assessments and optional assessments conducted as part of the study should be recorded on the unscheduled visit eCRFs.	Administrative change for clarity

Location(s)	Description of Change	Brief Rationale
Section 6.2.3.3	**6.2.3.3 Urgent Safety Measures and Non-Suspected Unexpected Serious Adverse Reaction Reporting** … The reporting collection period for urgent safety issues is the period from the signing of the ICF until the Year 10 Visit or the subject withdraws from the study.	Administrative change for clarity
Section 6.5.3	**6.5.3 Potential Cases of Drug-Induced Liver Injury** … ~~Any subject enrolled in a study reveals an increase of AT to > 3 × ULN (to > 5 × ULN in subjects with liver metastases) or TBL > 2 × ULN should undergo detailed testing for liver enzymes (including at least alkaline phosphatase, ALT, AST and TBL). Testing should be repeated within 72 hours of notification of the test results.~~ For studies for which a central laboratory is used, alerts will be generated by the central laboratory regarding moderate and severe liver abnormality to inform the Investigator and study team. Subjects should be ~~asked if they have any~~ further evaluated for additional symptoms suggestive of hepatobiliary dysfunction.	To align with the current procedure for liver enzyme testing
Section 7.4.2.2.2	**7.4.2.2.2 Other Secondary Efficacy Endpoints** **…** The number and percentage of subjects attaining age-appropriate clinically relevant gross motor function milestones through Week 24, and subjects achieving full ventilator independence ~~in the absence of acute illness and perioperatively~~ at Week 24, will be summarized for low dose and high dose.	Administrative change
Section 8.1	**8.1 Ethical Conduct of the Study** … This study will be conducted in accordance with the following: • United States (US) CFR sections that address clinical research studies • European Union (EU) Directive 2001/20/EC and EU Clinical Trial Regulation 536/2014~~its updates~~	Administrative changes to reflect the regulation changes
Section 10	**10 REFERENCES** Adeboye A, Alkhatib D, Butt A, et al. A review of the role of imaging modalities in the evaluation of viral myocarditis with a special focus on COVID-19-related myocarditis. Diagnostics (Basel). 2022;12(2):549. ~~Appleby VJ, Hutchinson JM and Davies MH. Safety and efficacy of long-term nasobiliary drainage to treat intractable pruritus in cholestatic liver disease. Frontline Gastroenterol. 2015;6(4):252-4.~~ Arjomandnejad M, Dasgupta I, Flotte TR, et al. Immunogenicity of recombinant adeno-associated virus (AAV) vectors for gene transfer. BioDrugs. 2023;37(3):311-29. D'Amico A, Longo A, Fattori F, et al. Correction to: Hepatobiliary disease in ~~xlmtm~~XLMTM: ~~A~~a common comorbidity with potential impact on treatment strategies ~~(with correction)~~. Orphanet J Rare Dis. ~~2021~~2022;17(1):18. Erratum for: Orphanet J Rare Dis. 2021;16(1):425. FDA. Toxicity Risks of Adeno-associated Virus (AAV) Vectors for Gene Therapy (GT). FDA Cellular, Tissue, and Gene Therapies Advisory Committee (CTGTAC) Meeting #70. 2021. Available from: https://www.fda.gov/media/151969/download.	Administrative changes to reflect more recent citations.

Location(s)	Description of Change	Brief Rationale
	~~Hegade VS, Krawczyk M, Kremer AE, et al. The safety and efficacy of nasobiliary drainage in the treatment of refractory cholestatic pruritus: A multicentre european study. Aliment Pharmacol Ther. 2016;43(2):294-302.~~ ~~Jannone G, Stephenne X, Scheers I, et al. Nasobiliary drainage prior to surgical biliary diversion in progressive familial intrahepatic cholestasis type ii. Eur J Pediatr. 2020;179(10):1547-52.~~ Lek A, Atas E, Hesterlee SE, et al. Meeting Report: 2022 Muscular Dystrophy Association summit on 'safety and challenges in gene transfer therapy'. J Neuromuscul Dis. 2023;10:327-36. ~~Mendell JR, Al-Zaidy S, Shell R, et al. Single-dose gene-replacement therapy for spinal muscular atrophy. N Engl J Med. 2017;377(18):1713-22.~~ ~~Nathwani AC, Reiss UM, Tuddenham EG, et al. Long-term safety and efficacy of factor ix gene therapy in hemophilia b. N Engl J Med. 2014;371(21):1994-2004.~~ ~~Nathwani AC, Tuddenham EG, Rangarajan S, et al. Adenovirus-associated virus vector-mediated gene transfer in hemophilia b. N Engl J Med. 2011;365(25):2357-65.~~ ~~Pasi J, Wong W, Rangarajan S, et al. Interim results of an open-label, phase 1/2 study of bmn 270, an aav5-fviii gene transfer in severe hemophilia a. Haemophilia. 2016(22 (Suppl. 4)):151.~~ Pilati M, Rebonato M, Formigari R, et al. Endomyocardial biopsy in pediatric myocarditis and dilated cardiomyopathy: a tool in search for a role. J Cardiovasc Dev Dis. 2022;9(1):24. Prasad S, Dimmock DP, Greenberg B, et al. Immune responses and immunosuppressive strategies for adeno-associated virus-based gene therapy for treatment of central nervous system disorders: current knowledge and approaches. Hum Gene Ther. 2022;33(23-24):1228-45. ~~Prednisolone USPI. Pediapred (prednisolone sodium phosphate) oral solution [united states package insert]. Manasquan, NJ: Royal Pharmaceuticals; 2013.~~ Shen W, Liu S and Ou L. rAAV immunogenicity, toxicity, and durability in 255 clinical trials: A meta-analysis. Front Immunol. 2022;13:1001263. Shieh PB, Bönnemann CG, Müller-Felber W, et al. Re:“Moving forward after two deaths in a genetherapy trial of myotubular myopathy” by Wilson and Flotte. Hum Gene Ther. 2020;31:787. Shieh PB, Kuntz NL, Dowling JJ, et al. Safety and efficacy of gene replacement therapy for X-linked myotubular myopathy (ASPIRO): a multinational, open-label, dose-escalation trial. Lancet Neurol. 2023 Dec;22(12):1125-39. ~~Spark Therapeutics. Spark therapeutics and pfizer present updated preliminary data from hemophilia b phase 1/2 trial suggesting consistent and sustained levels of factor ix activity at annual ash meeting [press release]. 2016~~ ~~Stapelbroek JM, van Erpecum KJ, Klomp LWJ, et al. Nasobiliary drainage induces long-lasting remission in benign recurrent intrahepatic cholestasis. Hepatology. 2006;43(1):51-3.~~ ~~Sun B, Yu D, Chen J, Tang Y and Wu H. Endoscopic biliary drainage management for children with serious cholangitis caused by congenital biliary dilatation. Pediatr Surg Int. 2018;34(8):897-901.~~ ~~Ursodiol USPI. Ursodiol (ursodeoxycholic acid) [united states prescribing information]. Bridgewater, NJ: Aptalis Pharma US, Inc.; 2013.~~	

Location(s)	Description of Change	Brief Rationale
	Urzua Fresno C, Sanchez Tijmes F, Shaw KE, et al. Cardiac imaging in myocarditis: current evidence and future directions. Can Assoc Radiol J. 2023 Feb;74(1):147-59. Whiteley LO. An Overview of nonclinical and clinical liver toxicity associated with AAV gene therapy. Toxicol Pathol. 2023:1926233231201408. ~~Zellos A, Lykopoulou L, Polydorou A, et al. Nasobiliary drainage in an episode of intrahepatic cholestasis in a child with mild abcb11 disease. J Pediatr Gastroenterol Nutr. 2012;55(1):88-90.~~	
Section 11	~~I confirm that Audentes Therapeutics, Inc. will comply with all obligations as detailed in all applicable regulations and guidelines. I will ensure that the Investigators and subjects/parents/legal guardians are informed of important information that becomes available during the conduct of this study.~~ PPD PPD ~~Date~~ PPD PPD ~~Date~~ Required sponsor signatures as required by ICH GCP 4.5.1 are located in the first attachment. Attachment 1 | Electronic Sponsor Signatures	Administrative change