X-linked myotubular myopathy

A prospective international natural history study

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Abstract

Objectives

Because X-linked myotubular myopathy (XLMTM) is a rare neuromuscular disease caused by mutations in the *MTM1* gene with a large phenotypic heterogeneity, to ensure clinical trial readiness, it was mandatory to better quantify disease burden and determine best outcome measures.

Methods

We designed an international prospective and longitudinal natural history study in patients with XLMTM and assessed muscle strength and motor and respiratory functions over the first year of follow-up. The humoral immunity against adeno-associated virus serotype 8 was also monitored.

Results

Forty-five male patients aged 3.5 months to 56.8 years were enrolled between May 2014 and May 2017. Thirteen patients had a mild phenotype (no ventilation support), 7 had an intermediate phenotype (ventilation support less than 12 hours a day), and 25 had a severe phenotype (ventilation support 12 or more hours a day). Most strength and motor function assessments could be performed even in very weak patients. Motor Function Measure 32 total score, grip and pinch strengths, and forced vital capacity, forced expiratory volume in the first second of exhalation, and peak cough flow measures discriminated the 3 groups of patients. Disease history revealed motor milestone loss in several patients. Longitudinal data on 37 patients showed that the Motor Function Measure 32 total score significantly decreased by 2%. Of the 38 patients evaluated, anti-adeno-associated virus type 8 neutralizing activity was detected in 26% with 2 patients having an inhibitory titer >1:10.

Conclusions

Our data confirm that XLMTM is slowly progressive for male survivors regardless of their phenotype and provide outcome validation and natural history data that can support clinical development in this population.

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Glossary

AAV = adeno-associated virus; CHOP-INTEND = Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders; FEV₁ = forced expiratory volume in the first second of exhalation; FVC = forced vital capacity; MEP = maximum expiratory pressure; MFM = Motor Function Measure; MHFMS = Modified Hammersmith Functional Motor Scale; MIP = maximum inspiratory pressure; NSAA = North Star Ambulatory Assessment; PCF = peak cough flow; XLMTM = X-linked myotubular myopathy.

X-linked myotubular myopathy (XLMTM, OMIM 310400) is a rare disease of skeletal muscle affecting approximately 1 in 50,000 male newborns.¹ XLMTM is caused by mutations in the *MTM1* gene, which encodes the ubiquitous phosphatase myotubularin. Myotubularin is implicated in the phosphatidylinositol 3-kinase pathway that regulates intracellular membrane trafficking and vesicular transport processes.^{2–6}

Patients with XLMTM are classified as having mild, intermediate, or severe phenotype based on the amount of ventilator support required.⁷ Much milder phenotypes have also been described.^{8,9} Most patients have the severe form^{10,11} and typically present at birth with severe hypotonia and inability to establish spontaneous respiration, often leading to death within the first months of life. Long-term survivors with the severe phenotype are often nonambulant and need ventilation support.^{10,12} Patients with the intermediate and mild phenotypes breathe independently at least a few hours per day, and become ambulant.^{7,12,13} Among comorbidities, liver abnormalities are often reported.^{10–12}

Several therapeutic strategies are under investigation including gene therapy (NCT03199469),^{14,15} enzyme replacement therapy,¹⁶ dynamin-2 modulation,^{17,18} and PIK3C2B inhibition.¹⁹ To ensure clinical trial readiness and to better quantify disease burden in patients with XLMTM, we designed an international prospective and longitudinal natural history study to assess muscle strength and pulmonary and motor function using standardized measures. We also tested for the presence of neutralizing antibodies against adeno-associated virus (AAV) type 8 in serum, as humoral immune response to AAV can prevent AAV vector transduction.^{20,21}

Methods

Patients and study design

We conducted an international, multicenter, prospective, and longitudinal study on patients with XLMTM (NatHis-MTM) sponsored by Valerion Therapeutics, LLC (Concord, MA). This 3-year study was performed at 14 investigational sites located in the United States, Canada, France, Spain, Italy, Germany, and Belgium. All patients with XLMTM, males and symptomatic females, not treated with an experimental therapy other than pyridostigmine and not having another disease that might substantially interfere with XLMTM assessments, were allowed to take part in this study with no restrictions on

age. This report focuses on the data obtained on male patients over a 1-year period.

Patients younger than 2 years were assessed quarterly, patients aged between 2 and 6 years were assessed every 6 months, and older patients were assessed every 6 months during the first year and annually thereafter. Depending on the enrollment date, patient participation lasted between 12 and 36 months. Disease history, psychomotor development, cardiac, respiratory, liver, kidney, coagulation, and hematologic functions, and feeding and orthopedic status were obtained from the available medical files and patient/parent interviews. A standard liver ultrasound was performed every year and serum chemistries were collected from the available medical files. A 24-hour urine collection every 6 months measured the amount of creatinine excreted as a marker of the skeletal muscle mass.²² At each visit, the physician performed a complete physical examination, noted concomitant treatments and adverse events, measured vital signs, weight and height, and assessed ophthalmoplegia and ptosis. Likewise, at each visit, certified physiotherapists assessed motor function, upper limb strength, and respiratory function. These standardized evaluations were adjusted according to age, ambulatory status, and respiratory support needed.

Grip and pinch strength were assessed with the MyoGrip and MyoPinch devices in patients older than 6 years, as previously described.²³ To limit fatigue, patients were tested only on the dominant side. Results are expressed as percent of predicted values based on age using predictive equations constructed with normative data aggregated at the Institute of Myology:

For patients aged 6 to 16 years:

$$\text{Grip strength} = \text{Grip} \times 100 / 3.3996 \times \text{EXP}(0.1527 \times \text{age})$$

$$\text{Pinch strength} = \text{Pinch} \times 100 / 1.9647 \times \text{EXP}(0.0828 \times \text{age})$$

For patients 17 years and older:

$$\text{Grip strength} = \text{Grip} \times 100 / 45.662 \times \text{EXP}(-0.001 \times \text{age})$$

$$\text{Pinch strength} = \text{Pinch} \times 100 / 7.98 \times \text{EXP}(-0.001 \times \text{age})$$

Patients older than 6 years without tracheostomy underwent pulmonary function tests measuring forced vital capacity (FVC), forced expiratory volume in the first second of exhalation (FEV₁), peak cough flow (PCF), and maximum

expiratory and inspiratory pressure (MEP and MIP). FVC, FEV₁, and PCF were measured using the Vitalograph spirometer and software (Lenexa, KS). MEP and MIP were captured using the MicroRPM device. Results are expressed as percent of predicted values using previously published equations based on age and height.^{24–26} Percentage of FVC and FEV₁ were computed using a calculator (biostat.jhsph.edu/courses/bio622/misc/calculate.htm) based on information presented in ref. 24.

To assess motor function, children younger than 2 years of age performed the Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP-INTEND) scale²⁷ and the Modified Hammersmith Functional Motor Scale (MHFMS).²⁸ For safety reasons, the last 2 items of the CHOP-INTEND were not performed. Patients older than 2 years of age performed the Motor Function Measure (MFM)²⁹ scale. Children aged from 2 to 5 years performed the MHFMS and short version of MFM (20 items vs 32). Patients turning 6 years of age during the study continued to be evaluated with the MFM20.³⁰ Patients older than 6 years performed the MFM32, and their dominant upper limb motor function was assessed by the MoviPlate device, as previously described.²³ Ambulant patients older than 6 years performed the North Star Ambulatory Assessment (NSAA) scale and timed tests including 6-minute walk distance, 10-m run/walk (NSAA, item 17), and time to rise from floor (NSAA, item 11), as previously described.^{31–33}

Patients were given 2 attempts to perform testing with MoviPlate and 3 attempts to measure grip and pinch strength and each pulmonary function. If they continuously improved, further tests were conducted until patients achieved their best result, which was retained for statistical analysis.

We used the Patient Reported Outcomes Measurement Information System Short Form Instruments for Open Distribution to assess the patients' quality of life and asked 3 questions on the number of social events, work, or school days missed because of XLMTM.

All assessments were administered according to standardized procedures. The same physiotherapist assessed patients in France, Italy, and Spain. She fully trained Belgian and German physiotherapists and assisted them during first assessments. Assessments in North America were performed by physiotherapists trained at an organized session by the European physiotherapist. Study monitors regularly evaluated protocol and Good Clinical Practice compliance as well as quality, completeness, and consistency of the data collected in the case report forms at all sites.

The neutralizing antibody assay on serum samples of patients was performed using the GenoSafe procedure. Briefly, HEK293T cells were plated on day 1 in 96-well plates and were allowed to rest for 24 ± 1 hour at 35°C to 38°C in 5% CO₂ before transduction. After serial dilution (1:1–1:405) in

normal seronegative human pooled serum, dilutions and quality controls were incubated together with a recombinant AAV8-CMV-luciferase vector for 1 hour ± 15 minutes at 35°C to 38°C. AAV8 transduction inhibition titers were determined as the reciprocal dilution of plasma interpolated at the titer cut point set at 61.60% of transduction.

Standard protocol approvals, registrations, and patient consents

This study was approved by local ethics/institutional review boards and the French health authority (ANSM) and was registered on clinicaltrials.gov (NCT02057705). All enrolled patients (or parents for patients younger than 18 years) signed an informed consent before any study-related procedure.

Statistical analyses

Statistical analyses were performed following a prespecified statistical analysis plan. Mild, intermediate, and severe phenotypes were prespecified for comparison analysis. Characteristics of the 3 groups were computed by descriptive analyses. To determine outcome measures able to differentiate patients with different disease levels, proportions were compared using the χ^2 test and quantitative variables using the Kruskal-Wallis 1-way analysis of variance. Post hoc tests based on the Dunn-Bonferroni method were performed in case of significance. Correlation between creatinuria excreted in 24 hours and standardized assessments for muscle strength and pulmonary and motor functions were assessed with the Spearman correlation coefficient. Changes over time were assessed using a Wilcoxon signed-rank test. All analyses were performed using SPSS Statistics 22 statistical software (IBM Corp., Armonk, NY). The limit of statistical significance was set to 0.05.

Data availability

Deidentified participant data are available on request.

Results

Patient characteristics

Demographics

Forty-five male patients from 3.5 months to 56.8 years of age were enrolled between May 2014 and May 2017 (figure 1A). Nine patients were enrolled in North America (5 in Canada, 4 in the United States) and 36 in Europe (15 in France, 10 in Germany, 7 in Italy, 2 in Spain, and 2 in Belgium). At inclusion, 27% of patients were younger than 2 years old, 20% were aged between 2 and 5 years, and 53% were 6 years or older (table 1).

Clinical characteristics at baseline

Twenty-nine percent of patients presented with a mild phenotype, 16% with an intermediate phenotype, and 55% with a severe phenotype (figure 1A and table 1). All patients, including patients with a mild phenotype had abnormal

Figure 1 Patient flowchart and mutations detected in the *MTM1* gene

(A) Patient flowchart. Age is expressed in years. (B) Mutations detected in the *MTM1* gene. Mutations written in black, light blue, and dark blue correspond to mild, intermediate, and severe phenotype, respectively. Mutations predicted to be linked to reduction or absence of functional protein are indicated in bold (frameshift, nonsense, splicing, in-frame exon deletion, large deletion, and start codon loss). A protein with residual function may be present for missense mutations and in-frame small deletions. Numbers in parentheses are number of patients with the same mutation and phenotype.

pulmonary function with FVC, FEV₁, MEP, MIP, and PCF lower than 80% of normal. FVC, FEV₁, and PCF were the most different parameters between patient groups (table 2 and figure 2). Motor function as assessed by MFM and distal muscle strength as assessed by MyoGrip and MyoPinch were also abnormal in all groups with a strong group effect toward lower values in the intermediate and severe phenotypes. Distal muscle weakness was correlated with reduced muscle mass as assessed by the 24-hour excreted creatinuria (Spearman coefficient of 0.727 and 0.656 for grip and pinch strength, respectively) (figure 3 and table 2).

Scoliosis was present in 60% of patients and was more frequently encountered in patients with the severe phenotype ($p = 0.007$). Four patients required spinal surgery before the study. The angle of scoliosis was less than 30° for 12 of the 19

remaining patients. Contractures were also more frequent in patients with the severe phenotype but the difference did not reach statistical significance (64% vs 43% and 38% in the intermediate and mild phenotypes, respectively; $p = 0.118$).

Eighty-nine percent of patients reported feeding difficulties mainly characterized by swallowing, sucking, and chewing difficulties (76%, 58%, and 56%, respectively), and 76% of patients had feeding tubes (16% nasogastric tube, 60% gastrostomy) (table 1). Gastrostomies were more frequent in patients with severe phenotype (84% vs 57% and 23% in the intermediate and mild phenotypes, respectively; $p = 0.005$) (table 1).

Ophthalmoplegia and ptosis were common in all phenotypes and present in 96% and 78% of patients, respectively.

Table 1 Patient characteristics

Patient no.	Age at inclusion, y	Weight, ^a percentile	Length/height, ^a percentile	Mutation (NM_000252.2)	Predicted effect	Ambulatory status	Breathing support	Feeding support	AAV8 inhibitory titer
Mild (i.e., no ventilation support)									
1^b	0.8	<1	37.7	c.1210G>T	p.(Glu404*)	NA	None	NGT	<1
2^b	1.7	21.2	92.4	c.205C>T	p.(Arg69Cys)	NA	None	PEG	27.51
3^b	4.2	16.4	76.9	c.205C>T	p.(Arg69Cys)	A	None	None	<1
4^b	4.9	14.7	52.3	c.63+2T>C	Destruction donor splice site exon 2	NA	None	NGT	<1
5^b	6.5	29.1	92.5	c.205C>T	p.(Arg69Cys)	A	None	None	<1
6^b	7.7	28.8	85.9	c.205C>T	p.(Arg69Cys)	A	None	None	3.91
7^b	12.6	4.5	32.9	c.1210G>A	p.(Glu404Lys)	A	None	None	<1
8^b	13.7	19.2	17.9	c.205C>T	p.(Arg69Cys)	NA	None	PEG	<1
9^b	14	3.1	50.1	c.575A>G	p.(Tyr192Cys)	A	None	None	<1
10^b	15.3	19.3	<1	c.1496G>T	p.(Trp499Leu)	A	None	PEG	8.49
11^b	33.7	2.1	25	c.1233G>T	p.(Trp411Cys)	A	None	None	<1
12^b	53.4	98.3	90.1	c.208C>T	p.(Leu71Phe)	A	None	None	<1
13^b	56.8	90.1	42.5	c.575A>G	p.(Tyr192Cys)	A	None	None	<1
Intermediate (i.e., ventilation support <12 h/d)									
14^b	0.5	<1	9.8	c.1490C>A	p.(Ser497Tyr)	NA	NIV: 10 h	PEG	<1
15	1.2	0.3	96.4	c.721C>T	p.(Arg241Cys)	NA	IV: 10 h	PEG	<1
16^b	3.9	6	79.9	c.567-569delTAA	p.(Asn189del)	NA	IV: 8 h	PEG	<1
17^b	5	1.4	72.7	c.139_141delAAA	p.(Lys47del)	NA	NIV: 10 h	PEG	<1
18^b	14.2	59.7	62.4	c.676C>T	p.(Pro226Ser)	NA	NIV: 8 h	NGT	<1
19	18.4	29.8	80.7	c.695A>G	p.(His232Arg)	NA	NIV: 8 h	NGT	4.1
20^b	50.8	92.9	56.4	c.194T>A	p.(Ile65ASN)	A	NIV: 10 h	None	>405

Continued

Table 1 Patient characteristics (continued)

Patient no.	Age at inclusion, y	Weight, ^a percentile	Length/height, ^a percentile	Mutation (NM_000252.2)	Predicted effect	Ambulatory status	Breathing support	Feeding support	AAV8 inhibitory titer
Severe (i.e., ventilation support ≥12 h/d)									
21^b	0.3	<1	71.5	c.99_105delGGCTGTT	p.(Glu33Aspfs*9)	NA	IV: 20 h	PEG	3.71
22	0.8	4.3	90	c.1054-2A>G	Destruction acceptor splice site exon 11	NA	IV: 24 h	PEG	<1
23^b	1.3	89	91.4	c.670C>T	p.(Arg224*)	NA	IV: 24 h	PEG	UNK
24	1.6	9.7	88.6	c.614C>T	p.(Pro205Leu)	NA	IV: 24 h	PEG	UNK
25^b	1.6	20.9	>99	c.1505T>A	p.(Ile502Lys)	NA	IV: 24 h	PEG	<1
26^b	1.6	29.2	>99	c.1505T>A	p.(Ile502Lys)	NA	IV: 24 h	PEG	<1
27	1.7	67	>99	c.205C>T	p.(Arg69Cys)	NA	IV: 18 h	PEG	UNK
28	1.9	56.9	99.6	c.32C>A	p.(Ser11*)	NA	IV: 24 h	PEG	UNK
29^b	2.2	0.5	96.9	c.141-144delAGAA	p.(Glu48Leufs*24)	NA	IV: 24 h	PEG	<1
30	2.3	6.7	91.7	Deletion all <i>MTM1</i>	Deletion all <i>MTM1</i>	NA	IV: 24 h	PEG	UNK
31^b	2.6	36.5	98.5	c.98_103delAGGCTG	p.(Glu33_Ala34del)	NA	IV: 24 h	PEG	<1
32^b	4.7	55.9	>99	c.1A>T	p.? ^c	NA	IV: 23 h	PEG	3.25
33^b	5.2	50.9	82.9	c.1264dupA	p.(Ile422Asnfs*5)	NA	IV: 16 h	PEG	<1
34	6.6	48.5	80	c.679G>A	p.(Val227Met)	NA	IV: 24 h	PEG	<1
35^b	7.1	22.9	65.5	Exon 5 deletion ^d	Exon 5 deletion	NA	IV: 12 h	PEG	<1
36^b	10.3	4.6	21.8	Deletion exons 11–15 ^d	Deletion exons 11–15	NA	IV: 24 h	PEG	<1
37^b	11.8	36	72.6	c.205C>T	p.(Arg69Cys)	NA	IV: 24 h	PEG	2.69
38^b	12.3	48.7	55.4	c.595C>T	p.(Pro199Ser)	NA	NIV: 13 h	None	<1
39^b	13.6	41.4	70.5	c.232-1G>A	Destruction acceptor splice site exon 5	NA	IV: 24 h	PEG	UNK
40^b	15	<1	<1	c.1420C>T	p.(Arg474*)	NA	IV: 24 h	PEG	<1
41^b	15.8	41	40.2	c.139_141delAAA	p.(Lys47del)	NA	IV: 24 h	None	<1

Continued

Table 1 Patient characteristics (continued)

Patient no.	Age at inclusion, y	Weight, ^a percentile	Length/height, ^a percentile	Mutation (NM_000252.2)	Predicted effect	Ambulatory status	Breathing support	Feeding support	AAV8 inhibitory titer
42 ^b	18	6.9	43.6	c.141_144delAGAA	p.(Glu48Leufs*24)	NA	IV: 24 h	PEG	1.96
43 ^b	19.1	5.9	<1	c.520delT	p.(Arg174Glyfs*12)	NA	IV: 24 h	PEG	UNK
44 ^b	19.7	<1	17.1	c.721C>T	p.(Arg241Cys)	NA	NIV: 12 h	None	3.77
45 ^b	27	3.9	<1	c.232-26_232-23delGACT	Branch point mutation potentially interfering with exon 5 splicing	NA	IV: 24 h	NGT	<1

Abbreviations: A = ambulant; AAV8 = adeno-associated virus type 8; IV = invasive ventilation; NA = nonambulant; NGT = nasogastric tube; NIV = noninvasive ventilation; PEG = percutaneous endoscopic gastrostomy; UNK = unknown.

^a Weight, length, and height in percentiles were computed by different calculators: for those younger than 2 years of age, we used infantchart.com/ based on the "WHO Child Growth Standards." For those between 2 and 20 years of age, we used cdc.gov/growthcharts/ based on the National Health and Nutrition Examination Survey data. Hyattsville, MD: US Department of Health and Human Services, Centers for Disease Control and Prevention, 2008. Patients older than 20 years of age were considered to be 20 for the purpose of these calculations.

^b Followed over 12 months.

^c Consequence should be a start codon loss with no protein production, but a residual amount of protein could be produced from an alternate initiation codon.

^d The exact nucleotide and amino acid numbers spanning the deletions were not indicated because of the absence of break point determination.

Genotype/phenotype correlation

The 45 mutations detected were distributed throughout the *MTM1* gene. Mutations were mainly missense (53.3%) and in-frame small deletions (8.9%) that may be compatible with a residual normal function of the protein. Those mutations represent 90% of the mutations in the mild and intermediate groups but only 40% in the severe group. The remaining 37.8% of mutations are predicted to result in reduction or absence of functional protein (frameshift, nonsense, splicing, in-frame exon deletion, large deletion, and start codon loss) and were mainly present in the severe group (figure 1B).

History of the disease

Hypotonia and respiratory distress were common at birth (43/45 and 41/45), requiring breathing support in 82% of patients and leading to long hospital stays during the first year of life. The median hospital stays were 22, 75, and 142 days for the mild, intermediate, and severe groups, respectively. Breathing support duration at birth was longest in the severe group. Nevertheless, independent breathing occurred in 31 of 45 patients shortly after birth and was secondarily lost in 9 patients in the severe group at the median age of 6 months (figure 4).

Motor milestones acquired by most of the patients included the ability to bring hand to mouth, independent head control, and the abilities to roll on one side and to sit independently when placed in a sitting position. Milestone acquisitions were delayed and previously acquired milestones were secondarily lost mostly in patients with the severe phenotype (figure 4).

In addition, 30 patients reported at least one episode of lower respiratory tract infection. Physicians reported contracture in 25 patients, areflexia in 13, high arched palate in 11, and chest dysmorphism in 5 patients. Severe patients were often affected by cryptorchidism (17/25) and phimosis (5/25). Three patients reported cholelithiasis and 5 nephrolithiasis. Conduction and heart rhythm abnormalities were reported in 5 and 7 patients, respectively. Seven had abnormal heart structure (ventricular hypertrophy, patent foramen ovale, and ventricular septal defect). Episodes of abnormal bleeding were reported in 7 patients, abnormal clotting in 2 patients, anemia in 9 patients, and thrombocytopenia and hypertension in 5 patients. Hepatomegaly was observed in 4 patients; steatosis, hyperechogenicity, and hepatic cysts in 3 patients; and portal and hepatic vessel modification in one patient. No intellectual disabilities were reported.

Disease burden

A feeding tube was used by 82% of patients (table 1). Sixty-nine percent of patients older than 2 years of age were non-ambulant and required assistive devices such as manual or powered wheelchairs, standers, orthotics, or splints. Sixty-eight percent of patients with scoliosis needed a corset or a lumbar belt. The number of hospitalizations normalized by the life-year number varied between 0.1 and 4.2 with a median number of 0.8. The total duration of hospitalization was

Table 2 Clinical characteristics at baseline

	All (n = 45)		Mild (n = 13)		Intermediate (n = 7)		Severe (n = 25)		Phenotype effect, <i>p</i> value ^a
	No.	Median (min–max)	No.	Median (min–max)	No.	Median (min–max)	No.	Median (min–max)	
Age, y	45	6.6 (0.3–56.8)	13	12.6 (0.8–56.8)	7	5.0 (0.5–50.8)	25	5.2 (0.3–27.0)	0.361
Height, percentile	45	72.6 (<1–>99)	13	50.1 (<1–92.5)	7	72.7 (9.8–96.4)	25	80.0 (<1–>99)	0.350
Weight, percentile	45	19.3 (<1–98.3)	13	19.2 (<1–98.3)	7	6.0 (<1–92.9)	25	22.9 (<1–89)	0.820
Creatinuria, mg/kg/24 h	21	3.1 (0.1–22.3)	6	10.6 (5–22.3)	2	6.9 (3.5–10.4)	13	1.7 (0.1–3.9)	0.001 ^b
Diastolic BP, mm Hg	43	61 (10–104)	13	59 (10–79)	6	62 (52–80)	24	65 (46–104)	0.428
Systolic BP, mm Hg	43	103 (81–147)	13	110 (84–147)	6	103.5 (88–123)	24	102.5 (81–142)	0.691
Heart rate, bpm	43	107 (58–155)	12	111.5 (58–138)	6	107.5 (66–148)	25	105 (70–155)	0.978
Respiratory rate, bpm	39	25 (14–70)	11	22 (14–37)	5	24 (15–70)	23	27 (14–48)	0.502
Hours on ventilation	31/31	24 (8–24)	—	—	7	10 (8–10)	24	24 (12–24)	—
No. of respiratory infections/y	32/32	1 (1–6)	9	2 (1–6)	5	2 (1–5)	18	1 (1–4)	0.106
FVC, %	12/15	33.35 (6.3–61.9)	8/9	44.4 (30.4–61.9)	3/3	25.3 (18.4–26.5)	1/3	6.3	0.022 ^b
FEV ₁ , %	12/15	37.7 (6.8–62.3)	8/9	40.3 (27.6–62.3)	3/3	18.9 (16–28.1)	1/3	6.8	0.033 ^b
MEP, %	13/15	25.1 (11.7–76.7)	8/9	30.8 (18.3–76.7)	3/3	23.0 (13.5–54.6)	2/3	12.3 (11.7–13.0)	0.065
MIP, %	13/15	34.0 (4.9–83.9)	8/9	42.8 (0.2–83.9)	3/3	22.3 (21.9–45.7)	2/3	11.7 (4.9–18.5)	0.055
PCF, %	12/15	35.6 (21.8–61.3)	8/9	45.1 (30.7–61.3)	3/3	26.0 (23.0–26.1)	1/3	21.8	0.022 ^b
Grip strength, %	19/24	21.7 (0.4–97.7)	9/9	24.4 (4.8–97.7)	3/3	53.1 (17.1–61.7)	7/12	4.6 (0.4–25.4)	0.016 ^b
Pinch strength, %	21/24	25.6 (0.6–105.7)	9/9	45.2 (8.7–105.7)	3/3	74.4 (34.7–84.0)	9/12	7.1 (0.6–47.3)	0.010 ^b
MF20 total, %	9/9	28.3 (16.7–66.7)	2/2	65.8 (65.0–66.7)	2/2	29.2 (26.7–31.7)	5/5	25.0 (16.7–33.3)	0.102
MF32 total, %	22/24	31.3 (0–93.8)	9/9	69.8 (47.9–93.8)	2/3	39.1 (22.9–55.2)	11/12	15.5 (0–31.25)	0.000 ^b
MoviPlate, n	19/24	51 (11–108)	9/9	66 (31–108)	3/3	68 (65–71)	7/12	33 (11–64)	0.030 ^b
CHOP-INTEND, n	12/12	35.5 (20–47)	2/2	41 (40–42)	2/2	37 (33–41)	8/8	27.5 (20–47)	0.452
MHFMS, n	21/21	2 (0–32)	4/4	13.5 (2–32)	4/4	2 (0–5)	13/13	1 (0–6)	0.027 ^b
NSAA, n	8/9	18 (6–32)	8/8	18 (6–32)	0/1	—	—	—	—
6MWD, m	9/9	302 (185–644)	8/8	351.5 (185–644)	1/1	235	—	—	—

Continued

Table 2 Clinical characteristics at baseline (continued)

	All (n = 45)			Mild (n = 13)			Intermediate (n = 7)			Severe (n = 25)			Phenotype effect, p value ^a
	No.	Median (min-max)		No.	Median (min-max)		No.	Median (min-max)		No.	Median (min-max)		
Time to run/walk 10 m, s	6/9	7.4 (2.5–10.9)		6/8	7.4 (2.5–10.9)		0/1			—			—
Time to rise from floor, s	2/9	3.4 (2.1–4.7)		2/8	3.4 (2.1–4.7)		0/1			—			—

Abbreviations: BP = blood pressure; CHOP-INTEND = Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders; FEV₁ = forced expiratory volume in the first second of exhalation; FVC = forced vital capacity; MEP = maximum expiratory pressure; MFM = Motor Function Measure; MHFMS = Modified Hammersmith Functional Motor Scale; MIP = maximum inspiratory pressure; NSAA = North Star Ambulatory Assessment; PCF = peak cough flow; 6MWD = 6-minute walk distance.
^a Kruskal-Wallis Test.
^b Significant.

higher in more severely affected patients but the difference did not reach statistical significance (median durations of 89, 120, and 154 days in mild, intermediate, and severe phenotypes, respectively). During the 1-year follow-up, 37 patients reported between 1 and 11 adverse events, and 37 patients reported using concomitant treatment (median number of 5).

Patient Reported Outcomes Measurement Information System T scores for emotional distress, fatigue and pain interference averaged 80% of that of the general population. It was surprising that mild adult patients appeared more affected than other groups. Except for 2 ambulant children, patients reported a severe impairment in physical function, although social functions were within normal limits or mildly or moderately affected (data available from Dryad, figure e-1, doi.org/10.5061/dryad.c392v5q). Patients reported a median number of 2 social events missed because of the disease with some severe patients reporting up to 30.

Clinical changes over 1 year

We focused on changes in upper limb strength and motor and pulmonary function using standardized assessments. Forty patients completed their 12-month visit. Three of the 12 patients enrolled before the age of 2 years died during the first year of the study due to cardiorespiratory arrest for 2 severe patients and probable sepsis for the third intermediate patient. Despite variability between patients, pulmonary function scores were stable over the 1-year period with standardized response means lower than 0.3 (figure 2 and table 3). The most reproducible tests were the FVC and MEP.

Grip and pinch strengths were numerically decreased over the year in the intermediate and severe groups, but changes did not reach statistical significance (figure 3A and table 3). Functional scales MFM20 and MHFMS were stable in the infant group. MFM32 significantly decreased by 2.3 points over 1 year (figure 3B and table 3). Timed tests performed in ambulant patients were stable over 1 year but we observed a large variability between patients in the 6-minute walk distance (table 3).

No changes were observed in physical examination and ophthalmoparesis. The degree of respiratory support did not change appreciably over 1 year except in 2 patients: one from the mild group who required nighttime respiratory support 12 months after enrollment, and the other from the severe group who changed ventilation from invasive to noninvasive. Similarly, the feeding status was stable over 1 year, and no specific changes in liver abnormalities were noted except in 2 children with a mild phenotype who changed from upper limit normal to hepatomegaly status. Lastly, there was no indication that patients treated with pyridostigmine had different outcomes.

Seroprevalence of anti-AAV8 neutralizing antibodies

Anti-AAV8 neutralizing activity (≥1:1) was detected in 10 of 38 available XLMTM serum samples, which represents

Figure 2 Pulmonary function

Pulmonary function in percentage of predicted value for age at baseline and 12-month visit. FEV₁ = forced expiratory volume in the first second of exhalation; FVC = forced vital capacity; MEP = maximum expiratory pressure; MIP = maximum inspiratory pressure; PCF = peak cough flow.

a prevalence of 26.3% (table 1). Among those 10 samples, 2 were associated with an inhibitory titer greater than 1:10; these samples were from 1.7- and 50.8-year-old patients. Total immunoglobulin G levels specific for AAV8 were not quantified.

Discussion

We report 1-year follow-up data regarding male patients with XLMTM aged from 3.5 months to 56.8 years evaluated by standardized assessments adjusted according to age and ambulatory and respiratory status. Of note, the MFM32 revealed a significant drop over 1 year, making it an attractive outcome measure for future clinical trials. Many additional outcomes (e.g., pulmonary function tests or grip and pinch strength) remained stable over the 1-year observation period.

Knowledge of the natural history of a disease is essential to build an effective clinical development program, especially when developing drugs to treat rare diseases like XLMTM. The Food and Drug Administration, in the 21st Century Cures Act, noted the importance of natural history in understanding the full spectrum of a disease, including the need for a description of genotypic and phenotypic manifestations and distinct subpopulations affected. In addition, natural history studies improve understanding of how the condition progresses and how it can be most effectively treated. In the

Duchenne field, the understanding of outcome measures and natural history available after the first large international pivotal trials underlined how clinical development is affected by the absence of good knowledge of the natural history. Indeed, drisapersen demonstrated clinical efficacy in a phase 2 trial conducted in younger and stronger patients,³⁴ but in phase 3, testing did not show efficacy in a more rapidly declining population.³⁵ In contrast, study of the natural history of patients with spinal muscular atrophy type 1³⁶ allowed a better appreciation of progress in an open label trial³⁷ and paved the way for a successful phase 3 trial. Gaining solid natural history data through a study that meets industry and regulatory standards is a strategy that was successfully used in patients with spinal muscular atrophy types 2 and 3,³⁸ Pompe disease,³⁹ and hypophosphatasia.⁴⁰

Our international natural history study in patients with XLMTM was designed to collect data using standardized procedures with quality matching interventional studies. We chose standardized assessments regularly used in clinical trials for neuromuscular disorders such as Duchenne muscular dystrophy and spinal muscular atrophy since no outcome measure is currently validated in XLMTM. We limited the number of evaluators by recruiting a dedicated physiotherapist who assessed half of the enrolled patients and carefully trained the other participating physiotherapists to ensure uniformity. In addition, all data were quality controlled by regular site visits.

Figure 3 Muscle strength and motor function

(A) Upper limb muscle strength and (B) motor function at baseline and 12-month visit. CHOP-INTEND = Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders; MFM = Motor Function Measure; MHFMS = Modified Hammersmith Functional Motor Scale.

Clinical characteristics of the population described here are consistent with previous cohorts described.^{7,10–12,41,42} Hypotonia and respiratory distress at birth were common, leading to long hospital stays during the first years of life. A quarter of patients enrolled before 2 years of age did not survive the first year of the study, which is consistent with data found in one study¹⁰ but less than half the number reported in other retrospective studies.^{7,11,41} This difference may be explained by the fact that we enrolled living patients and may also reflect improvements in standard of care and more aggressive treatment in the neonatal period. As expected, long-term survivors were often nonambulant and ventilator-dependent and feeding tube-dependent. Nevertheless, ambulation was conserved in 29% of patients enrolled at over 2 years of age. Motor milestone acquisitions were generally delayed regardless of phenotype. Comorbidities were similar to those previously described^{7,10,12,42} with a predominance of genitourinary disorders, facial muscle weakness, frequent lower respiratory tract infections,

and scoliosis, all of which were more prevalent in the most severe patients.

One of the main findings of this study is that XLMTM is clearly progressive. Contrary to observations made by others,¹² our data establish that substantial motor milestones previously acquired were lost by several patients including those mildly affected. In addition, motor function assessed consistently by the general function scale MFM32 decreased significantly over 1 year in long-term survivors older than 6 years. It must be noted that a 2-point decrease in MFM may not be clinically relevant. Over a longer term, however, the retrospective assessment of motor development clearly demonstrates that major function may be lost during the life of a patient with XLMTM, even if years of observation are needed before changes are clinically meaningful. Standardized pulmonary function test, motor function test other than MFM32, and upper limb strength test scores remained stable over 1 year, confirming the very slow progression of the disease.

Figure 4 Motor milestones

Median ages of acquisition and loss of motor milestones. Ages are expressed in months.

The fact that some patients lost previously acquired motor milestones suggests that XLMTM disease progression presents as do many neuromuscular diseases, such as Duchenne muscular dystrophy and spinal muscular atrophy type 3, with an initial period of positive evolution followed by a progressive loss of muscle strength and motor function. The maximal ability achieved and the timing of decline differs among patients, ranging from nearly no motor acquisition to full motor development. The major determinant of this evolution is certainly the genotype. Genotype-phenotype correlation is consistent with the previous observation that some mutations are expected to be associated with a mild or intermediate phenotype, probably because of a residual function of the mutated myotubularin, whereas complete loss of function is linked to the most severe phenotype.⁴³ In addition, a mutated amino acid may be a predictor of severity when located in a functional domain of the protein. Indeed, missense mutations that alter the phosphatase consensus active site (PTP) in exon 11 or mutations located in the nearby SET interaction domain (SID domain) in exons 12 and 13 are mainly

associated with the severe phenotype.⁴⁴ In our study, duplications and nonsense mutations in exons 12 and 13 were indeed associated with a severe phenotype, but 2 missense mutations and one nonsense mutation in exon 11 were associated with a mild phenotype. Mutations could induce an abnormal protein structure that is not obviously predicted. Point mutations may act as missense mutations or may affect splice sites or exonic splicing enhancers or silencers that are important for splicing modulation. Indeed, the mutation c.205C>T, which is predicted to lead to the missense p.Arg69Cys, may also alter splicing patterns and lead to variation in the amount of residual function of the mutated protein among patients, explaining the associations with mild and severe phenotypes.⁴⁵

Despite the population heterogeneity, our study provides some data on potential outcome measures applicable for analyzing the effects of future therapeutics. The MFM32 scale was sensitive to change over 1 year, and thus may constitute a suitable outcome for therapeutics that aim to stabilize patients. The MFM32 scale was particularly

Table 3 Changes over 1 year

Median changes over 1 y ^a	All			Mild			Intermediate			Severe		
	No.	Median (min to max)	SRM	No.	Median (min to max)	SRM	No.	Median (min to max)	SRM	No.	Median (min to max)	SRM
FVC, %	11	0.1 (–23.9 to 8.9)	0.29	8	0.8 (–23.9 to 8.9)	0.21	2	–5.5 (–9.0 to –2.0)	—	1	6.3	—
FEV₁, %	11	–1.4 (–13.5 to 14.2)	0.18	8	–1.3 (–13.5 to 14.2)	0.05	2	–6.2 (–10.1 to –2.2)	—	1	0.4	—
MEP, %	12	–1.2 (–6.2 to 14.2)	0.04	8	–2.0 (–5.4 to 14.2)	0.07	2	–2.5 (–6.2 to 1.2)	—	2	–0.9 (–1.2 to –0.6)	—
MIP, %	12	2.3 (–29.6 to 47.1)	0.24	8	6.8 (–29.6 to 47.1)	0.28	2	0.3 (–7.3 to 7.9)	—	2	0.5 (–1.5 to 2.5)	—
PCF, %	11	–1.4 (–14.6 to 9.2)	0.23	8	–1.8 (–14.6 to 9.2)	0.20	2	–2.2 (–5.3 to 0.9)	—	1	0.0	—
Grip strength, %	17	0.0 (–24.4 to 13.5)	0.09	9	0.6 (–5.8 to 13.5)	0.31	2	–12.7 (–24.4 to –1.0)	—	6	–0.6 (–1.9 to 3.4)	0.12
Pinch strength, %	19	0.0 (–26.2 to 16.3)	0.15	9	1.6 (–7.8 to 16.3)	0.35	2	–10.2 (–26.2 to 5.8)	—	8	–2.1 (–9.4 to 1.0)	0.78
MFM20 total, n	8	6.7 (–15 to 11.7)	0.41	2	3.3 (–1.7 to 8.3)	—	2	5.8 (0 to 11.7)	—	4	7.5 (–15 to 11.7)	0.24
MFM32 total, n	19	–2.3 ^b (–9.4 to 5.2)	0.68	9	–2.1 (–9.4 to 5.2)	0.53	1	–5.2	—	9	–2.3 (–6.3 to 1.0)	0.73
MoviPlate, n	16	3 (–30 to 15)	0.13	9	9 (–30 to 15)	0.26	2	–1 (–5 to 3)	—	5	–2 (–9 to 3)	0.32
CHOP-INTEND, n	3	3.0 (–12 to 4)	0.19	1	4.0	—	1	3.0	—	1	–12.0	—
MHFMS, n	13	0.0 (–3 to 6)	0.46	4	4.5 (4 to 8)	2.77	2	0.0 (0 to 0)	—	7	0.0 (–3 to 4)	0.13
NSAA, n	9	1 (–4 to 7)	0.27	8	1 (–4 to 7)	0.38	1	–2	—	—	—	—
6MWD, m	8	9 (–122 to 56.5)	0.01	7	25 (–122 to 56.5)	0.01	1	–7	—	—	—	—
Time to run/walk 10 m, s	7	0 (–0.9 to 0.3)	0.38	7	0 (–0.9 to 0.3)	0.36	—	—	—	—	—	—
Time to rise from floor, s	2	0.7 (0.1 to 1.3)	—	2	0.7 (0.1 to 1.3)	—	—	—	—	—	—	—

Abbreviations: CHOP-INTEND = Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders; FEV₁ = forced expiratory volume in the first second of exhalation; FVC = forced vital capacity; MEP = maximum expiratory pressure; MFM = Motor Function Measure; MHFMS = Modified Hammersmith Functional Motor Scale; MIP = maximum inspiratory pressure; NSAA = North Star Ambulatory Assessment; PCF = peak cough flow; 6MWD = 6-minute walk distance; SRM = standardized response mean.

^a Wilcoxon test.

^b $p < 0.05$.

interesting for the adult population in whom growth is not a confounding factor.

Pulmonary function tests established that the pulmonary function of patients with XLMTM was abnormal even in ventilator independent patients, which is consistent with previously published data.⁴⁶ The stability over 1 year and the universally decreased respiratory function among phenotypes make pulmonary function testing a suitable outcome for therapeutics aiming to improve patient respiratory status. The same applies for MyoGrip and MyoPinch, which could be used in most patients and for which no ceiling effect was observed.

Since gene therapy using an AAV8 is under development for myotubular myopathy^{15,47} and currently tested in a clinical trial (NCT03199469), we monitored the levels of neutralizing antibodies against AAV8 in patients as humoral immunity may jeopardize efficient gene transfer. We found that about 25% of patients had detectable levels of neutralizing antibodies and only 2 patients had high inhibitory levels, meaning that at least 5% of patients (for a threshold titer of 1:10) would not be treatable by an AAV8-based gene therapy. These seroprevalence values were lower than those observed in patients affected by Duchenne muscular dystrophy,⁴⁸ probably because of the lower mean age of patients in our cohort.

Our study has some limitations. First, patients were highly heterogeneous leading to the need to stratify the population for analyses resulting in small and unbalanced sample sizes and therefore the necessity of using non-parametric statistical analyses that are highly conservative. This limitation is intrinsic to this disease and prospectively following a much larger cohort would be very difficult since this cohort was gathered through a large international effort. Second, we observed high variability between and within patients over 1 year, potentially masking a general trend. This may reflect the variability of the disease evolution between patients as some patients are in their decline phase, whereas others are still progressing. Alternatively, it may reflect the poor reliability of measures performed in very weak patients or a learning effect. In the protocol, we balanced test and retest assessments against fatigue, which is a well-known symptom in patients with XLMTM who present with neuromuscular junction symptoms.^{49,50} Follow-up for a longer time period will help address this issue. Another limitation is that pulmonary function was not assessed in patients with tracheostomy. Consequently, it was assessed in only a few severe patients, thus reducing the sample size for accurate comparison. Lastly, the cohort was skewed toward moderate and mild patients, but most studies suggest that 80% of all patients with XLMTM have the severe form. This is probably attributable to the design of the study. Indeed, the study aimed to include all living patients with a genetically confirmed diagnosis of XLMTM. Since diagnosis

of XLMTM is frequently delayed, many patients are beyond the neonatal period when confirmatory genetic testing is performed and some severe patients died before receiving a molecular diagnosis. In addition, we did not declare neonatal intensive care units as investigational sites and patients requiring intensive care were likely not seen in declared neuromuscular centers and enrolled in the natural history study unless the intensive care unit was located in the same hospital. This study should thus be considered as a natural history study of patients surviving beyond the neonatal period, since very few patients in the neonatal intensive care unit were included. In this view, this analysis is complementary to the Inceptus study (NCT02704273) that covers younger patients with respiratory involvement. Our analysis demonstrated the slow progressive nature of the disease and identified disease burden and outcome measures suitable for clinical trials in male patients with XLMTM who survive the neonatal period.

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Disclosure

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Appendix (continued)

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