Journal of Neurology (2021) 268:923–935
https://doi.org/10.1007/s00415-020-10223-9

ORIGINAL COMMUNICATION

Nusinersen treatment significantly improves hand grip strength, hand motor function and MRC sum scores in adult patients with spinal muscular atrophy types 3 and 4

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Received: 15 July 2020 / Revised: 30 August 2020 / Accepted: 8 September 2020 / Published online: 15 September 2020

Abstract

Background Nusinersen recently became available as the first treatment for Spinal Muscular Atrophy (SMA) and data on its effectiveness and safety in adult SMA patients are still scarce.

Methods We evaluated the effectiveness and safety of nusinersen treatment during 14 months in 16 adult patients with SMA types 3 and 4 in a prospective study, and retrospectively detailed the natural history of 48 adult SMA patients types 2, 3 and 4.

Results Hand grip strength ($p=0.03$), hand motor function ($p=0.04$) as assessed by a sub-score of the Revised Upper Limb Module (RULM) and the Medical Research Council (MRC) sum score ($p=0.04$) improved significantly at month 14. Importantly, the MRC sum score had declined significantly ($p<0.01$) prior to start of treatment in these patients. A minimal clinically important difference (MCID) in the Hammersmith Functional Motor Scale Expanded (HFMSE) and RULM scores was achieved in 31% and 50% of the patients, respectively, but the mean changes from baseline failed to reach significance. Forced Vital Capacity (FVC) transiently increased at month 6 ($p=0.01$), whereas the Peak Expiratory Flow (PEF) did not. The Activity Limitations scale declined significantly prior to start of treatment ($p<0.01$) and showed an improvement with nusinersen which was not significant. The safety evaluation did not reveal serious adverse events and no signs of nephrotoxicity or antisense oligonucleotide (ASO)-mediated inflammation.

Conclusions We conclude that hand grip strength and hand motor function, as well as MRC sum scores improved significantly in nusinersen-treated adult patients with SMA types 3 and 4.

Keywords SMA · Spinraza® · Muscle strength · ActivLim · Outcome measures · Natural history

Abbreviations

SMA	Spinal Muscular Atrophy
SMN	Survival of motor neuron protein
FAGG	Belgian Federal Agency for Medicines and Health Products
CT	Computed Tomography
MRC	Medical Research Council
FVC	Forced Vital Capacity
PEF	Peak Expiratory Flow
6MWD	Six-minute walk distance

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CPAP	Continuous positive airway pressure
OSAS	Obstructive sleep apnoea syndrome
HFMSE	Hammersmith Functional Motor Scale Expanded
RULM	Revised Upper Limb Module
SF-36	36-Item Short Form Health Survey
ActivLim	Activity Limitations scale
PROM	Patient-reported outcome measure
CK	Creatine kinase
MCID	Minimal clinically important difference
PPH	Post-puncture headache

Introduction

Spinal Muscular Atrophy (SMA) is a rare, progressive, autosomal recessive, hereditary neuromuscular disease affecting motor neurons in the spinal cord, leading to denervation and consequent loss of skeletal muscle function and atrophy. SMA encompasses a wide spectrum of severity and is classified into types 1 through 4 according to age of symptom onset and achievement of motor milestones [1]. SMA type 1 is characterized by symptom onset in the first 6 months of life and never achieving the sitting position, whereas in SMA type 2 symptoms start later, up to 18 months, and the motor milestone of sitting upright unsupported ('sitters') is reached. SMA type 3 patients are often referred to as 'walkers' and experience first symptoms between 18 months and early adulthood, whereas symptoms in SMA type 4 are usually milder and start after the age of 30 years [2]. SMA is caused by a homozygous deletion of the *SMN1* gene (90%), or a combination of a deletion and an afunctional hybrid *SMN1-SMN2* gene (5%), or *SMN1* point mutations (4%), leading to reduced levels of survival of motor neuron (SMN) protein [3]. The SMN protein has an important role in the maintenance of motor neurons and is produced mainly by the *SMN1* gene and only for 10–15% by the *SMN2* gene [4]. *SMN2* differs from *SMN1* by a single nucleotide in exon 7, leading to predominant skipping of exon 7 in *SMN2* and the production of a dysfunctional SMN protein [5].

Recently, nusinersen became available as a first treatment for SMA [6]. Nusinersen is an intrathecally injected antisense oligonucleotide (ASO) that binds to exon 7 of *SMN2*, thereby correcting *SMN2* exon 7 splicing and increasing production of functional SMN protein [7]. The efficacy of nusinersen has initially been demonstrated in SMA type 1 infants of maximum 7 months of age at treatment initiation and children with SMA types 2 and 3 between the ages of 2 and 9.5 years old at first dose [8, 9]. Very recently, functional improvements have also been reported in adult SMA patients types 2, 3 and 4 treated with nusinersen in two studies [10, 11]. However, the studied patient populations differed, another set of outcome measures was used and the results of both studies did not completely coincide. Furthermore, some reported improvements were only transient and certain relevant outcome measures were not included in these studies.

Here, we prospectively evaluated the effectiveness of nusinersen treatment in a cohort of adult patients with SMA types 3 and 4 and studied, besides similar outcome parameters, several additional outcome measures, such as bilateral hand grip strength and patient-reported outcome measures (PROM). For comparative purposes, we also performed a retrospective analysis in SMA patients types 2, 3 and 4 from adolescence through adulthood. Based on our study results and previously available data on nusinersen-treated adult SMA patients, we suggest which outcome measures may be preferably used in future clinical trials in SMA adults. Finally, we conducted for the first time a comprehensive screening for ASO-mediated inflammation and nephrotoxicity in adult SMA patients treated with nusinersen.

Patients and methods

Patients and study design

We included 48 adult patients with genetically confirmed 5q SMA types 2, 3 and 4 in this study. The patients were followed at the Neuromuscular Reference Centre of the Neurology Department at University Hospitals Leuven, Belgium. The study contained a retrospective and a prospective part. We included all 48 patients in the retrospective study, with the objective to investigate the natural history of muscle strength, muscle function and respiratory function in adult SMA patients. Sixteen of these patients additionally participated in a prospective, observational study to examine the effectiveness and safety of intrathecal nusinersen (Spinraza®) treatment in adult SMA patients.

Prospective study

Inclusion criteria for the prospective study evaluating treatment with nusinersen were as follows: genetically confirmed 5q SMA, adult age at the time of enrolment (≥ 18 years), patients with SMA type 3 or 4, the use of an adequate contraceptive by female patients for the whole duration of the study and patients' written informed consent. We employed as exclusion criterion any (relative) contra-indication for lumbar puncture, such as a history of scoliosis surgery ($n = 20$). Patients with untreated scoliosis were allowed to participate. Four patients refused informed consent and five patients were deceased at the start of this study. After applying these criteria, 18/48 patients were potentially eligible. One of these patients was additionally excluded because of active alcoholism without motivation for abstinence. For

safety reasons, we performed a baseline brain Computed Tomography (CT) scan in the remaining 17 patients, which led to the exclusion of one more patient because of an incidentally diagnosed, hitherto unknown Chiari malformation type 1. Ultimately, 16 patients were included in the prospective study.

Treatment schedule and outcome variables

Patients received intrathecal administrations of 12 mg of nusinersen following the standard of care dosing schedule, for a study duration of 14 months. The study duration was chosen in analogy with previous reports to allow a more accurate comparison with these studies [10, 11]. We performed intrathecal injections by standard access, except in two patients with severe scoliosis. In these patients, we injected the medication under imaging control, consisting of a lumbar spine CT scan to localize the area for access, followed by radioscopy-guided intrathecal injection.

We measured selected outcome variables at baseline, month 6 and month 14 (Table 1). To evaluate muscle strength, we measured hand grip strength at both sides using a handheld dynamometer, and determined the 60-point Medical Research Council (MRC) sum score [12, 13]. The MRC sum score is a compound score (0–60 points) of strength in 6 individual muscle groups: shoulder abduction, elbow flexion, wrist extension, hip flexion, knee extension and foot dorsiflexion [14]. To monitor changes in skeletal muscle function, we assessed the six-minute walk distance (6MWD), Hammersmith Functional Motor Scale Expanded (HFMSE), Revised Upper Limb Module (RULM), Forced Vital Capacity (FVC) and Peak Expiratory Flow (PEF) [15–17]. For standardization purposes, each patient visit was scheduled in the late morning and outcome measures were assessed in the same order, starting with the MRC sum score. The lumbar puncture for nusinersen administration was performed after all outcome measures were assessed. The MRC sum score was assessed by the authors BDW or KGC. To correct for potential inter-rater variability in scoring the HFMSE and RULM tests, both were recorded on video and reassessed at the end of the study. There were two assessors for each test (AS and EVC for HFMSE; EG and GVK for RULM), who received training from the same experts prior to the start of the study. Pulmonary function tests were performed with Jaeger® spirometers by a group of trained professionals at the pulmonology department at UZ Leuven. All patients received the same instructions and only the best result of three consecutive measurements was noted. For measurements in sitting position, non-ambulatory patients were allowed to stay in their wheelchair and were positioned in front of the spirometer. Measurements in supine position in non-ambulatory patients were performed after transferring them onto a gurney. Reference values of the Global Lung Function Initiative were used (GLI 2012) [18]. The 6MWD was also performed by trained personnel at the pulmonology department, who have extensive experience with this test.

Furthermore, we studied two PROM's: the Rasch-built measure of Activity Limitations (ActivLim) scale and the 36-Item Short Form Health Survey (SF-36) [19, 20]. The first allows patients to assess their own functional status and the latter their quality of life. Finally, we measured the serum creatine kinase (CK) level at every visit to evaluate its potential as a serum biomarker.

Table 1 Overview of selected outcome measures in the prospective study

Outcome variables	Baseline	Month 6	Month 14
Handgrip strength left and right	x	x	x
MRC sum score	x	x	x
6MWD	x	x	x
HFMSE	x	x	x
RULM	x	x	x
Spirometry (FVC and PEF, sitting and supine)	x	x	x
ActivLim	x		x
SF-36		x	x

MRC medical research council, *6MWD* six-minute walk distance, *HFMSE* Hammersmith functional motor scale expanded, *RULM* revised upper limb module, *FVC* forced vital capacity, *PEF* peak expiratory flow, *ActivLim* activity limitations scale, *SF-36* 36-item short form health survey

Safety

At every visit, we examined blood and urine samples and vital parameters (blood pressure, heart rate and temperature) and assessed a custom-made patient-reported questionnaire for side effects of nusinersen treatment. We assessed a complete blood count, electrolytes, liver tests, creatinine and eGFR in blood and protein in urine samples. At month 6 we also measured antinuclear antibodies (ANA), antineutrophil cytoplasmic antibodies (ANCA) and complement factors in serum to screen for ASO-mediated inflammation [21]. To further screen for potential ASO-related nephrotoxicity, we performed a renal ultrasound with measurements of cortical thickness and overall size, cystatin C in serum and urinary protein electrophoresis and immunofixation at month 6 [22–24].

Retrospective study

Besides the prospective study, we also performed a retrospective natural history analysis in all 48 SMA patients, including the following data from adolescence through

adulthood collected from the patients' medical files: age at symptom onset, age at genetic diagnosis, age and cause of death, family history, SMA type, ambulatory status, presence of contractures, MRC sum score, history of scoliosis surgery, comorbidities, genetic defects in the *SMN1* gene, copy number of *SMN2*, Cobb angles from spine radiographies, FVC and PEF. The assessment of the MRC sum score and pulmonary function tests were done according to the standard procedures in our centre, in the same manner as described for the prospective study (see above).

Statistical analysis

We used RStudio® Desktop (Open Source License, version 1.2.5001) for statistical analyses. Descriptive statistics are stated as averages (minimum–maximum) and percentages. We applied paired *t* tests for comparison of outcome variables between baseline and visits at months 6–14. If assumptions for normality were not met, non-parametric equivalents (Wilcoxon signed-rank test or sign-test) were applied. Linear Mixed Models were used to analyse retrospective longitudinal data, with patient identification as a random effect to avoid pseudoreplication. The same models were applied for the retrospective analysis of pre-treatment deterioration in patients in the prospective study as for the analysis of the retrospective study group. We modelled each retrospectively analysed variable (MRC sum score, FVC and ActivLim) against patient's age (included as a fixed effect). Only in post hoc analyses the additional variable of interest was added as a fixed effect each time. A multiple linear regression model was performed to assess the correlation of pulmonary function with spinal deformation and was calculated as FVC versus patient's age, Cobb angle and history of scoliosis surgery in one linear model. Significance level was determined at $\alpha = 0.05$.

Results

Demographic, genetic and clinical patient characteristics

Patient characteristics of both the prospective ($n = 16$) and retrospective ($n = 48$) study group are summarized in Table 2. Note that the 16 patients included in the prospective study are also included in the retrospective study.

The prospective study group consisted of ten males and six females, aged between 22 and 66 years, with a mean symptom onset at 6.5 years of age (range 1–30y). The average age at genetic diagnosis was 20.8 years (range 0–45y). Fourteen patients had SMA3 and two SMA4. *SMN2* copy numbers varied between 3 and 5. Seven patients still retained the ability to walk, with a mean six-minute walk distance (6MWD) of 296 m, and nine had lost ambulation, on average at the age of 24.6 years (range 10–48y). Four patients presented mild flexion contractures of the hip, knee or ankles.

In the retrospective study of all 48 SMA patients, five of them had died at ages between 27 and 63 years: the youngest died due to respiratory failure and the other four due to unknown causes. Eleven patients had a positive family history for SMA, two of them were siblings of which one was treated with nusinersen and the other refused treatment. All patients harboured a homozygous deletion of *SMN1*, except for two patients who had a deletion on one allele and a fused afunctional *SMN1/SMN2* chimere on the other, resulting in a clinical status equivalent to a homozygous deletion [3]. The latter two patients were only included in the retrospective study. Finally, we noted as comorbidities a high rate of one or more post-traumatic bone fractures (50%), osteopenia/osteoporosis (31.3%), depression (31.3%), sub-luxation of the hip (12.5%) and overactive bladder syndrome (10.4%).

Prospective study: nusinersen treatment effects in 16 adult patients with SMA types 3 and 4

Handheld dynamometry measurements showed significant improvements in hand grip strength in both hands at months 6 and 14, compared to baseline (Table 3). On average, hand grip strength in the left hand increased by 37% (2.3 kg) at month 6 and 30% (1.9 kg) at month 14. The right hand showed a similar evolution, with an increase of 35% (2.4 kg) at month 6 and 29% (2.0 kg) at month 14. All patients were right-handed and baseline strength measurements were significantly higher in the dominant hand, by on average 0.7 kg or 11% ($p = 0.01$).

MRC sum scores improved on average 2.4–2.5 points at months 6–14, respectively. Even though the improvement from baseline was similar at months 6 and 14, only the latter was significant ($p = 0.04$). This can be explained by missing data of the MRC sum score at month 6, which led to the fact that only 11/16 patients could be included in the 6-month analysis, whereas 15/16 patients could be included in the 14-month analysis.

In the seven ambulatory patients, the 6MWD increased by 5% (average of 16 m) at month 6, which failed to reach significance ($p = 0.06$). At month 14, the increase from baseline was only 2% (7 m on average).

The HFMSE increased 8% at month 6 from baseline (on average 2.1 points; with 8 patients numerically improving, 2 deteriorating and 6 remaining stable) and this increase was maintained at month 14. However, both improvements were not significant compared to baseline. The RULM slightly improved throughout the study, with an average increase from baseline of 3% at month 6 and 4% at month 14, but both failed to reach significance. Nine patients numerically improved and two deteriorated by month 6, whereas by

Table 2 Demographic, genetic and clinical patient characteristics in the prospective and retrospective study groups

Demographics	Prospective study group ($n=16$)	Retrospective study group ($n=48$)
Gender		
Male (*n*)	10 (62.5%)	22 (45.8%)
Female (*n)*	6 (37.5%)	26 (54.2%)
Age at symptom onset (years)	6.5 [range 1–30]	4.2 [range 0–30]
Age at genetic diagnosis (years)	20.8 [range 0–45]	20.2 [range 0–63]
Current age (years)	37.5 [range 22–66]	37.1 [range 20–66]
SMA type (*n*)		
2	0	15 (31.3%)
3	14 (87.5%)	30 (62.5%)
4	2 (12.5%)	3 (6.3%)
SMN2 copy number (n)		
2	0	5 (10.4%)
3	13 (81.3%)	34 (70.8%)
4	2 (12.5%)	6 (12.5%)
5	1 (6.3%)	1 (2.1%)
NA	0	2 (4.2%)
Current BMI (kg/m^2)	22.9 [range 16–35]	22.7 [range 13–48]
Non-ambulatory (*n*)	9 (56.3%)	38 (79.2%)
Age at loss of ambulation (years)[a]	24.6 [range 10–48]	19 [range 3–43]
Contractures (*n*)	4 (25%)	29 (60.4%)
Arthrodesis for scoliosis (*n*)	0	20 (41.7%)
Age at arthrodesis (years)	NA	11.5 [range 5–16]
NIV (*n*)[b]		
CPAP	3 (18.8%)	4 (8.3%)
BiPAP	0	5 (10.4%)

All adult SMA patients were included in the retrospective study, and 16 of those patients were also included in the prospective study

SMA spinal muscular atrophy, *n* number, *y* years, *SMN2* survival of motor neuron 2 gene, *BMI* body mass index, *NIV* non-invasive ventilation, *CPAP* continuous positive airway pressure, *BiPAP* bilevel positive airway pressure

[a]Age at loss of ambulation is calculated only in those patients who were ambulatory at some point in their lives (only SMA 3–4 patients)

[b]The reason for CPAP treatment was obstructive sleep apnoea syndrome (OSAS) in all cases. All patients treated with BiPAP suffered from hypoventilation (three were treated nightly, one only by day and one continuously)

month 14 ten patients improved and three deteriorated compared to baseline. Because the hand grip strength increased by about 30% in this study, we evaluated post hoc if the subdomains of the RULM related to hand function (tests C, D, G, H and I) also improved throughout the study [17]. These 5 tests encompass, as follows: drawing a path, picking up tokens, pushing a button light, tearing folded paper and opening a Ziploc container. A sub-score on a total of nine points was calculated with these results for each patient. We established a significant improvement of this sub-score at month 14 ($p=0.04$). This indicates that not only hand grip strength but also hand function improved with nusinersen treatment.

Pulmonary function, as measured by FVC and PEF, remained stable over the course of the treatment, both for measurements in sitting and in supine position. We calculated a minimal (0.12L) but significant improvement at month 6 ($p=0.01$) of FVC in sitting position, but not in supine position. However, this effect disappeared at month 14, when no difference with the baseline FVC could be detected anymore ($p=0.11$). We also compared baseline FVC and PEF in sitting and supine position, to determine if testing in both positions may be useful to follow up adult SMA patients. For FVC, no significant difference could be established. However, PEF was significantly lower (on average 0.44L) when measured in supine position ($p<0.01$).

Table 3 Treatment effect of nusinersen in adult SMA patients types 3 and 4

Outcome measures	Baseline values	6-month analysis		14-month analysis	
	Mean score ± SD	Mean score ± SD (difference vs. baseline; 95% CI)	*p* values	Mean score ± SD (difference vs. baseline; 95% CI)	*p* values
Hand grip strength (right) [Kg]	6.94 ± 8.84	9.38 ± 8.73 (2.44; 0.88–3.99)	**< 0.01**	9.00 ± 7.76 (2.06; 0.27–3.85)	**0.03**
Hand grip strength (left) [Kg]	6.25 ± 9.31	8.56 ± 9.40 (2.31; 0.76–3.87)	**< 0.01**	8.19 ± 8.78 (1.94; 0.18–3.69)	**0.03**
MRC sum score	36.9 ± 10.3	39.3 ± 9.60 (2.40; − 4.93–8.93)	0.55 *	39.4 ± 8.40 (2.53; 0.18–4.88)	**0.04**
6MWD [m]	296 ± 199	312 ± 203 (16; NA)	0.06	303 ± 211 (7; − 38.42–53.27)	0.71
HFMSE [score]	27.3 ± 19.8	29.4 ± 19.3 (2.1; NA)	0.11	29.4 ± 19.9 (2.1; NA)	0.31
RULM [score]	27.1 ± 8.10	28 ± 8.54 (0.90; NA)	0.10	28.2 ± 8.41 (1.06; − 0.79–2.91)	0.24
FVC [L]	4.03 ± 1.16	4.15 ± 1.20 (0.12; 0.03–0.21)	**0.01**	4.12 ± 1.20 (0.09; − 0.02–0.20)	0.11
PEF [L]	7.19 ± 1.91	7.28 ± 1.87 (0.09; − 0.21–0.39)	0.52	7.34 ± 1.94 (0.15; − 0.21–0.51)	0.39
ActivLim [logits]	− 1.80 ± 2.68	NA	NA	− 1.44 ± 2.59 (0.36; − 0.20–0.94)	0.18

Significant *p* values are marked in bold. For non-parametric tests no confidence interval on the difference from baseline is given. FVC and PEF results are in sitting position

*Due to missing MRC sum score data, at the 6-month analysis only 11/16 patients could be included and at the 14-month analysis 15/16. This explains why for a similar difference vs. baseline the *p* values for these analyses differ. For all other outcome measures there were no missing data

SMA spinal muscular atrophy, *SD* standard deviation, *CI* confidence interval, *MRC* medical research council, *6MWD* six-minute walk distance, *HFMSE* Hammersmith functional motor scale expanded, *RULM* revised upper limb module, *FVC* forced vital capacity, *PEF* peak expiratory flow, *ActivLim* activity limitations scale

Analysing the PROM's included in the study, the ActivLim score revealed an improvement of 0.36 logits at month 14, but this difference was not significant ($p = 0.18$). The SF-36 quality of life questionnaire comparison between months 6 and 14 (no baseline data available) showed a general stabilization, with a significant improvement in 'role limitations due to physical health' ($p = 0.02$), no change in 6 out of 8 sub-scores (physical functioning, energy/fatigue, emotional wellbeing, social functioning, pain and general health) and a significant deterioration in 'role limitations due to emotional problems' ($p = 0.02$).

Next, we evaluated the evolution of serum CK values at each administration of nusinersen, and no significant changes could be measured ($p = 0.67$).

Finally, we evaluated the effect of the range in severity in our patient population on the outcome, by comparing the improvements in ambulatory versus non-ambulatory patients in a sub-analysis. No significant differences in results were found for hand grip strength, HFMSE, RULM and ActivLim. However, the MRC sum score only improved significantly in the non-ambulatory sub-group at months 6 and 14 ($p = < 0.01$ and 0.02, respectively) and the FVC measurement in sitting position only improved significantly in the ambulatory sub-group at month 6 ($p = 0.04$).

Evolution of MRC sum score and FVC before treatment versus under treatment ($n = 16$)

Since no control group was available in this study, we compared patients' historical disease progression (before treatment) to their evolution under treatment, thereby making them their own control group.

Previous MRC sum score measurements were available for 14/16 SMA patients included in the prospective study. We observed an annual decline of 0.48 points on the MRC sum score between the ages of 18–64 years before treatment was started ($p < 0.01$). This deterioration is in sharp contrast with the significant improvement of MRC sum scores under nusinersen treatment noted in our study (Table 3; Fig. 1). Likewise, historical FVC data (from the age of 16 years onward) were available for 12/16 patients before start of treatment. However, no significant change of FVC was observed in adult SMA patients over time ($p = 0.44$), which correlates with the relative stability of FVC noted under treatment with nusinersen.

Natural history analysis of MRC sum score, FVC and ActivLim in all 48 adult SMA patients

In order to corroborate our results of historical disease progression in the 16 patients receiving nusinersen treatment, we retrospectively evaluated the natural disease progression in the entire set of 48 adult SMA patients types 2, 3 and 4 between the ages of 16–65 years.

First, we confirmed an annual deterioration of MRC sum score ($p < 0.01$). Non-ambulatory patients had a significantly lower MRC sum score at any age ($p < 0.01$), but loss of ambulation also did not alter the rate of deterioration with ageing. Additional post hoc analyses yielded the

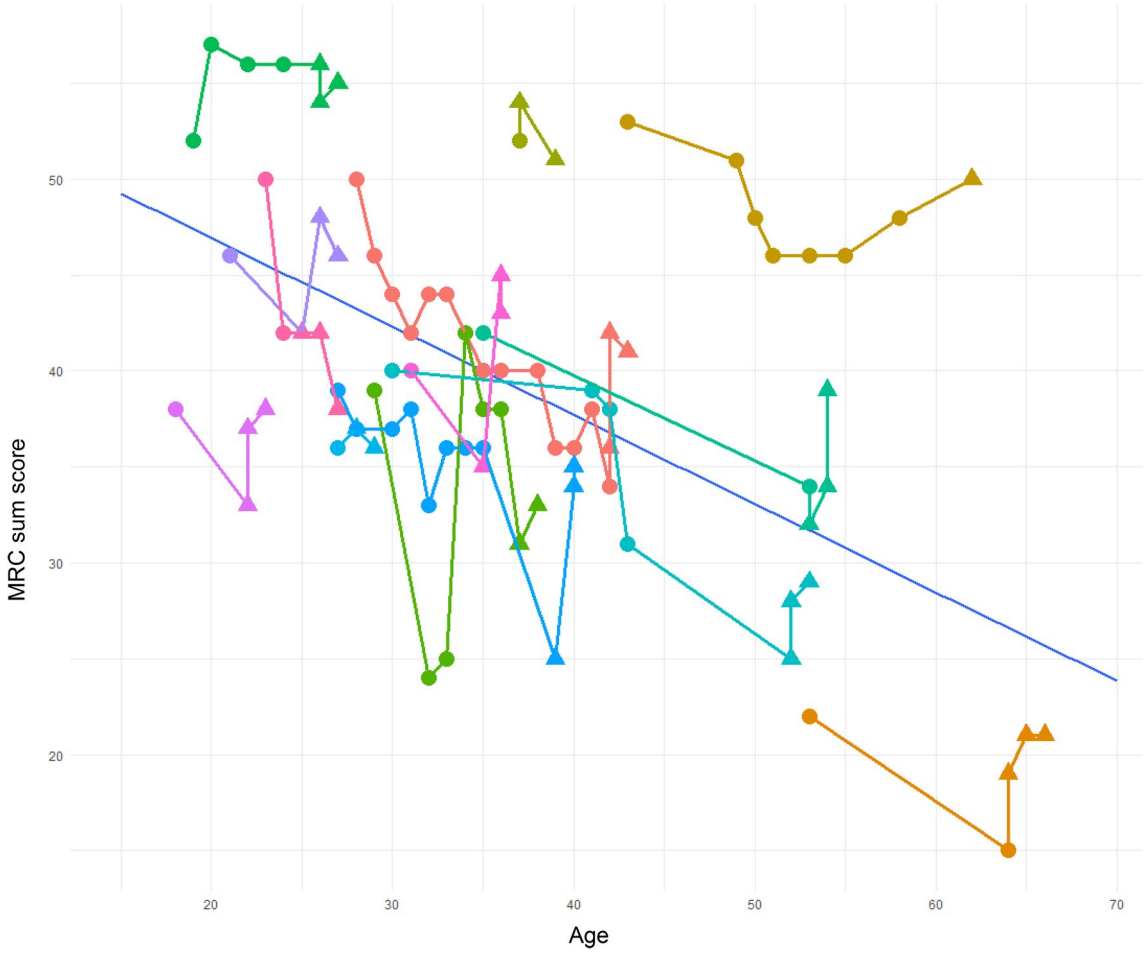

Fig. 1 Evolution of Medical Research Council (MRC) sum score with age. The 16 nusinersen-treated patients are each depicted by a different colour. Pre-treatment values are indicated by dots and values under treatment are shown by triangles. The blue regression line (calculated by a Linear Mixed Model of MRC sum score vs. age and a dummy variable for treatment) indicates the deterioration of the MRC sum score with ageing. Despite the significant increase of MRC sum score after start of treatment, this regression line still shows a deterioration because the positive effect of one year of treatment does not outweigh a lifetime of deterioration

following results: the rate of MRC sum score deterioration was not different for patients younger versus older than 40 years ($p=0.63$) nor was it influenced by patient's gender ($p=0.70$), patients with an *SMN2* copy number of 3, 4 or 5 had significantly higher MRC sum scores than those with two *SMN2* copies ($p=0.02$, $p<0.01$ and $p=0.04$, respectively) and patients with SMA type 3 or 4 had significantly higher MRC sum scores than SMA2 ($p<0.01$ for both).

Second, we confirmed that adult SMA patients types 2, 3 and 4 did not experience a significant decline of pulmonary function as measured by the FVC ($p=0.35$). However, we discovered a considerable variability in our dataset, with some patients remaining stable at relatively normal FVC values and others at notably lower FVC values (Fig. 2). In an attempt to explain this variability, we looked at the effect of ambulatory status, *SMN2* copy number and SMA type on patients' FVC in post hoc analyses. Patients who retained ambulation ($p<0.01$), patients with four *SMN2* copies ($p=0.03$, but not those with three copies) and patients with SMA types 3 and 4 ($p<0.01$ and $p<0.01$, respectively) all had a significantly higher FVC. However, when these three factors were corrected for each other, the SMA type was the only significant differentiating factor between normal and low FVC values (Fig. 2). Next, we evaluated the correlation of FVC with the degree of scoliosis measured by the Cobb angle at the patients' most recent spine X-rays. We established that an increasing severity of scoliosis is correlated with a significantly decreasing FVC ($p<0.01$). Additionally, patients with a spinal arthrodesis had a significantly lower FVC, irrespective of their remaining degree of scoliosis after surgery ($p<0.01$).

Finally, we evaluated longitudinal measurements of the ActivLim score in our cohort of adult SMA patients. This was scored annually in every patient since 2010. Repeated

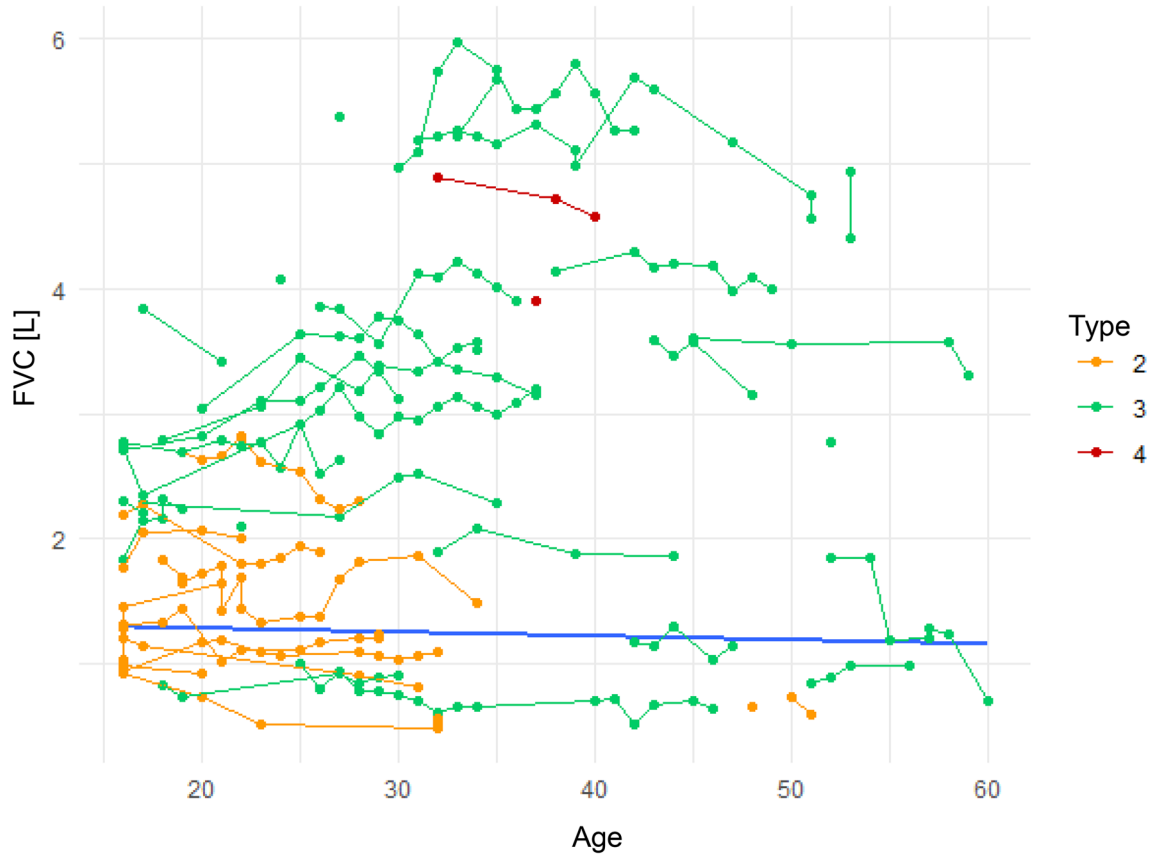

Fig. 2 Changes in Forced Vital Capacity (FVC) in relation to age. Data derived from the retrospective study. Historic FVC measurements were available in 42/48 patients. Each patient is represented by a separate line. The patients with SMA types 2 (orange), 3 (green) and 4 (red) are indicated in different colours. Patients with SMA types 2, 3 and 4 experience stable FVC values throughout their adult lives, indicated by the Linear Mixed Model prediction line in blue (modelled as FVC vs. age and SMA type), which showed a slightly decreasing but non-significant trend ($p = 0.262$). However, SMA type 2 patients consistently have a lower FVC, while SMA3 patients experience more variability, but frequently have a (quasi) normal FVC

measurements were available in 36/48 patients. The ActivLim score deteriorated significantly by 0.13 logits per year ($p < 0.01$).

Safety of intrathecal nusinersen treatment

In total, we administered 112 intrathecal nusinersen injections and no serious adverse events occurred (Table 4). None of the patients discontinued treatment. Blood pressure, heart rate and body temperature were monitored at every visit and did not show abnormalities for the duration of the study. The most frequently occurring side effect was back pain (64.3%), which endured for 3.6 days on average, with a 5/10 pain score on a visual analogue scale, and only requiring analgesics in 15.2%. Following 25% of the injections, patients reported headache, which was compatible with post-puncture headache in 9.8%: of a moderate to severe intensity, worsening in upright position and improving in supine position. Three of these cases (2.7%) resulted in a visit to the emergency department and subsequent treatment with a blood patch. In one of these three patients, a brain CT was performed, which did not show any abnormalities, including no communicating hydrocephalus [25]. Headache occurred notably more frequently in the first two months of the study, when intervals between lumbar punctures were shorter. A similar trend could be seen for an increased appetite and nausea, but not for the other adverse events, such as back pain. Patients reported an increased appetite more frequently (22.3%) than nausea (10.7%), even though the latter is a more well-known adverse effect of nusinersen treatment. Myalgia was reported in the limbs in 15 cases (13.4%) and diffusely in 2 cases (1.8%). All reported adverse events are listed in Table 4.

As expected, eGFR and creatinine measurements in blood were not very informative in these patients because of severe muscle atrophy, making it difficult to detect renal dysfunction (patients' average eGFR and creatinine were very high 138 ml/min/1.73m^2 and low 0.24 mg/dl, respectively).

Table 4 Adverse events after intrathecal nusinersen administrations in adult SMA patients

Adverse events	Baseline	Week 2	Week 4	Week 8	Month 6	Month 10	Month 14		Total
Back pain	10 (62.5%)	9 (65.3%)	9 (65.3%)	12 (75%)	10 (62.5%)	10 (62.5%)		12 (75%)	72 (64.3%)
Headache	6 (37.5%)	5 (31.3%)	5 (31.3%)	5 (31.3%)	3 (18.8%)	1 (6.3%)		3 (18.8%)	28 (25%)
PPH	3 (18.8%)	2 (12.5%)	2 (12.5%)	3 (18.8%)	1 (6.3%)	0		0	11 (9.8%)
Blood patch	1 (6.3%)	0	0	2 (12.5%)	0	0		0	3 (2.7%)
Fatigue	6 (37.5%)	6 (37.5%)	5 (31.3%)	8 (50%)	7 (43.8%)	7 (43.8%)		6 (37.5%)	45 (40.2%)
Increased appetite	5 (31.3%)	4 (25%)	4 (25%)	4 (25%)	4 (25%)	2 (12.5%)		2 (12.5%)	25 (22.3%)
Myalgia	1 (6.3%)	3 (18.8%)	1 (6.25%)	3 (18.8%)	3 (18.8%)	3 (18.8%)		7 (43.8%)	21 (18.8%)
Agitation	3 (18.8%)	5 (31.3%)	4 (25%)	2 (12.5%)	2 (12.5%)	3 (18.8%)		3 (18.8%)	22 (19.6%)
Nausea	3 (18.8%)	3 (18.8%)	2 (12.5%)	3 (18.8%)	1 (6.3%)	0		0	12 (10.7%)
Dizziness	1 (6.3%)	1 (6.3%)	1 (6.3%)	3 (18.8%)	2 (12.5%)	2 (12.5%)		0	10 (8.9%)
Proteinuria	0	1 (6.3%)	1 (6.3%)	1 (6.3%)	2 (12.5%)	1 (6.3%)		0	6 (5.4%)

PPH post-puncture headache

Cystatin C protein was measured in serum at month 6 in 14/16 patients as a more objective biomarker of renal function and revealed normal eGFRCysC ranges in all of them. Discretely lowered bicarbonate levels occurred transiently in 5/16 patients, potentially indicating proximal renal tubular acidosis (no urinary pH available), but recovered spontaneously. Proteinuria (> 0.20 g/L) was detected in two patients but was transient in both and did not coincide with lowered bicarbonate levels. Urinary protein electrophoresis was only possible in two patients because of the low rate of proteinuria and was normal. Urinary immunofixation (available in 13/16 patients) also did not display significant abnormalities. Renal ultrasounds were performed in 9/16 patients at month 6 and were normal. Based on these results we can conclude that none of the treated patients suffered from short-term nephrotoxicity. None of the patients had thrombocytopenia or hyponatremia. Finally, no clinical or biochemical arguments for ASO-mediated inflammation (potentially leading to vasculitis or glomerulonephritis) were noted; in particular, patients showed no rash and measurements of ANA, ANCA and complement factors revealed no significant abnormalities.

Discussion

We showed both effectiveness and safety of nusinersen treatment in 16 adult SMA patients types 3 and 4 in a prospective study over the course of 14 months. Hand grip strength improved significantly at both months 6 and 14 and a sub-score of the RULM pertaining to hand motor function improved significantly at month 14. Additionally, the 60-point MRC sum score improved after 14 months of treatment. Finally, no serious adverse events occurred and a comprehensive safety analysis including Cystatin C measurements and renal ultrasounds excluded renal toxicity in our patient cohort.

Previous studies of nusinersen treatment in adult SMA patients and gaps

Very recently, two other prospective nusinersen treatment studies in adult SMA patients were published [10, 11]. Walter et al. studied 17 adult SMA type 3 patients and established significant improvements in 6MWD and RULM at month 10 and a transient significant improvement of Peak Cough Flow (PCF) at month 6. The HFMSE, a non-classic MRC score (different than the classic 60-point MRC sum score), FVC and Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS) did not improve significantly [10]. Important to note is that not all patients completed all the evaluations at month 10. For example, the HFMSE and RULM scores were collected in only 12 and 16 patients, respectively, at this final time point in the study of Walter et al. [10, 11] Hagenacker and colleagues evaluated only the HFMSE, RULM and 6MWD, and reported significant improvements in all three outcome measures after 14 months of treatment in 57 adult patients with SMA types 2, 3 and 4 [11]. In these studies, hand strength and hand function were not evaluated and screening for nephrotoxicity beyond serum creatinine measurements was not performed.

In the randomized controlled CHERISH trial, the authors compared intrathecal nusinersen treatment versus a sham procedure in children with SMA type 2. They found a significant increase of HFMSE scores in the treated group, compared to a decrease in scores in the sham group, which was also further discussed in a recent Cochrane review of randomized controlled trials evaluating treatments for SMA

[9, 26]. In contrast to this earlier study in SMA2 children, both previous studies in adult populations, as well as the current study, did not use control groups to assess the efficacy of nusinersen treatment, although a randomized controlled trial would provide superior evidence and justification of this costly treatment. However, since nusinersen was already approved and reimbursed as treatment for SMA in adult patients in Belgium prior to the start of this study, it was not possible nor justified to conduct a placebo-controlled trial.

Current study in nusinersen-treated SMA adults: significant improvement in hand grip strength and hand function

We showed for the first time that hand grip strength improved significantly in adult SMA patients types 3 and 4 who were treated with nusinersen. Importantly, patients not only experienced an increase of around 30% in hand grip strength bilaterally but hand function also increased significantly on a sub-score of the RULM. For most SMA patients, hand function is very relevant, since the use of a computer or (electric) wheelchair is central to their daily activities, especially after loss of ambulation. Indeed, it was shown previously in SMA type 2 patients that the ability to produce a measurable amount of hand grip strength is correlated with increased independence in activities of daily living (ADL), mobility and hand function [27]. Furthermore, as muscle strength is often best preserved in the hands in SMA, this is a relevant outcome measure, even in patients with severe loss of motor function [28, 29]. Importantly, it has been established that untreated, non-ambulatory SMA patients types 2 and 3 experience a significant annual decline in hand grip strength (median − 0.2 kg/y) from the age of 14 years old, leading to important morbidity, which highlights the impact of our findings even more [13].

At month 14, a slight decrease in mean hand grip strength was observed in comparison with month 6, which was also seen in 6MWD and FVC scores. Although these minimal differences were not significant, a decrease in the mean 6MWD and PCF after initial improvements was also noted in the study by Walter et al. [10] In order to clarify whether this may be the start of a stabilization or whether there is another explanation, long-term follow-up studies in nusinersen-treated adult SMA patients are needed.

Current study in nusinersen-treated SMA adults: significant improvement in MRC sum scores

Next to hand grip strength, we established for the first time that MRC sum scores also improved significantly in nusinersen-treated adult SMA patients types 3 and 4. This finding is exceptional when compared to the historical annual decline in MRC sum scores in this group of patients before initiation of treatment (Fig. 1). Indeed, before treatment was started, a significant annual decline of 0.5 points on the MRC sum score was observed, whereas 14 months after initiation of treatment, we detected a significant improvement of 2.5 points. This is a fivefold larger increase compared to the decrease in MRC sum scores they would normally have experienced in this year. A potential explanation for the fact that Walter et al. did not observe a significant improvement is that they used a compound MRC score of a wide range of muscles as opposed to the classic 60-point MRC sum score used in this study [10]. The study by Hagenacker et al. did not include the MRC sum score as an outcome measure [11].

Outcome measures of muscle function in adult SMA patients treated with nusinersen

The lack of a significant improvement in 6MWD in our study is very likely due to the small proportion of treated patients who retained ambulation, as well as lower baseline values compared to the other two studies. Indeed, the study by Walter et al. reported an improvement of 8 m at month 10 in 11 patients as significant, which is half the improvement noted in the seven ambulatory patients in this study (16 m at month 6) [10].

The significant increase in muscle strength measured with the MRC sum score in this study was not accompanied by significant increases in the HFMSE or RULM scores. This appears to contradict the results of the two aforementioned adult SMA nusinersen treatment studies, but actually our results are very similar [10, 11]. Although only Hagenacker et al. discovered a statistically significant improvement in HFMSE scores (3.1 points at month 14), the improvement in absolute values from baseline was comparable to Walter et al. (4.3 points at month 10) and this study (2.1 points at month 14). This might be explained by the larger sample size in the study of Hagenacker et al. Improvements in the RULM score were also very similar in all studies: 1.1 points at month 14 in Hagenacker et al. 0.7 points at month 10 in Walter et al. and 1.1 points at month 14 in this study. Probably sample size is again the differentiating factor here, since only Hagenacker et al. achieved distinctly significant results. Indeed, with equal smaller sample sizes, the RULM improvement in Walter et al. only just reached significance and the improvement in this study narrowly failed to do so.

Do statistical improvements in functional scores actually translate into a meaningful clinical improvement for adult SMA patients?

Often, the minimal clinically important difference (MCID) is estimated at an increment of three points for the HFMSE and two points for the RULM. However, it has been proposed that increases in the HFMSE or RULM scales of as

little as one point each could represent meaningful changes for patients [30, 31]. In our study, 5/16 patients (31%) experienced a clinically important improvement (of ≥ 3 points) of the HFMSE score at month 14. These results are again very similar to those of Hagenacker et al. where 40% (of SMA type 3 patients) experienced such a MCID at month 14. For the RULM score, we even observed a MCID (of ≥ 2 points) in 8/16 patients (50%). Furthermore, it is plausible that these scales are not sensitive enough to detect small but meaningful functional changes in the measured domains [32]. Finally, patients also often report improvements that are not measured by one of these functional scales, such as increased energy and endurance.

Outcome measures of respiratory function in adult SMA patients

Our study suggests that FVC and PEF measurements are not valid outcome measures in adult SMA patients. The natural history analysis showed no further deterioration of pulmonary function at adult age, which corresponds with the relatively stable values measured under treatment with nusinersen in the prospective study. This finding is in line with a recent natural history analysis by Wijngaarde et al. and the stable FVC values and only transient improvement in PCF in the nusinersen treatment study by Walter et al. [10, 33] The study by Wijngaarde et al. also did not detect a difference between FVC measurements in sitting or supine position in SMA patients types 1–4 of all ages. We confirmed this finding and added that the PEF is significantly lower when measured in supine position.

The potential of ActivLim scores as a PROM in adult SMA patients

Conversely, our results indicated that the ActivLim could be a good outcome measure in adult SMA patients. Our retrospective study revealed a significant decline of 0.13 logits per year, whereas patients in the prospective study improved by 0.36 logits at month 14 of treatment. Although this improvement failed to reach significance, this threefold increase in logits that patients historically lose every year might very well reach significance in a larger patient group. Very recently, another study in nusinersen-treated adult SMA patients also concluded that PROM's are an important measure to assess treatment effects [34].

Other potential outcome measures

Radiological outcome measures are increasingly being used as objective markers of disease progression or treatment effect. A recent study using Diffusion Tensor Imaging (DTI) noted a significant increase in muscle fibre tracks and significant reduction of Fractional Anisotropy (FA) values after 10 and 24 months of nusinersen treatment in two adult SMA patients [35]. Another larger study with 31 adult SMA 2 and 3 patients showed that both FA and fat fraction (Dixon Magnetic Resonance Imaging) correlated with HFMSE and MRC sum scores [36]. Future studies should try to incorporate such novel and objective biomarkers.

Analysis of safety of nusinersen treatment in adult SMA patients

Finally, we established that nusinersen is a safe treatment, without serious adverse events. We particularly focussed on possible renal toxicity in this study, which could not be observed in any of the patients. Although tubular lesions and glomerulonephritis have not been observed in patients treated with nusinersen so far, renal toxicity is regarded as a class effect of oligonucleotides following incidents with other ASOs, such as drisapersen and mipomersen [24, 37]. Mild (and transient) proteinuria, such as was noted in this study, is most likely due to tubular changes and as such markedly more benign. Conversely, severe proteinuria (> 1.5 g/L), complement activation or presence of large molecular weight proteins point more towards a glomerular toxicity, but were not detected in this study. Conventionally, monitoring of serum creatinine, eGFR and urea are advised in patients treated with oligonucleotides [24]. However, we advocate the measurement of Cystatin C in future studies, as it is an objective surrogate measure for renal function in patients with neuromuscular diseases, since this serum protein is independent from muscle mass [23].

Limitations

The sample size of our prospective study was relatively small and follow-up limited to 14 months. Future studies reporting data from a larger group of patients, during a longer follow-up period and including a wider set of outcome measures, are warranted to evaluate the long-term effectiveness and safety of nusinersen treatment in adult SMA patients.

Conclusion

Nusinersen is an efficacious and safe treatment for adult SMA patients types 3 and 4. We showed for the first time that hand grip strength and hand function, as well as MRC sum scores improve significantly under treatment with nusinersen. Although improvements in HFMSE and RULM scores compared to baseline did not reach significance in this study, a large proportion of patients experienced a MCID in these functional tests. Spirometry parameters are not useful as outcome measures because they remain stable in adult

SMA patients, but the ActivLim score as a patient-reported outcome measure may hold high potential in a larger patient cohort.

Acknowledgements The authors thank the patients for their participation in the study. They are grateful to the colleagues from the Radiology Department at UZ Leuven for their imaging support during some of the nusinersen injections. They thank Eveline Dewaege, Elise Lenaerts, Chris Schrevens, Kimberly Tielens, Cindy Thys, Leonie Vandenhoudt, Ann Van Der Vennet and Marielle Verbeek for their administrative and/or technical support. KGC is Chairholder of the Emil von Behring Chair for Neuromuscular and Neurodegenerative Disorders by CSL Behring. KGC is member of the European Reference Network for Rare Neuromuscular Diseases (ERN Euro-NMD) and of the European Reference Network for Rare Neurological Diseases (ERN-RND).

Funding This study was partially funded by Biogen.

Data availability The supporting datasets analysed during the current study and the RStudio code used for statistical analyses are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflicts of interest KGC received advisory board honoraria from Alnylam, Biogen, CSL Behring, Sanofi-Genzyme and travel reimbursement from Sanofi-Genzyme, unrelated to this work. The other authors report no conflicts of interest.

Ethical standards Written informed consent was obtained from the patients. Both the Ethics Committee Research UZ/KU Leuven and the Belgian Federal Agency for Medicines and Health Products (FAGG) approved the study (EudraCT-nr: 2019-005007-40), which has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

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