ORIGINAL RESEARCH

Nusinersen safety and effects on motor function in adult spinal muscular atrophy type 2 and 3

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Received 11 May 2020
Revised 7 August 2020
Accepted 10 August 2020

► http://dx.doi.org/10.1136/jnnp-2020-324036

To cite: Maggi L, Bello L, Bonanno S, *et al*. *J Neurol Neurosurg Psychiatry* Epub ahead of print: [*please include* Day Month Year]. doi:10.1136/jnnp-2020-323822

ABSTRACT

Objective To retrospectively investigate safety and efficacy of nusinersen in a large cohort of adult Italian patients with spinal muscular atrophy (SMA).

Methods Inclusion criteria were: (1) clinical and molecular diagnosis of SMA2 or SMA3; (2) nusinersen treatment started in adult age (>18 years); (3) clinical data available at least at baseline (T0-beginning of treatment) and 6 months (T6).

Results We included 116 patients (13 SMA2 and 103 SMA3) with median age at first administration of 34 years (range 18–72). The Hammersmith Functional Rating Scale Expanded (HFMSE) in patients with SMA3 increased significantly from baseline to T6 (median change +1 point, p<0.0001), T10 (+2, p<0.0001) and T14 (+3, p<0.0001). HFMSE changes were independently significant in SMA3 sitter and walker subgroups. The Revised Upper Limb Module (RULM) in SMA3 significantly improved between T0 and T14 (median +0.5, p=0.012), with most of the benefit observed in sitters (+2, p=0.018). Conversely, patients with SMA2 had no significant changes of median HFMSE and RULM between T0 and the following time points, although a trend for improvement of RULM was observed in those with some residual baseline function. The rate of patients showing clinically meaningful improvements (as defined during clinical trials) increased from 53% to 69% from T6 to T14.

Conclusions Our data provide further evidence of nusinersen safety and efficacy in adult SMA2 and SMA3, with the latter appearing to be cumulative over time. In patients with extremely advanced disease, effects on residual motor function are less clear.

INTRODUCTION

Spinal muscular atrophy (SMA) is an autosomal-recessive lower motor neuron disease causing progressive muscular atrophy and weakness. The clinical spectrum of SMA is heterogeneous, and it is divided into four subtypes according to age of symptoms onset (from infancy to adulthood) and achieved motor milestones (from inability to sit unsupported in SMA1, to very mild adult-onset phenotypes in SMA4).[1] In about 96% of cases, SMA is caused by homozygous deletions in the survival motor neuron 1 (*SMN1*) gene located on chromosome 5q13.2.[1] The paralogue *SMN2* gene differs from *SMN1* for a C>T substitution in exon 7 that interferes with RNA splicing of exon 7. Therefore, SMN protein expressed from the *SMN2* gene is about 90% truncated and non-functional, and the small amount of full-length SMN protein is insufficient to prevent the disease.[2] However, *SMN2* copy number is variable and acts as a genetic modifier of disease severity.[3 4]

Nusinersen (Biogen, Cambridge, Massachusetts, USA) is an antisense oligonucleotide administered intrathecally, able to modify the pre-mRNA splicing of *SMN2*, increasing functional SMN protein levels.[5 6] Nusinersen improved motor function in phase III randomised-controlled trials in infants with SMA1 and in children with later onset SMA, modifying SMA natural history.[7–9] In 2017, despite the lack of clinical trials in adult SMA, nusinersen was approved in Italy for any 5q SMA. Data on nusinersen efficacy in adults are limited to one large observational study and a few smaller case series.[10–15]

We aim to investigate safety and efficacy on motor function of nusinersen in a large cohort of adult Italian patients with SMA2 and SMA3.

METHODS

Patients

In this retrospective cohort study, inclusion criteria were the following: (1) clinical and molecular diagnosis of SMA2 or SMA3; (2) nusinersen treatment started >18 years of age and (3) clinical data available at least at baseline (beginning of treatment) and 6 months (T6). Of 149 screened patients, 5 were excluded because of age <18 years at baseline; 4

because of disease onset >18 years; 2 due to not completing the treatment loading phase because of side effects (abducens nerve palsy 1 week after T0, subarachnoid haemorrhage after T0 with transforaminal approach);[16] 1 due to shifting to a pharmacological clinical trial and 21 patients because T6 had not been reached at the time of data collection. We thus included 116 patients (13 SMA2, 103 SMA3).

Nusinersen administration

All patients were treated with intrathecal loading doses of 12 mg nusinersen at baseline (T0), day 14, day 28 and day 63, followed by maintenance doses every 4 months: T6 at 6 months from T0 (n=116 patients), T10 at 10 months (n=84 patients) and T14 at 14 months (n=54 patients), according to the standard protocol. Intrathecal injections were primarily performed with standard lumbar access in 85/103 (82.5%), via CT-guided in 4 (3.9%) and via X-ray-guided procedure in 14 (13.6%) patients with SMA3; 7/85 (8.2%) patients needed a shift from manual to imaging-guided techniques during treatment. Only 1/13 (7.7%) patients with SMA2 was managed without imaging guidance, 7 (53.8%) with CT-guided and 5 (38.5%) with X-ray-guided approaches.

Clinical assessments

Clinical, motor function and safety data were collected at each time point. According to the international recommendations on SMA standard of care,[1] the following primary outcome measures were assessed by trained evaluators: the Hammersmith Functional Rating Scale Expanded (HFMSE),[17 18] consisting of 33 items, each scored from 0 to 2, up to a maximum of 66 points with higher scores indicating better motor performance; Revised Upper Limb Module (RULM),[19] including 20 items with maximum score of 37 and higher scores indicating better upper limb motor function; 6 min walk test (6MWT).[20] Secondary outcome measures included timed-function tests (TFTs: timed run/walk 10 m, timed rise from floor, timed rise from chair and timed climb four standard steps, all expressed as velocities)[20] and per cent-predicted forced vital capacity and forced expiratory volume in 1 s (FVC% and FEV1%). In addition, at each time point starting from T6, patients were asked if they felt subjectively improved, stable or worsened compared with baseline.

The definition of 'walkers' was patients able to take at least a few steps independently or with aids (eg, cane) but without the assistance of others.

Safety evaluations included vital signs, clinical and laboratory findings and patient-reported adverse events (AEs), categorised by severity and relationship to nusinersen.

Statistical analyses

'Responders' were defined as patients who improved from baseline by at least 3 HFMSE points, 2 RULM points or 30 m in the 6MWT. These cut-offs correspond to definition of 'clinically meaningful' changes from the literature and have been adopted in clinical trials.[18 21–23] Responders in at least one of the three outcomes were defined 'overall responders'.

Variables were summarised as mean±SD or median (range) as appropriate. Distributions of quantitative and ordinal variables between groups were compared with the Wilcoxon-Mann-Whitney or Student's t test as appropriate. Correlations between quantitative and/or ordinal variables were tested with the Spearman method. Distributions of categorical variables were compared by χ^2 test. Logistic regression was used to identify effects of predictor variables (age, sex, *SMN2* copy number) on treatment response. Statistical significance was set at $p<0.05$. No formal correction for multiple testing was adopted, but we distinguished between nominal ($0.05>p>0.01$) and strong ($p<0.01$) statistical significance in presenting results. Analyses were performed with R V.3.5.3.

Standard protocol approvals, registrations and patient consents

The study involved 18 Italian secondary or tertiary care centres for SMA and was approved by Ethics Committees at each centre (ID: SMADU; approved by the Ethics Committee of Fondazione IRCCS Istituto Neurologico 'Carlo Besta', the coordinator centre, on 10 July 2019). Written informed consent was obtained from all participants, according to the Helsinki declaration.

RESULTS

Median age at onset of the 116 included patients (68 males and 48 females) was 3.0 years (range 0.5–17), while median age at T0 was 34 years (range 18–72). Thirteen (11.2%) patients had SMA2 and 103 (88.8%) SMA3, divided into 51 'sitters' and 52 'walkers'. Clinical, molecular features and results of baseline motor function assessments are shown in table 1. Comorbidities are listed in online supplemental table S1.

Nusinersen effect on motor function

Summary statistics for the main study outcomes are shown in table 2; distributions and changes for all outcomes are detailed in online supplementary table S2.

HFMSE

HFMSE score in SMA3 increased from baseline by a median of +1 point (range −5 to 8) at T6 ($p<0.0001$), +2 (–3 to 9) at T10 ($p<0.0001$) and +3 (–3 to 11) at T14 ($p<0.0001$; table 2). HFMSE changes were independently significant in 'sitter' and 'walker' SMA3 subgroups: at T14, a 3-point median increase was observed in sitters ($p=0.0014$) and a 2-point median increase in walkers ($p=0.00016$) (figure 1B-C). HFMSE improvements were also significant between intermediate time points (T6–T10 and T10–T14; online supplementary table S2).

In SMA2, no significant HFMSE change was found between T0 and the following time points (figure 1A; table 2).

RULM

In SMA3, median RULM remained unchanged between T0–T6 and T6–T10, but increased by a median +0.5 points (−6 to 6) between T0 and T14 (nominally significant, $p=0.012$). In sitter patients with SMA3, nominally significant changes were observed at T10 (+1, range −6 to 5, $p=0.021$) and T14 (+2, range −6 to 5, $p=0.018$)(figure 1E). RULM did not change in walker patients with SMA3, who showed a 'ceiling' effect (figure 1F; table 2).

In SMA2, median RULM did not change between baseline and T6, T10 and T14, but a positive trend was observed (median +2 points at T14, range 0–3; table 2)(figure 1D).

6MWT and timed tests

6MWT distance increase was strongly significant at T6 (median +11 m; $p=0.0005$), T10 (+25 m; $p=0.00019$) and nominally significant at T14 (+20 m; $p=0.016$; table 2) (figure 2A).

Among TFTs (online supplementary table S2; figure 2B-E), rise from chair velocity increase was nominally significantly at T6 ($+0.02\,s^{-1}$; $p=0.026$) and T10 ($0.04\,s^{-1}$; $p=0.016$) and strongly significant at T14 ($0.06\,s^{-1}$; $p=0.0067$). Ten-metre

Table 1 Clinical features and results of motor function assessments at baseline

Variable		n*	All SMA	SMA2			SMA3 'sitters'	SMA3 'walkers'	
Age at onset (years)		116	3 (0–17)	13	0.8 (0.5–12)	51	3 (0.3–15)	52	8 (0–17)
Age at T0 (years)		116	34 (18–72)	13	24 (19–41)	51	40 (18–72)	52	33 (18–68)
Disease duration at T0 (years)		116	29 (3–63)	13	22.5 (7–40.5)	51	37 (14–63)	52	26 (3–51)
Gender (F/M)		116	48/68	13	3/10	51	15/36	52	30/22
SMN2 copies†	Two copies	116	5 (4.3%)	13	3 (23.1%)	51	2 (3.9%)	52	0 (0%)
	Three copies		36 (31.0%)		6 (46.2%)		16 (31.4%)		14 (26.9%)
	Four copies		54 (46.6%)		2 (15.4%)		21 (41.2%)		31 (59.6%)
	Unknown		21 (18.1%)		2 (15.4%)		12 (23.5%)		7 (13.5%)
Salbutamol (%)		116	27 (23.3%)	13	5 (38.5%)	51	9 (17.8%)	52	13 (25.0%)
Ventilatory support at T0 (%)		116	21 (18.1%)	13	10 (76.9%)‡	51	8 (15.7%)§	52	3 (5.8%)
Surgery for scoliosis (%)		116	16 (13.8%)	13	8 (61.5%)	51	7 (13.7%)	52	1 (1.9%)¶
Clinical assessments									
HFMSE score		116	22.5 (0–64)	13	0 (0–9)	51	9 (0–40)	52	50.5 (17–64)
RULM score		114	29 (0–37)	12	2.5 (0–22)	51	20 (0–34)	51	37 (25–37)
6MWT (m)		NA	NA	0	NA	0	NA	48	322 (14–588)
Rise from floor (s^{-1})		NA	NA	0	NA	0	NA	28	0.1 (0.01–0.33)
Rise from chair (s^{-1})		NA	NA	0	NA	0	NA	31	0.25 (0.06–1)
Climb four steps (steps/s)		NA	NA	0	NA	0	NA	35	0.8 (0.17–2)
Run/walk 10 m (m/s)		NA	NA	0	NA	0	NA	40	1.12 (0.09–2.08)
FVC (% of predicted)		86	88.5 (11–139)	7	20 (11–74)	40	83 (30–128)	39	102 (40–139)
FEV1 (% of predicted)		76	92.5 (16–134)	5	20 (16–55)	35	84.3 (35–120)	36	103 (47–134)

Summary values are presented as median (minimum–maximum). All patients carried homozygous *SMN1* exon 7 deletions, except 3: one with a nonsense and two with a missense mutation on the other allele.

*HFMSE was available for all patients, remaining assessments were not available for all patients.

†SMN2 copy number was not available in 21 patients.

‡A further patient stopped ventilatory support before T0 due to poor tolerance.

§2 patients used ventilatory support due to obstructive sleep apnea and a further patient refused ventilatory support although indicated.

¶Patient able to walk for few steps with cane.

F, female; FEV_1, forced expired volume in 1 s; FVC, forced vital capacity; HFMSE, Hammersmith Functional Motor Scale Expanded; M, male; 6MWT, 6 min walk test; NA, not available; RULM, Revised Upper Limb Module; SMA, spinal muscular atrophy; *SMN2*, survival motor neuron 2 gene.

run/walk speed increased only at T6, with nominal significance (+0.07 m/s; p=0.02).

Pulmonary function tests

There was a nominally significant FVC% increase at T14 (median +7%; p=0.031) in the SMA3 'walker' subgroup only (figure 2F). To a lesser degree, FEV1% improved between baseline and T14 in the whole SMA3 population (+3%; p=0.0499). In SMA2, small sample size hindered statistical comparisons.

Responder rates

Fifty-three per cent of the whole cohort were considered 'overall responders' at T6, increasing to 63% at T10 and to 69% at T14, with higher rates for SMA3 than SMA2 (figure 3). When looking at responder status for individual outcomes, there were more HFMSE-defined than RULM-defined responders. HFMSE responder rate was higher in SMA3 (41% at T10% and 52% at T14) than in SMA2 (11% at T10% and 20% at T14), with similar rates in 'sitter' and 'walker' subgroups at T10, but higher frequency in 'sitters' at T14 (58% vs 48%). Conversely, RULM-defined responder rate was higher in SMA2 (56% at T10% and 60% at T14) than in SMA3 (24% at T10% and 32% at T14), with SMA3 sitters (39% at T10% and 53% at T14) behaving more similarly to SMA2, whereas the 'walker' subgroup showed very low RULM-defined responder rates (11% at T10% and 16% at T14). In SMA3 'walkers', HFMSE-defined responder rates (43% at T10% and 48% at T14) and 6MWT-defined responder rates (46% at T10% and 42% at T14) were quite similar, with a low frequency of RULM responders.

Multivariate logistic regression models looking for concurrent effects of age, sex and *SMN2* copy number on clinically meaningful responses did not identify significant predictors, except for a barely significant, negative effect of age in 'sitter' SMA3 patients (OR 0.92 per-year of age, p=0.048).

Subjective clinical improvement was reported by 61/104 (58.7%) patients at T6, 47/79 (59.5%) at T10 and 28/49 (57.1%) at T14, at a difference with the responder rate at T10 and T14 (see online supplementary table S3). No significant association was found between clinically meaningful responses and patient-reported subjective improvement at each time point, despite a trend towards concordance.

In SMA2, RULM improvement positively correlated with higher functional status at baseline (T6: rho 0.62, p=0.033; T10: rho=0.88, p=0.002 and T14: rho=0.97, p=0.005). Figure 1 shows that six patients with SMA2 with 0 or 1 RULM scores at baseline showed no improvement, whereas five out of six patients, starting from higher baseline scores, did show improvement. Conversely, RULM improvements in SMA3 'sitters' were larger than in SMA3 'walkers'; this observation is probably due to the ceiling effect of RULM in SMA3 walkers. At T14, 6MWT in 12 SMA3 'walker' patients with four SMN2 copies improved by 34.7±48.4 m, as compared with five with three SMN2 copies, who lost −25.4±50.5 m (p=0.027). HFMSE changes at T6, T10 and T14 showed no significant correlation with age, sex, *SMN2* copy number or baseline functional performance, in the overall population as well as in SMA2 and SMA3 subgroups.

Table 2 Functional changes at T6, T10 and T14 in patient subgroups with SMA2 and SMA3

		SMA2				SMA3 'sitters'				SMA3 'walkers'				SMA3 total			
Variable		N	Mean ±SD	Median (min–max)	Paired Wilcoxon P value	N	Mean ±SD	Median (min–max)	Paired Wilcoxon P value	N	Mean ±SD	Median (min–max)	Paired Wilcoxon P value	N	Mean ±SD	Median (min–max)	Paired Wilcoxon P value
T0-T6 change	HFMSE	13	0.15 ±2.08	0 (–5 to 5)	n.s.	51	1.37 ±2.02	1 (–4 to 6)	**<0.0001**	52	1.58 ±2.52	1 (–5 to 8)	**<0.0001**	103	1.48 ±2.28	1 (–5 to 8)	**<0.0001**
	RULM	12	0.8 ±1.95	0 (–1 to 6)	n.s.	51	0.63 ±2.48	0 (–8 to 6)	0.056	51	0 ±1.23	0 (–4 to 3)	n.s.	102	0.31 ±1.97	0 (–8 to 6)	0.093
	6MWT	0	NA	NA	NA	0	NA	NA	NA	48	14.66 ±27.57	11 (–42.2 to 96)	**0.0005**	NA	NA	NA	NA
	FVC%	4	–0.25 ±2.06	0 (–3 to 2)	n.s.	19	0 ±9.04	1 (–19 to 28)	n.s.	16	1.16 ±6.16	0.5 (–9 to 16)	n.s.	35	0.53 ±7.77	1 (–19 to 28)	n.s.
T0–T10 change	HFMSE	9	1 ±2	0 (0–6)	n.s.	35	2.51 ±2.94	1 (–3 to 9)	**<0.0001**	40	2.38 ±2.71	2 (–3 to 8)	**<0.0001**	75	2.44 ±2.8	2 (–3 to 9)	**<0.0001**
	RULM	9	1.67 ±1.8	2 (0–5)	0.057	33	1 ±2.45	1 (–6 to 5)	**0.021**	38	0.26 ±1.66	0 (–4 to 6)	n.s.	71	0.61 ±2.08	0 (–6 to 6)	**0.011**
	6MWT	0	NA	NA	NA	0	NA	NA	NA	35	26.45 ±34.6	25 (–53 to 90)	**0.00019**	NA	NA	NA	NA
	FVC%	4	0.75 ±2.5	0.5 (–2 to 4)	n.s.	7	3.3 ±7.83	4.1 (–10 to 16)	n.s.	10	5.8 ±14.26	4.5 (–10 to 39)	n.s.	17	4.77 ±11.79	4.1 (–10 to 39)	n.s.
T0–T14 change	HFMSE	5	1.2 ±2.68	0 (0–6)	n.s.	19	3.53 ±3.67	3 (–3 to 11)	**0.0014**	27	2.37 ±2.22	2 (–2 to 6)	**0.00016**	46	2.85 ±2.93	3 (–3 to 11)	**<0.0001**
	RULM	5	1.6 ±1.52	2 (0–3)	n.s.	19	1.47 ±2.5	2 (–6 to 5)	**0.018**	25	0.4 ±1.83	0 (–3 to 6)	n.s.	44	0.86 ±2.18	0.5 (–6 to 6)	**0.012**
	6MWT	0	NA	NA	NA	0	NA	NA	NA	24	23.11 ±51.2	20 (–101 to 111)	**0.016**	NA	NA	NA	NA
	FVC%	0	NA	NA	NA	8	4.25 ±8.55	1 (–4 to 19)	n.s.	7	9 ±9.95	7 (–1 to 29)	**0.031**	15	6.47 ±9.22	4 (–4 to 29)	**0.020**

Significant p values are highlighted in bold.
FVC%, per cent-predicted forced vital capacity; HFMSE, Hammersmith Functional Motor Scale Expanded (score); 6MWT, 6 min walk test distance (m); NA, not available; RULM, Revised Upper Limb Module (score); SD, Standard Deviation; SMA, Spinal Muscular Atrophy.

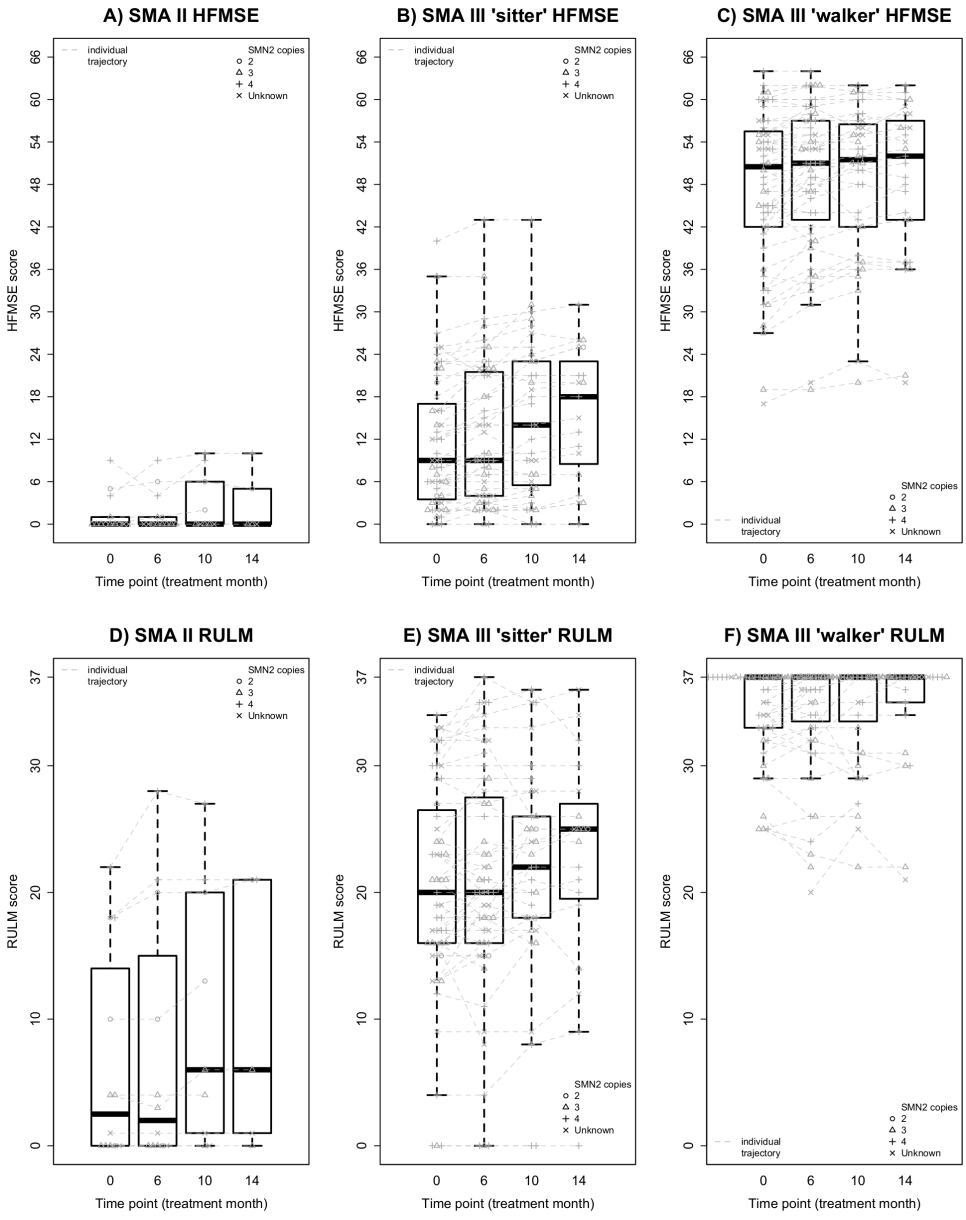

Figure 1 Box-whisker-beeswarm plots of (A–C) HFMSE scores and (D–F) RULM scores across time points. Data for SMA2 are shown in panels A and D, while data for SMA3 sitters are shown in panels B and E, and those for SMA3 'walkers' in panels C and F. Boxes identify first to third quartile range in the distribution, thick horizontal lines indicate median values, and whiskers indicate minimum/maximum values or first/third quartile ±1.5 * the IQR, whichever is the least extreme. 'Beeswarms', superimposed in grey, indicate all individual values for the 116 patients with longitudinal data. Different dot types identify *SMN2* copy number. Dashed lines describe individual patient trajectories. HFMSE, Hammersmith Functional Rating Scale Expanded; RULM, Revised Upper Limb Module; SMA, spinal muscular atrophy.

Safety

Two (1.7%) patients with SMA3 stopped nusinersen treatment after T6, due to lack of subjective benefit and poor tolerability of repeated lumbar puncture. Both patients had received nusinersen through imaging-guided administration.

AEs were reported in 48 (41.4%) patients (6 SMA2 and 42 SMA3); the most frequent AE was postprocedure headache, observed at least once in 43/116 (37.1%) patients. Headache was orthostatic, mild to moderate in intensity and spontaneously resolved in a few days, except for five patients (four SMA3 and one SMA2) who required hospitalisation. Headache was associated in 34/43 (79.1%) cases with manual procedures and in 9/43 (20.9%) with imaging-guided techniques, with no significant difference in headache probability between the two approaches.

Lumbar pain was reported in 10/116 (8.6%) patients, 7 of whom underwent imaging-guided lumbar punctures. Two patients with SMA3 reported transient (1–2 months) worsening of existing hand tremor, one after T0 and one after T14. One renal colic requiring hospitalisation occurred in a patient with SMA2 the day following T10. No relevant changes related to nusinersen treatment were observed in laboratory tests, including serum creatinine. Except for cases requiring hospitalisation, AEs were mild or moderate and were judged not related to nusinersen itself, but rather to the administration procedure.

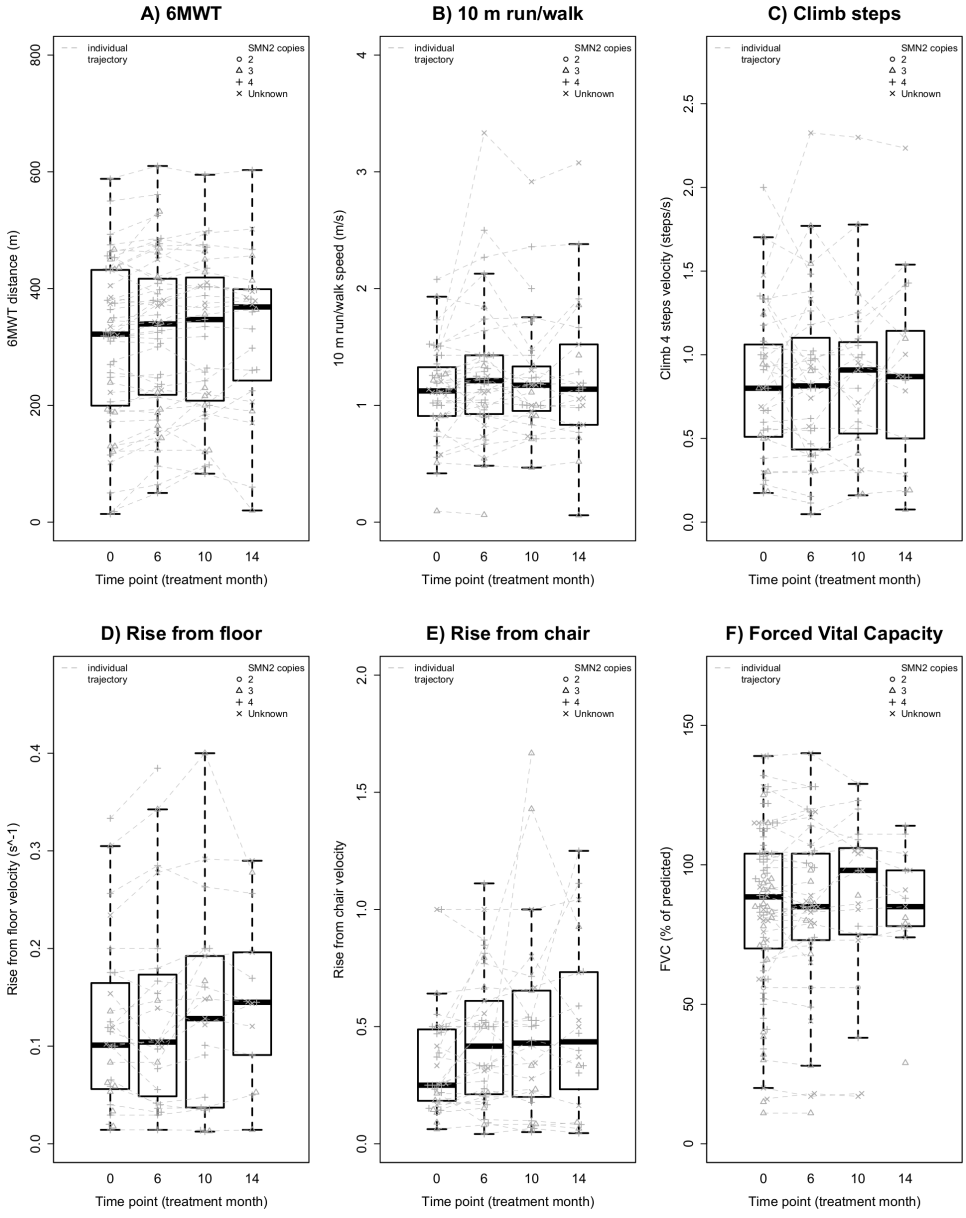

Figure 2 Box-whisker-beeswarm plots of (A) 6MWT distance, (B) 10 m run/walk velocity, (C) climb four standard step velocity, (D) rise from floor velocity, (E) rise from chair velocity and (F) FVC (% of predicted), across time points. Data for all patients with available measures are shown. Boxes identify first to third quartile range in the distribution, thick horizontal lines indicate median values and whiskers indicate minimum/maximum values or first/third quartile ±1.5 * the IQR, whichever is the least extreme. 'Beeswarms', superimposed in grey, indicate all individual values for the 116 patients with longitudinal data. Different dot types identify *SMN2* copy number. Dashed lines describe individual patient trajectories. FVC, forced expiratory volume; 6MWT, 6 min walking test.

DISCUSSION

Adults represent a relevant portion of the overall SMA population. However, compared with paediatric SMA, in adult SMA, the natural history is not as well defined;[24–26] outcome measures have been not as thoroughly standardised and validated;[27] targeted clinical trials are fewer and a higher rate of chronic musculoskeletal complications, due to longer disease duration, and age-specific comorbidities confound motor evaluations. For all these reasons, recommendations for adult SMA management are urgently due.[28]

Small cohort studies, and a recent multicentre observational study, have confirmed the benefit of nusinersen on motor function in adult SMA.[10–15] This study further supports nusinersen safety and efficacy in a similar real-life setting. However, our cohort included more patients with SMA3 than the cohort reported by Hagenacker *et al*[13] and represents the largest adult SMA3 cohort investigated to date. Our main finding is a strongly significant improvement of HFMSE in SMA3. This was more marked in SMA3 'sitters' than SMA3 'walkers', whereas RULM significantly improved only in 'sitters', probably due to a ceiling effect of this measure in 'walker' patients.[13]

Clinical improvements detected at T6 were maintained and further increased at follow-up for both RULM and HFMSE, suggesting that the efficacy of nusinersen may be cumulative, at least throughout the first 14 months of treatment. The entity of clinical improvement in our SMA3 cohort was slightly different compared with Hagenacker *et al*; mean HFMSE change (table 2) was lower at T6 (+1.5 vs +2.4), T10 (+2.4 vs +3.4) and T14

		All SMA			SMA II			SMA III			SMA III "Sitters"			SMA III "Walkers"		
		T6	T10	T14	T6	T10	T14	T6	T10	T14	T6	T10	T14	T6	T10	T14
HFMSE	n of responders	33	32	25	1	1	1	32	31	24	14	14	11	18	17	13
	Total n	116	84	51	13	9	5	103	75	46	51	35	19	52	40	27
	Responder %	28%	38%	49%	8%	11%	20%	31%	41%	52%	27%	40%	58%	35%	43%	48%
RULM	n of responders	24	22	17	3	5	3	21	17	14	15	13	10	6	4	4
	Total n	114	80	49	12	9	5	102	71	44	51	33	19	51	38	25
	Responder %	21%	28%	35%	25%	56%	60%	21%	24%	32%	29%	39%	53%	12%	11%	16%
6MWT	n of responders	NA	NA	NA	NA	NA	NA	14	16	10	NA	NA	NA	14	16	10
	Total n	NA	NA	NA	NA	NA	NA	48	35	24	NA	NA	NA	48	35	24
	Responder %	NA	NA	NA	NA	NA	NA	29%	46%	42%	NA	NA	NA	29%	46%	42%
Overall	responder n	61	53	35	3	5	3	58	48	32	26	21	15	32	27	17
	total n	116	84	51	13	9	5	103	75	46	51	35	19	52	40	27
	responder %	53%	63%	69%	23%	56%	60%	56%	64%	70%	51%	60%	79%	62%	68%	63%

Figure 3 Heatmap/table of clinically meaningful functional improvements during treatment. Red colour code corresponds to population size at given time points for different subgroups (SMA2, SMA3, sitters, walkers), with intense red corresponding to relatively large populations. Green colour code corresponds to % of responders (ie, patients with clinically meaningful improvement) at a given time point, with intense green corresponding to high responder rates. Responders are defined as at least 3-point HFMSE score change from T0, at least 2-point RULM score change from T0 and at least 30-m 6MWT distance change from T0. 'Overall' response is defined as clinically meaningful response in at least one measure. HFMSE, Hammersmith Functional Motor Scale–Expanded; 6MWT, 6 min walking test; RULM, Revised Upper Limb Module; SMA, spinal muscular atrophy.

(+2.9 vs +4.2).[13] These discrepancies may be due to our cohort including a larger proportion of patients with SMA3 (89% vs 62%) with a worse motor performance at baseline shown by baseline 6MWT (308.5 vs 321.8 m; baseline HFMSE scores for patients with SMA3 was not reported in Hagenacker *et al*).[13]

In our patients with SMA2, HFMSE and RULM did not increase significantly, despite a positive trend for RULM at T14. Interestingly, the mean T14 improvement was +1.6, identical to the German SMA2 cohort.[13] The small size of our SMA2 subgroup does not allow definite conclusions, but considering available natural history data, we cannot exclude that the stabilisation of motor function over the follow-up period may be considered as a positive effect of nusinersen.[23 24 29 30]

We observed an improvement of FVC% in SMA3 'walkers' at T14, suggesting a mild, but slow beneficial effect of nusinersen on respiratory function. This had been hinted at by Walter *et al*, who failed to demonstrate FVC change in a smaller cohort at 10 months.[10]

We investigated possible predictors of a clinically meaningful response, which could assist clinicians in selecting and advising patients, in view of the high costs and invasive route of administration of nusinersen. We recognise that a clinically meaningful response is hard to define objectively, but we adhered to definitions derived from natural history studies and already applied in relevant clinical trials.[18 21–23] In SMA3, we observed a steady increase of 'overall' responders (as defined in Methods section), from 56% at T6, to 64% at T10, and finally to 70% at T14, reinforcing the impression of a cumulative beneficial effect. Despite caution imposed by the small sample size, responders were also frequent in SMA2, suggesting that even patients with poor motor function at baseline may benefit from nusinersen. HFMSE-defined responder rate was slightly higher in our SMA3 cohort compared with the German cohort, in particular at T14 (52% vs 41%).[13] We recognise a methodological limitation in comparing clinically meaningful changes between populations with very different baseline functional performance, such as SMA3 'sitters' vs 'walker'. Indeed, a 3-point HFMSE change in these populations denotes the modification of very different motor functions, and probably has a different impact on overall functioning. Therefore, it may be useful to redefine clinically meaningful improvements separately, in distinct classes of adult patients with SMA3.

We were unable to identify clear predictors of clinically meaningful HFMSE improvement in SMA3, except for a marginal, negative effect of age in 'sitters'. RULM, conversely, increased in patients with low baseline scores, except for those with *very* low scores ('floor' effect). More sensitive outcome measures, targeted to advanced disease stages and assessing also respiratory and bulbar functions or including patient-reported outcomes, are needed.

So far, our data support nusinersen efficacy in non-ambulatory SMA adults with some residual upper limb function (ie, SMA3 'sitters'), while they do not allow definite conclusions about adult patients with SMA2, because of low sample size in this category.

Similar to RULM, 6MWT also showed greater improvement in patients with relatively worse performance at baseline (approximately 200 m or less). Interestingly, at T14, patients carrying 4 *SMN2* copies improved strikingly more than to those carrying three copies. A larger *SMN2* copy number, targeted by nusinersen, may be responsible of a reduction of fatigue with long-term treatment, that is best captured by the endurance-based 6MWT than by other measures.

Unlike the paediatric population, our study confirms that the effect of nusinersen is not clearly correlated with disease duration in adults, consistent with the German study.[13] In addition, we confirm that patients with less advanced disease at baseline showed greater motor improvement, although this was more evident with RULM than with HFMSE.[13] In particular, our data stress the relevance of residual motor function at baseline in predicting response to nusinersen.

No significant association was found between the rates of clinically meaningful response, defined by objective outcome measure changes, and of subjective, patient-reported improvement. Although not included in the present paper, specific patient-reported outcomes, focused on quality of life or daily activities, may be valuable outcome measures in adult SMA and may be useful in future studies.[31 32]

This study confirms safety and feasibility of intrathecal administration of nusinersen. Only two patients dropped out because of insufficient perceived balance between benefit and adverse effects. Postprocedural headache was the most frequent AE, observed in 37% of the patients, as expected.[10 13] AEs were almost exclusively related to lumbar punctures and not to the drug itself.

We recognise several limitations of our study: the retrospective design, the small SMA2 sample size and missing data for some of the variables. Retrospective studies, however, present real-world data outside the rigid setting of a clinical trial. In fact, the few included patients with SMA2 reflect the small prevalence of this condition in adults. Missing data were mostly limited to timed and pulmonary function tests, while the strongest conclusions

were drawn from HFMSE, RULM and 6MWT. Last, some results were only supported by 'nominal' statistical significance, but this observational study had an open recruitment and was not formally powered for efficacy. Therefore, we deem that in interpreting these observational data, compared with a clinical trial, less importance should be given to p value thresholds, than to the magnitude of described changes and their clinical implications.

In conclusion, our study strongly supports the safety and efficacy of nusinersen in adult patients with SMA3. The small amount of adult patient with SMA2 did not allow definite conclusions in this category, despite positive trends. Follow-up studies are warranted, in order to investigate the efficacy and safety of nusinersen in the long term.

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Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests LM has received honoraria for speaking and compensation for congress participations from: Sanofi Genzyme and Biogen. LB reports speaker honoraria from PTC Therapeutics, participation in advisory boards for PTC Therapeutics and Sarepta Therapeutics and participation in research sponsored by Santhera Pharmaceuticals. SB received funds for travel and congress participation from Sanofi Genzyme and Biogen. LP has received compensation for congress participations from: Sanofi Genzyme and Biogen. MG received honoraria for speaking and grants to attend scientific meetings from Sanofi-Genzyme and Pfizer. VB compensation for congress participations from Biogen and Sanofi Genzyme. LC received compensation for congress participations from Biogen. RP has received compensation for congress participation from Sanofi Genzyme and Biogen. RT has received compensation for congress participations from Sanofi Genzyme, Biogen and Roche. ES has received compensation for congress participations from: Biogen. GR has received compensation for congress participations from Sanofi Genzyme, CSL Behring, Santhera and Biogen. RZ has received funds for travel and congress participation from Biogen. MS has received speaker's honoraria from ITALFARMACO, PIAM, Boheringer and Novartis Pharmaceuticals. LP received compensation for congress participations from Biogen Inc, Italy. AS received honoraria for speaking and grants to attend scientific meetings from Sanofi-Genzyme, Alnylam Pharmaceuticals, Akcea Therapeutics and Pfizer. LV has received compensation for congress participation from Sanofi Genzyme and Biogen. RM received compensation for participating on advisory boards in relation to MG clinical trial design, congress participations and research support in the last 5 years from: Catalyst Pharmaceuticals, Alexion Pharmaceuticals, ARGENX Pharma, Biomarin. RL has received: Lecture fees from LT3 s.r.l., NICO s.r.l., SUMMEET s.r.l. and GALEN SYMPOSION s.r.o.; fees for consultancy (Advisory Board) from LT3 s.r.l. and PREX s.r.l.; fees for scientific meeting organization from I & C s.r.l.; Chair meeting fees from PLANNING CONGRESSI s.r.l. GC in the last two years has received compensation for advisory board compensation from: Sanofi Genzyme, Spark, Roche, Lupin and Santhera, congress participations from: Sanofi Genzyme, CSL Behring, Sarepta and Biogen. ILS received compensation for congress participation from Biogen, Italy. TM has received compensation for Scientific Board participations from Sanofi Genzyme; for congress participation from Biogen. GC received compensation for participation ad advisory board for Roche, Sarepta and Italfarmaco. EP reports personal fees from Sarepta, grants and non-financial support from Santhera, personal fees and non-financial support from PTC Pharmaceuticals, non-financial support from Genzyme, personal fees from Roche, outside the submitted work.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request. All anonymised data from this study will be shared by request from any qualified investigator. Data are available from ORCID identifier is 0000-0002-0932-5173. Data reuse is permitted only for academic purposes.

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