European Journal of Neurology

european journal of neurology
the official journal of the european academy of neurology

ORIGINAL ARTICLE OPEN ACCESS

Longitudinal Assessment of 4-Year HFMSE Changes in SMA II and III Patients Treated With Nusinersen

Giorgia Coratti[1,2] | Francesca Bovis[3] | Marika Pane[1,2] | Amy Pasternak[4] | Emilio Albamonte[5] | Irene Mizzoni[6] | Allan M. Glanzman[7] | Simone Morando[8] | Jacqueline Montes[9] | Ilaria Cavallina[10] | Sally Dunaway Young[11] | Tina Duong[11] | Enrica Rolle[10] | Matthew Civitello[12] | Roberto De Sanctis[1,2] | Chiara Bravetti[2] | Federica Ricci[10] | Giulio Gadaleta[13] | Tiziana Mongini[13] | Maria Sframeli[14] | Maria Carmela Pera[1,2,15] | Sonia Messina[14] | Adele D'Amico[6] | Michela Catteruccia[6] | Noemi Brolatti[8] | Michio Hirano[9] | Zarazuela Zolkipli-Cunningham[16] | Basil T. Darras[4] | Enrico Bertini[6] | Claudio Bruno[8] | John Day[11] | Valeria A. Sansone[5] | Richard S. Finkel[12] | Eugenio Mercuri[1,2] | the iSMAC/international SMA consortium

[1]Pediatric Neurology Unit, Catholic University of Sacred Heart, Rome, Italy | [2]Centro Clinico Nemo, U.O.C. Neuropsichiatria Infantile, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome, Italy | [3]Department of Health Sciences (DISSAL), University of Genoa, Genoa, Italy | [4]Neuromuscular Program, Boston Children's Hospital, Harvard Medical School, Boston, Massachusetts, USA | [5]The NEMO Center in Milan, Neurorehabilitation Unit, University of Milan, ASST Niguarda Hospital, Milan, Italy | [6]Unit of Neuromuscular and Neurodegenerative Disorders, Translational Paediatrics and Clinical Genetics, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy | [7]Department of Physical Therapy, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, USA | [8]Center of Translational and Experimental Myology and Department of Neuroscience, Rehabilitation, Ophthalmology Genetics, Maternal and Child Health, IRCCS Istituto Giannina Gaslini and University of Genoa, Genoa, Italy | [9]Columbia University Irving Medical Center, New York, New York, USA | [10]Section of Child and Adolescent Neuropsychiatry, Department of Public Health and Paediatric Sciences, University of Turin, Turin, Italy | [11]Department of Neurology and Clinical Neurosciences, Stanford University, Palo Alto, California, USA | [12]Center for Experimental Neurotherapeutics, Department of Pediatric Medicine, St. Jude Children's Research Hospital, Memphis, Tennessee, USA | [13]Neuromuscular Unit, Department of Neurosciences 'Rita Levi Montalcini', University of Turin, Turin, Italy | [14]Department of Clinical and Experimental Medicine, University of Messina, Messina, Italy | [15]Department of Medicine and Surgery, Child Neuropsychiatry Unit, University of Parma, Parma, Italy | [16]Division of Human Genetics, Children's Hospital of Philadelphia, Department of Pediatrics, University of Pennsylvania, Philadelphia, Pennsylvania, USA

Correspondence: Eugenio Mercuri (eugeniomaria.mercuri@unicatt.it)

Received: 5 February 2025 | **Revised:** 31 May 2025 | **Accepted:** 16 June 2025

Funding: EM is funded by grant from the Italian Ministry of Health (RF-2019-12370334). GC is funded by grant from the Italian Ministry of Health (GR-2021-12374579). MCP is funded by grant from the Italian Ministry of Health (GR-2018-12365706). EB is funded by Ricerca Corrente. The registry and the associated studies are funded by Biogen, Novartis and Roche.

Keywords: Hammersmith functional motor scale expanded | long term results | motor function | nusinersen | spinal muscular atrophy

ABSTRACT

Background: The aim of this international retrospective study was to assess 4-year change using the Hammersmith Functional Motor Scale Expanded (HFMSE) in individuals with type II and III spinal muscular atrophy (SMA) treated with nusinersen and to establish predictors of HFMSE changes.

Methods: Individuals with type II or III SMA, and at least 4 years of nusinersen-only treatment were included. All were assessed using the HFMSE. Age at baseline, sex, motor function, *SMN2* copy number, and age of onset were also retrospectively collected. Linear mixed effect models were used to calculate yearly changes and trajectory predictors.

ISMAC/International SMA Consortium: Rafael. S Rodriguez-Torres, Nicola Forcina, Giulia Norcia, Giulia Stanca, Sara Carnicella, Lavinia Fanelli, Antonella Longo, Michela Nani, Laura Antonaci, Giacomo De Luca, Roberto Materia.

Results: We included 73 individuals with SMA type II (mean age 8.58 years, SD 7.91, IQR 3.04–10.70) and 111 type III (mean age 7.91 years, SD 17.83, IQR 8.15–34.42). Over 4 years, mean changes were + 4.18 (95% CI: 2.85–5.50) for SMA II and + 1.08 (95% CI: 0.12–2.04) for SMA III. Age (SMA II: −0.34\[−0.51 to −0.17]; SMA III: −0.13\[−0.20 to −0.06], $p < 0.001$) and baseline HFMSE (SMA II: 1.02\[0.70–1.34]; SMA III: 0.79\[0.71–0.87], $p < 0.001$) were the strongest predictors of progression, with younger age and higher baseline scores associated with better outcomes. Functional status was only predictive for type III (6.96\[4.26–9.66]).
Conclusion: Our results confirm that, given a follow up of 4 years, there is a persistent impact of nusinersen on clinical progression that is better observed in younger patients with higher HFMSE scores at baseline, especially during the first 2 years of treatment.

1 | Introduction

Spinal muscular atrophy (SMA) is a caused by mutations in the survival motor neuron 1 (*SMN1*) gene [1] with reduction of the SMN protein leading to progressive weakness. Historically, 5q-MA was classified into three main types with pediatric onset. Type I was associated with onset before 6 months and the inability to achieve the ability to sit unsupported [2]. Type II, with onset typically between 6 and 18 months, was associated with the ability to sit and occasionally to stand but not to walk independently. In type III, the onset of symptoms was generally after ambulation had been achieved (after 18 months), and in all cases, there was progressive weakness; within this type, Type IIIa refers to onset before the age of 3 years, often with faster progression of weakness, while Type IIIb refers to onset after the age of 3 years, typically with slower progression and a milder clinical course. The advent of disease-modifying therapies (DMTs) has dramatically changed the progression of all three types, not only in type I infants, with increased survival and often achievement of sitting, but also in types II and III. A number of studies have reported the impact of nusinersen, the first commercially available drug, on pediatric and adult type II and III SMA [3–10]. A recent meta-analysis shows there was a consistent improvement in all the measures examined across all studies reporting the use of nusinersen in types II and III, in comparison to the decrease in scores observed in the published natural history studies of the same measures in untreated patients [11]. In most cases, data were related to a relatively short follow-up. The question has arisen whether the changes observed in the first years of treatment will persist at longer follow-up at the age when natural history studies report further progressive loss of function.

The aim of this study was to assess 4-year changes using the Hammersmith Functional Motor Scale Expanded (HFMSE) in types II and III individuals treated with nusinersen and to establish possible associations between HFMSE changes and several variables, including age at baseline, sex, motor function, *SMN2* copy number, and age of symptom onset.

2 | Methods

The study includes a retrospective analysis of prospectively collected data from the Pediatric Neuromuscular Clinical Research Network for SMA in the United States and the Italian network, associated with the International SMA Consortium (iSMAc) [12].

All individuals with a confirmed genetic diagnosis of SMA, a clinical diagnosis of type II or III SMA, and a nusinersen-only treatment history of at least 4 years were considered for inclusion in the study. This included all participants who had been evaluated at the neuromuscular clinics of the participating centers, following the same criteria used in our previous collaborative study on progression patterns [7, 13, 14]. Patients who received other DMTs besides nusinersen or who switched from another DMT to nusinersen were excluded from the analysis. Baseline data was collected before the initiation of nusinersen, using the HFMSE value closest to the start of treatment (mean time difference −0.20, SD 0.24 years).

All participants and their legal guardians provided written informed consent or assent, as applicable. This process was approved by the respective institutional review boards.

2.1 | Hammersmith Functional Motor Scale Expanded (HFMSE)

The HFMSE [15] consists of 33 items that evaluate a child's ability to engage in various activities. Each item is assessed using a 3-point scoring system: a score of 2 indicates the child can perform the activity without modifications, a score of 1 means the child can perform it with modifications or adaptations, and a score of 0 signifies that the child is unable to perform the activity. The total score is calculated by summing the scores of all individual items, ranging from 0, indicating failure in all activities, to 66, indicating successful completion of all activities. Assessments for all items must be conducted without the use of spinal jackets or orthoses.

2.2 | Training Sessions

A standardized HFMSE manual was distributed to all networks participating in the study. As part of their network activities, evaluators from each network received training during in-person meetings conducted in the United States and Europe [16]. The evaluators responsible for the assessments included in the current study were the same individuals who participated in the reliability studies. In both Europe and the United States, evaluators undergo regular annual refresher training that involves reviewing the scale items and scoring methods.

2.3 | Statistical Analysis

Baseline participants characteristics were described as proportions (percentages) for categorical variables and means with standard deviation (SD) for continuous variables, unless otherwise stated.

Due to the presence of missing at random values, we used multiple imputation (MI) to maximize the use of available

TABLE 1 | Baseline and clinical characteristics of the population analyzed according to the SMA type.

	Overall population	Type II	Type III
	***N*=184**	***N*=73**	***N*=111**
Age at baseline (years)			
Mean (SD)	16.66 (16.09)	8.58 (7.91)	7.91 (17.83)
Median (IQR)	11.09 (4.88–22.03)	6.04 (3.04–10.70)	15.75 (8.15–34.42)
Adults, *n*, (%)	56 (30.43%)	9 (12.33%)	47 (42.34%)
Sex, *n* (%)			
Male	105 (57.07)	43 (58.90)	62 (55.86)
Female	79 (42.93)	30 (41.10)	49 (44.14)
SMA function, *n* (%)			
Non-sitter	12 (6.52)	11 (15.07)	1 (0.90)
Sitter	95 (51.63)	62 (84.93)	33 (29.73)
Walker	77 (41.85)	0 (0.00)	77 (69.37)
HFMSE at baseline			
Mean (SD)	28.73 (20.75)	10.60 (9.04)	40.66 (17.36)
Median (IQR)	25.00 (9–48.50)	8.00 (3.00–15.00)	44.00 (29.00–55.00)
SMN2 copy number, *n* (%)			
2	26 (14.13)	15 (20.55)	11 (9.91)
3	94 (51.09)	49 (67.12)	45 (40.54)
≥4	49 (26.63)	2 (2.74)	47 (42.34)
Unknown	15 (8.15)	7 (9.59)	8 (7.21)
SMA type III subtype A/B, *n* (%)			
Type IIIA			53 (47.75)
Type IIIB			47 (42.34)
Unknown			11 (9.91)
Registry, *n* (%)			
Italy	129 (70.11)	46 (63.01)	83 (74.77)
USA	55 (29.89)	27 (36.99)	28 (25.23)
Treatment duration (years)			
Mean (SD)	4.01 (0.22)	4.02 (0.22)	4.00 (0.22)
Median (IQR)	4.01 (3.90–4.16)	3.99 (3.89–4.19)	4.02 (3.90–4.15)

information. Imputation for intermediate scores (year 1, 2 or 3 of follow-up) was performed on participants who had a baseline assessment and a treatment history of at least 4 years, using chained equations approach with 10 imputations, where each incomplete variable is imputed by a separate model and implemented through the Multiple Imputation by Chained Equation algorithm. In the imputation models we included as predictors age and HFMSE at baseline, sex, SMA type and functional level, and *SMN2* copy number.

Longitudinal changes in HFMSE were analyzed using linear mixed-effects models to account for repeated measures over the follow-up period. Cumulative changes at 1, 2, 3, and 4 years from baseline were estimated by treating time as a fixed effect and including a random intercept to accommodate individual differences. All analyses were adjusted for baseline HFMSE values and the registry in which participants were enrolled.

In line with previous literature findings, the cohort was further subdivided by functional level at baseline (non-sitters, sitters, walkers), by *SMN2* copy number (2, 3, 4+), and by age at baseline (SMA II: < 5, 5–17, ≥ 18; SMA III: < 7, 7–14, ≥ 15) [5, 14]. The ability to sit independently was defined as achieving a score of 2 on item 1 of the HFMSE, while the ability to walk independently

TABLE 2 | Yearly changes.

		Mean change from baseline to 1 year (95% CI)	Mean change from baseline to 2 year (95% CI)	Mean change from baseline to 3 year (95% CI)	Mean change from baseline to 4 year (95% CI)
SMA II	Whole cohort ($N=73$)	2.92 (1.60–4.24)	3.96 (2.63–5.29)	4.18 (2.85–5.50)	4.18 (2.85–5.50)
	<5 ($N=30$)	7.00 (4.74–9.26)	9.67 (7.41–11.93)	11.30 (9.04–13.56)	12.33 (10.07–14.59)
	5–17 ($N=34$)	0.03 (−0.78–0.84)	−0.26 (−1.07–0.54)	−1.12 (−1.93 to −0.31)	−1.94 (−2.75 to −1.13)
	≥ 18 ($N=9$)	0.22 (−0.48–0.92)	0.89 (0.19–1.59)	0.44 (−0.26–1.14)	0.11 (−0.59–0.81)
SMA III	Whole cohort ($N=111$)	1.87 (0.91–2.83)	2.33 (1.37–3.29)	1.47 (0.51–2.43)	1.08 (0.12–2.04)
	A ($N=53$)	2.09 (0.50–3.69)	3.32 (1.72–4.92)	2.81 (1.21–4.41)	2.49 (0.89–4.09)
	<7 ($N=20$)	5.40 (2.68–8.12)	8.35 (5.63–11.07)	9.30 (6.58–12.02)	9.90 (7.18–12.62)
	7–14 ($N=13$)	−1.38 (−4.11–1.34)	−0.77 (−3.49–1.95)	−2.15 (−4.88–0.57)	−2.15 (−4.88–0.57)
	≥ 15 ($N=20$)	1.05 (−0.43–2.53)	0.95 (−0.53–2.43)	−0.45 (−1.93–1.03)	−1.90 (−3.38 – −0.42)
	B ($N=47$)	1.79 (0.53–3.04)	1.51 (0.25–2.77)	−0.06 (−1.32–1.19)	−0.55 (−1.81–0.70)
	7–14 ($N=10$)	2.00 (−0.38–4.38)	2.90 (0.52–5.28)	1.00 (−1.38–3.38)	1.60 (−0.78–3.98)
	≥ 15 ($N=37$)	1.73 (0.28–3.18)	1.13 (−0.32–2.59)	−0.35 (−1.80–1.10)	−1.13 (−2.59–0.32)

Note: Linear mixed effect model was used to calculate yearly changes in the whole SMA II population; due to the limited sample size, subgroups should be mainly interpreted as descriptive.

was defined as the participant's capability to walk 10 m without assistance. The age cut-off points were selected based on previous observations of progression slopes in SMA type II and III at different ages [13, 14].

A linear mixed model analysis was conducted to explore the association between the annualized slopes of HFMSE change in participants with SMA type II and type III included in the longitudinal analysis and several variables: age at baseline, sex, motor function, *SMN2* copy number, and age of symptom onset (for SMA type III, IIIA, and IIIB only).

In this study, we applied the minimal clinically important difference (MCID) cut-off, previously established and published by our group based on caregiver perception of 1-year changes [17]. Therefore, MCID cut-offs were defined as follows: for SMA II, deterioration was indicated by scores ≤ -3.5 and improvement by scores ≥ 1.17; for SMA III, deterioration was indicated by scores ≤ -3.91 and improvement by scores ≥ 1.67. The application of the MCID cut-off point to the 4-year follow-up of HFMSE changes allowed us to establish the proportion of individuals meeting the MCID at annual intervals. More specifically, the annual MCID was applied to changes in HFMSE from baseline to 1 year, then year-to-year intervals up to 4 years (1–2 years, 2–3 years, and 3–4 years).

3 | Results

A total of 469 patients with a diagnosis of SMA type II or III, treated with nusinersen and with an available baseline HFMSE score, were identified in the registry. Among these, 184 individuals, including 73 with SMA type II and 111 with SMA type III, had completed 4-year follow-up.

Among the 111 SMA type III participants, 47.75% were classified as type IIIA, while 42.34% were classified as type IIIB. The remaining 10% were older adults in whom it was not possible to retrospectively establish the onset of clinical signs before or after 3 years.

The mean time from treatment initiation to baseline HFMSE assessment was −0.20 years (SD 0.24), with a median of −0.12 years and an interquartile range (IQR) of −0.28 to −0.02 years.

None of the patients included in the analysis underwent scoliosis surgery during the follow-up period.

Table 1 outlines the baseline characteristics of the populations.

Over the 4-year period, the overall change in the cohort with SMA type II was +4.18 points (95% CI: 2.85–5.50), corresponding to an estimated annualized change of +0.96 points (95% CI: 0.66–1.27) per year. In the cohort with SMA type III was +1.08 points (95% CI: 0.12–2.04), with an estimated annualized change of +0.18 points per year (95% CI: −0.66 to 0.40) (Table 2). Only 1 of the 77 (1.30%) SMA III walker patientsSMN2 at baseline lost ambulation within the 4-year follow-up period (Figure 1).

3.1 | Predictors of Disease Progression

A linear mixed model analysis was conducted to examine the association between variables and HFMSE scores in participants with SMA types II and III included in the longitudinal analysis.

For SMA type II, the model included sex, *SMN2* copy number, age at baseline, baseline HFMSE score, time since baseline, and

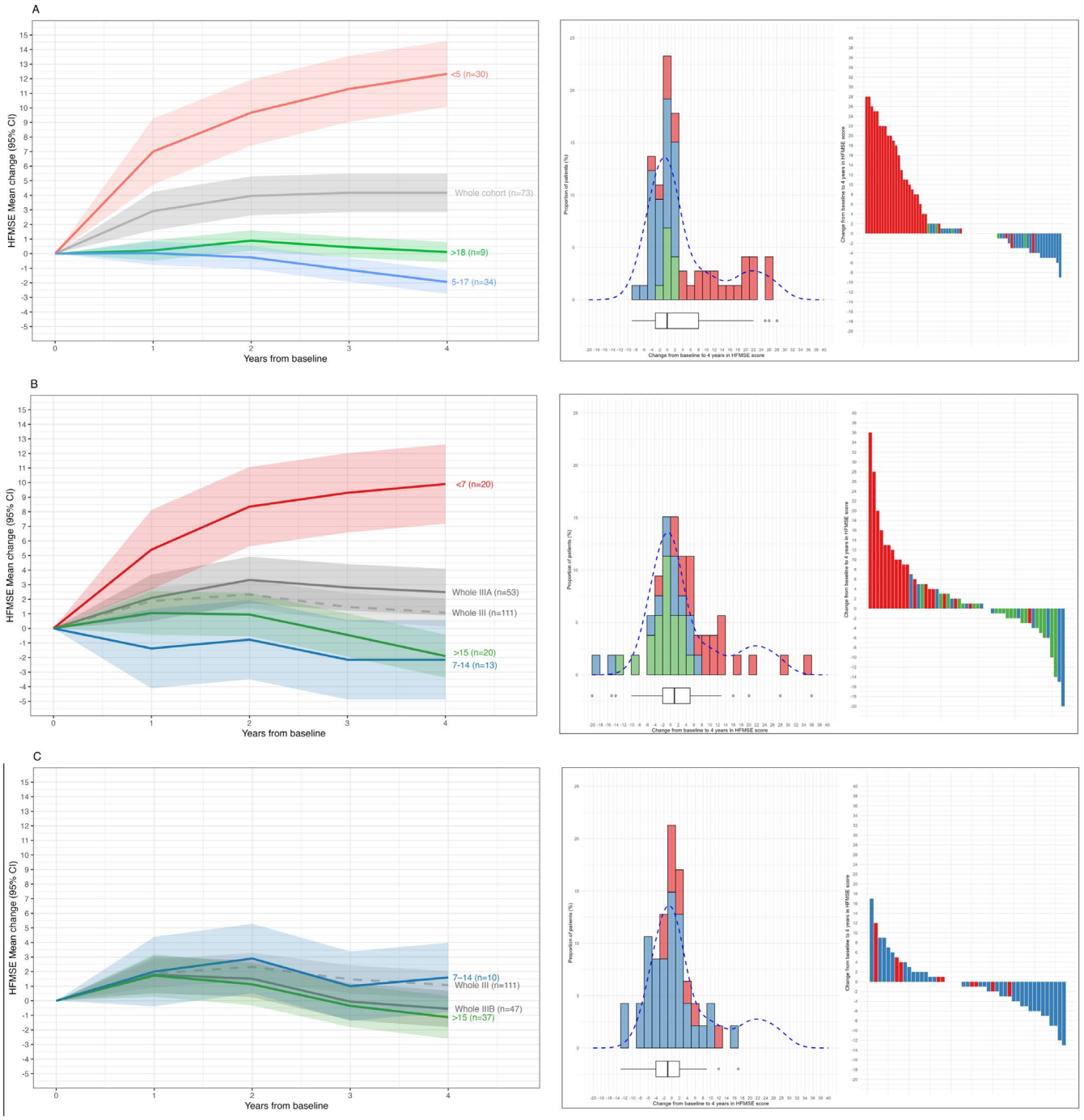

FIGURE 1 | Four-year progression in SMA Types II and III. (A) = SMA II; (B) = SMA IIIA; (C) = SMA IIIB. *Left panels*: Annual trajectories of HFMSE scores over 4 years, expressed as mean values with 95% confidence intervals. *Middle panels*: Distribution of changes in HFMSE scores from baseline to year 4. Each bar represents the proportion of patients who showed improvement or decline. The overlaid dashed line corresponds to the kernel density estimate of the distribution. Box-and-whisker plots indicate the median (central line), interquartile range (box), and values within 1.5 times the IQR (whiskers); individual outliers beyond this range are plotted as points. Mean values are marked with diamonds. *Right panels*: Individual patient-level changes in HFMSE scores from baseline. Each bar corresponds to one patient.

patient-specific random intercepts. For SMA type III, the model included sex, current functional status, *SMN2* copy number, age at baseline, subset (IIIA, IIIB), baseline HFMSE score, time since baseline, and patient-specific random intercepts.

Participants with unknown *SMN2* copy numbers and/or unknown onset (SMA II: $n = 7$; SMA III: $n = 8$) were excluded from the analysis presented in Table 3, Panel 1.

Table 3, Panel 2 presents data from the linear mixed model that was performed without using these predictors, allowing the inclusion of participants with unknown *SMN2* copy numbers and/or onset.

As a supplementary analysis to evaluate the perception of changes in HFMSE scores, previously published MCID thresholds [17] were applied to all annual intervals (baseline to 1 year,

TABLE 3 | Results of the two mixed models.

	SMA II[a] (*N* = 66)		SMA II[b] (*N* = 73)	
Variable	**Estimate (95% CI)**	***p***	**Estimate (95% CI)**	***p***
Time	1.02 (0.70–1.34)	< 0.0001*	0.96 (0.66–1.27)	< 0.0001*
HFMSE baseline	1.06 (0.91–1.22)	< 0.0001*	1.04 (0.89–1.19)	< 0.0001*
Age at baseline	−0.34 (−0.51 to −0.17)	< 0.0001*	−0.35 (−0.51 to −0.18)	< 0.0001*
Sex (Male vs Female)	−0.75 (−3.32–1.82)	0.566	−1.18 (−3.58–1.22)	0.334
Motor function (Sitter vs Non-sitter)	−1.78 (−5.54–1.97)	0.350	−2.00 (−5.56–1.56)	0.270
SMN2 Copy number		0.552		
3 vs 2	−1.38 (−4.37–1.61)			
4+ vs 2	−3.22 (−10.85–4.41)			
	SMA III[a] (*N* = 92)		**SMA III[b] (*N* = 100)**	
Variable	**Estimate (95% CI)**	***p***	**Estimate (95% CI)**	***p***
Time	0.14 (−0.12–0.39)	0.284	0.16 (−0.08–0.40)	0.184
HFMSE baseline	0.79 (0.71–0.87)	< 0.0001*	0.82 (0.75–0.89)	< 0.0001*
Age at baseline	−0.13 (−0.20 to −0.06)	0.0002*	−0.11 (−0.17 to −0.05)	0.0005*
Sex (Male vs Female)	−1.74 (−3.52–0.04)	0.055	−1.66 (−3.36–0.02)	0.053
Onset (B vs A)	1.05 (−1.33–3.43)	0.387	0.27 (−1.92–2.46)	0.807
Motor function (Walker vs Sitter)	6.96 (4.26–9.66)	< 0.0001*	6.46 (3.88–9.04)	< 0.0001*
SMN2 Copy number		0.736		
3 vs 2	0.95 (−1.85–3.73)			
4+ vs 2	1.12 (−1.73–3.96)			

[a]Linear mixed model analysis excluding patients with unknown onset and/or SMN2 copy number.
[b]Linear mixed model analysis including patients with unknown onset and/or SMN2 copy number, but excluding these as predictor variables; *$p < 0.05$.

1–2 years, 2–3 years, and 3–4 years). These results are presented in Figure S1.

3.2 | Sensitivity Analysis

An additional 163 individuals had a follow-up duration of less than 4 years after starting treatment, while 68 switched from nusinersen to risdiplam. Seven individuals discontinued treatment before reaching the 4-year mark, 20 participated in clinical trials, and in 27 cases, patients relocated from the referral center, making further follow-up unavailable. Therefore, 285 patients were not included in the main analysis.

Table 4 summarizes the key characteristics of patients with SMA II and SMA III, comparing those included in the main analysis (treated for at least 4 years) with those not included. The two groups were largely similar, with no significant differences in baseline values for age, sex, SMA function, or HFMSE scores.

As shown in Figure 2, which presents the mean HFMSE scores with 95% CI at each time point, the included patients (blue) and excluded patients (red) display nearly identical scores over time. The overlapping confidence intervals indicate no significant differences in HFMSE scores between the two groups at any time point.

4 | Discussion

The natural history of types II and III SMA with data collected before the advent of DMTs consistently shows progression of the disease. From the first studies in the early 90s [18, 19] to the most recent studies reporting data collected in the years before the therapies became available [20, 21], there is concordance that, even with the most recent standards of care, there was always some progression of functional impairment in both forms. Even the milder SMA phenotypes showed some decrease in function if a longer follow-up was considered [20, 22]. In a recent natural history study including 4-years of follow-up data from three large international networks, we reported an overall reduction in HFMSE scores over time in both SMA type II and III [23]. The study showed that the decline was age and baseline-dependent with older type II patients having already lost most of their HFMSE scores at baseline and therefore being unable to show much decline.

In the present study we report 4-year HFMSE changes following the initiation of nusinersen at the same centers in which the

TABLE 4 | Baseline demographic and clinical characteristics of the population.

	SMA II			SMA III		
	Included	Not included		Included	Not included	
	N = 73	*N* = 142	*p*	*N* = 111	*N* = 143	*p*
Age at baseline (years)						
Mean (SD)	8.58 (7.91)	10.09 (10.44)	0.474	21.98 (17.83)	19.88 (16.78)	0.304
Follow-up (years)						
Median (IQR)	5.47 (4.76–5.91)	1.88 (1.13–2.80)	< 0.0001	5.25 (4.54–5.64)	1.93 (1.04–2.86)	< 0.0001
Sex, *n* (%)						
Male	43 (58.90)	76 (53.52)	0.345	62 (55.86)	72 (50.35)	0.460
Female	30 (41.10)	66 (46.48)		49 (44.14)	71 (49.65)	
SMA function, *n* (%)						
Non-sitter	11 (15.07)	31 (21.83)	0.369	1 (0.90)	4 (2.80)	0.299
Sitter	62 (84.93)	110 (77.46)		33 (29.73)	47 (32.87)	
Walker	0 (0.00)	1 (0.70)		77 (69.37)	92 (64.34)	
HFMSE at baseline						
Mean (SD)	10.60 (9.04)	11.56 (10.75)	0.973	40.66 (17.36)	38.22 (18.39)	0.261
SMN2 copy number, *n* (%)						
1		1 (0.70)	0.114		1 (0.70)	0.029
2	15 (20.55)	13 (9.15)		11 (9.91)	7 (4.89)	
3	49 (67.12)	99 (69.72)		45 (40.54)	61 (42.66)	
≥ 4	2 (2.74)	4 (2.82)		47 (42.34)	46 (32.17)	
Unknown	7 (9.59)	25 (17.61)		8 (7.21)	28 (19.58)	
SMA type III subtype A/B, *n* (%)						
IIIA				53 (47.75)	90 (62.94)	0.052
IIIB				47 (42.34)	41 (28.67)	
Unknown				11 (9.91)	12 (8.39)	

Note: Age at baseline corresponds to time to treatment initiation.

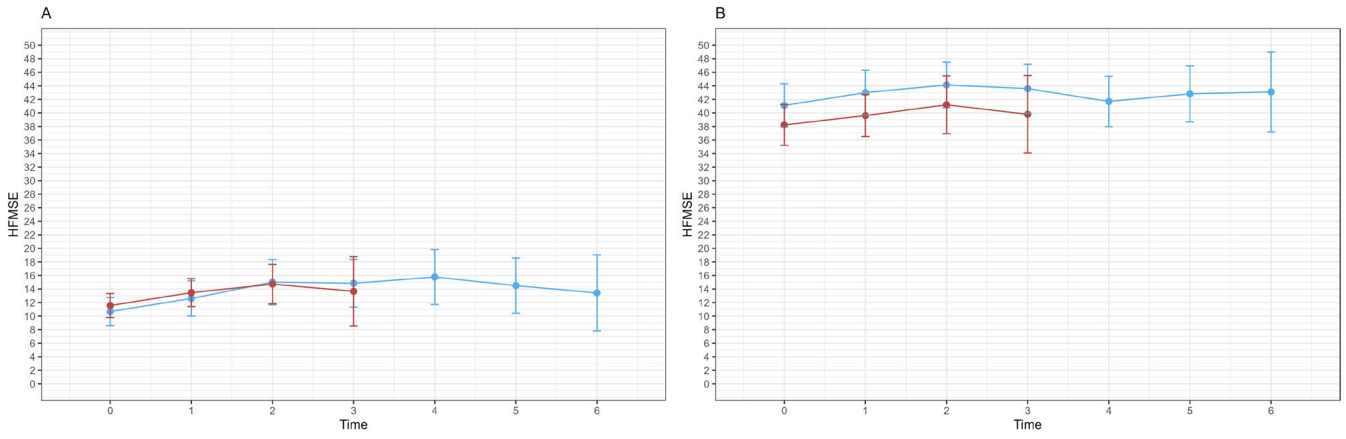

FIGURE 2 | Mean HFMSE scores with 95% CI at each time point in SMA II and SMA IIII subdivided by the included and excluded cohort. (A) = SMA II, (B) = SMA III. Blue solid line: Included patients, Red solid line: Excluded patients (red).

4-year natural history data were collected. Our findings show an overall increase of 4.18 points in the SMA type II cohort with a more obvious improvement in the first 2 years followed by a relative plateau in the third and fourth year. In SMA type III the overall increase following treatment was of 1.08 points with differences between IIIA and IIIB. While the improvement per se appears to be relatively small, this should be interpreted in relation to the loss of scores observed in natural history in the overall cohort of type III patients.

A potential limitation of this study is that we only included patients with at least 4 years of continuous treatment, which could introduce selection bias, as these individuals may only represent long-term responders. However, this risk is minimized by the sensitivity analysis conducted, which included patients with shorter follow-up or alternative treatment trajectories. The analysis showed no differences in HFMSE scores at any time point, with overlapping confidence intervals across groups, supporting the robustness of the main findings.

Not surprisingly, in agreement with previous findings from our group and from others in studies with shorter follow-up periods, the overall positive changes were mainly driven by the younger cohorts (below the age of 5 years in type II and below the age of 7 years in type III). In these age groups, their HFMSE scores showed a marked increase following the introduction of nusinersen that was well beyond the mild increase observed in our recent natural history study [23] or in any other natural history study published in which HFMSE data were available [11]. In the older treated patients, the scores remained stable or showed a tendency towards an overall mild decrease after the second year. These results are in line with the recently available long-term results from the SHINE study [24, 25]. This study, which assessed the long-term follow-up of the pivotal trials, also reported an overall impact of nusinersen on clinical progression in the whole cohort, with older patients often remaining stable or experiencing a loss of the initial gains in the fourth year. At variance with clinical trials that were performed in selected cohorts, our study includes a broader unselected cohort that also explains the wider variability of results observed at different ages and baseline values.

In our cohort, age and HFMSE scores at baseline were the best predictors of disease progression for both type II and type III. Functional status was a significant predictor only for type III, as ambulant type III individuals had better outcomes than those who had already lost ambulation at treatment initiation. Our results also highlight the importance of age when evaluating treatment outcomes and the need for careful consideration of treatment expectations. This was particularly true for those who were older at the time of treatment initiation. Older treated type II patients showed small or no HFMSE changes that were not different from those observed in the older type II untreated patients in our natural history cohort.

These findings could be partly explained by the reduced potential to rescue motor neurons progressively lost with increasing age, but they should be interpreted with caution given the limitations of the HFMSE in this age and functional subgroups. Many older patients included in the study presented with low baseline HFMSE scores or significant orthopedic complications such as scoliosis and joint contractures, which will affect the possibility of detecting meaningful changes on a gross motor scale such as the HFMSE. Other possible causes may not be directly related to SMN deficiency. Further studies using a wider range of measures, including upper limb and respiratory function and patient-reported outcome measures, may provide a more accurate evaluation across different functional aspects that may be more relevant for understanding the impact of the new therapies on different age and functional groups. These results could be better interpreted by comparing to large cohorts of untreated patients with methods such as propensity matching that could not be appropriately applied in this study.

Author Contributions

Giorgia Coratti: conceptualization, funding acquisition, writing – original draft, methodology, validation, writing – review and editing, formal analysis, data curation. **Francesca Bovis:** formal analysis, data curation, writing – original draft, writing – review and editing. **Marika Pane:** writing – review and editing, validation, supervision. **Amy Pasternak:** investigation, writing – review and editing, supervision. **Emilio Albamonte:** writing – review and editing, supervision, validation. **Irene Mizzoni:** investigation, writing – review and editing, supervision. **Allan M. Glanzman:** investigation, supervision, writing – review and editing. **Simone Morando:** investigation, writing – review and editing, supervision. **Jacqueline Montes:** investigation, writing – review and editing, supervision. **Ilaria Cavallina:** investigation, writing – review and editing, supervision. **Sally Dunaway Young:** investigation, writing – review and editing, supervision. **Tina Duong:** investigation, writing – review and editing, supervision. **Enrica Rolle:** investigation, writing – review and editing, supervision. **Matthew Civitello:** investigation, writing – review and editing, supervision. **Roberto De Sanctis:** investigation, writing – review and editing, supervision. **Chiara Bravetti:** writing – review and editing, supervision, data curation. **Federica Ricci:** writing – review and editing, supervision, validation. **Giulio Gadaleta:** validation, writing – review and editing, supervision. **Tiziana Mongini:** validation, writing – review and editing, supervision. **Maria Sframeli:** validation, writing – review and editing, supervision. **Maria Carmela Pera:** validation, writing – review and editing, supervision, data curation. **Sonia Messina:** validation, writing – review and editing, supervision. **Adele D'Amico:** validation, writing – review and editing, supervision. **Michela Catteruccia:** validation, writing – review and editing, supervision. **Noemi Brolatti:** validation, writing – review and editing, supervision. **Michio Hirano:** validation, writing – review and editing, supervision. **Zarazuela Zolkipli-Cunningham:** validation, writing – review and editing, supervision. **Basil T. Darras:** validation, writing – review and editing, supervision. **Enrico Bertini:** validation, writing – review and editing, supervision. **Claudio Bruno:** validation, writing – review and editing, supervision. **John Day:** validation, writing – review and editing, supervision. **Valeria A. Sansone:** validation, writing – review and editing, supervision. **Richard S. Finkel:** validation, writing – review and editing, supervision. **Eugenio Mercuri:** validation, writing – review and editing, supervision, conceptualization, funding acquisition, writing – original draft.

Acknowledgments

Doctor Coratti is supported by GR-2021-12374579 (Italian Health Ministry), Prof Mercuri is supported by RF-2019-12370334 (Italian Health Ministry) and Doctor Pera is supported by GR-2018-12365706 (Italian Health Ministry). Prof Bertini was supported by the Italian Ministry of Health with Current Research funds and by Ricerca Finalizzata RF-2019-12370334; E.B., A.D., M.C.; E.M., G.C., M.P., C.B. are members of the ERN (European Reference Network)-NMD. Data were available from the international SMA Registry (iSMAR), partly funded with a contribution from Biogen, Novartis and, as part

of the SMA-NH-LT study, by Roche Italia. Funders had no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication. Rafael. S. Rodriguez-Torres, Nicola Forcina, Giulia Norcia, Giulia Stanca, Sara Carnicella, Lavinia Fanelli, Antonella Longo, Michela Nani, Laura Antonaci, Giacomo De Luca, Roberto Materia.

Conflicts of Interest

Coratti G, Pane M, Pasternak A, Albamonte E, Pera MC, Glanzman A, Montes J, De Sanctis R, Duong T, Dunaway Young S, Civitello M, Sansone AV, D'Amico A, Bruno C, Messina S, Bertini E, Day J, Ricci F, Mongini T, Finkel R, and Mercuri E report personal fees for advisory boards, steering committees, speaker fees, or consultancies from BIOGEN S.R.L., ROCHE, AVEXIS and/or NOVARTIS outside the submitted work. Zolkipli-Cunningham Z reports support from CURE SMA outside the submitted work. Bovis F, Rohwer A, Darras BT, Hirano M, Sframeli M, Catteruccia M, Mizzoni I, Rolle E, Bravetti C, Cavallina I, Morando S, Brolatti N, and Salmin F have nothing to disclose.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. A. D'Amico, E. Mercuri, F. D. Tiziano, and E. Bertini, "Spinal Muscular Atrophy," *Orphanet Journal of Rare Diseases* 6 (2011): 71.

2. V. Dubowitz, "Chaos in Classification of the Spinal Muscular Atrophies of Childhood," *Neuromuscular Disorders* 1 (1991): 77–80.

3. F. Audic, S. M. Dubois, J. Durigneux, et al., "Effect of Nusinersen After 3 Years of Treatment in 57 Young Children With SMA in Terms of SMN2 Copy Number or Type," *Archives de Pédiatrie* 31 (2024): 117–123.

4. J. Cho, J. Lee, J. Kim, et al., "Nusinersen Demonstrates Effectiveness in Treating Spinal Muscular Atrophy: Findings From a Three-Year Nationwide Study in Korea," *Frontiers in Neurology* 14 (2023): 1294028.

5. M. Pane, G. Coratti, M. C. Pera, et al., "Nusinersen Efficacy Data for 24-Month in Type 2 and 3 Spinal Muscular Atrophy," *Annals of Clinical Translational Neurology* 9 (2022): 404–409.

6. R. H. Mendonca, G. J. Polido, C. Matsui, et al., "Real-World Data From Nusinersen Treatment for Patients With Later-Onset Spinal Muscular Atrophy: A Single Center Experience," *Journal of Neuromuscular Diseases* 8 (2021): 101–108.

7. M. C. Pera, G. Coratti, F. Bovis, et al., "Nusinersen in Pediatric and Adult Patients With Type III Spinal Muscular Atrophy," *Annals of Clinical Translational Neurology* 8 (2021): 1622–1634.

8. T. Hagenacker, C. D. Wurster, R. Gunther, et al., "Nusinersen in Adults With 5q Spinal Muscular Atrophy: A Non-Interventional, Multicentre, Observational Cohort Study," *Lancet Neurology* 19 (2020): 317–325.

9. E. Jochmann, R. Steinbach, T. Jochmann, et al., "Experiences From Treating Seven Adult 5q Spinal Muscular Atrophy Patients With Nusinersen," *Therapeutic Advances in Neurological Disorders* 13 (2020): 1756286420907803.

10. A. Veerapandiyan, K. Eichinger, D. Guntrum, et al., "Nusinersen for Older Patients With Spinal Muscular Atrophy: A Real-World Clinical Setting Experience," *Muscle & Nerve* 61 (2020): 222–226.

11. G. Coratti, C. Cutrona, M. C. Pera, et al., "Motor Function in Type 2 and 3 SMA Patients Treated With Nusinersen: A Critical Review and Meta-Analysis," *Orphanet Journal of Rare Diseases* 16 (2021): 430.

12. E. Mercuri, R. Finkel, M. Scoto, et al., "Development of an Academic Disease Registry for Spinal Muscular Atrophy," *Neuromuscular Disorders* 29 (2019): 794–799.

13. G. Coratti, S. Lucibello, M. C. Pera, et al., "Gain and Loss of Abilities in Type II SMA: A 12-Month Natural History Study," *Neuromuscular Disorders* 30 (2020): 765–771.

14. G. Coratti, S. Messina, S. Lucibello, et al., "Clinical Variability in Spinal Muscular Atrophy Type III," *Annals of Neurology* 88 (2020): 1109–1117.

15. J. M. O'Hagen, A. M. Glanzman, M. P. McDermott, et al., "An Expanded Version of the Hammersmith Functional Motor Scale for SMA II and III Patients," *Neuromuscular Disorders* 17 (2007): 693–697.

16. A. M. Glanzman, E. S. Mazzone, S. D. Young, et al., "Evaluator Training and Reliability for SMA Global Nusinersen Trials1," *Journal of Neuromuscular Diseases* 5 (2018): 159–166.

17. G. Coratti, F. Bovis, M. C. Pera, et al., "Determining Minimal Clinically Important Differences in the Hammersmith Functional Motor Scale Expanded for Untreated Spinal Muscular Atrophy Patients: An International Study," *European Journal of Neurology* 31 (2024): e16309.

18. K. Zerres, S. Rudnik-Schoneborn, E. Forrest, A. Lusakowska, J. Borkowska, and I. Hausmanowa-Petrusewicz, "A Collaborative Study on the Natural History of Childhood and Juvenile Onset Proximal Spinal Muscular Atrophy (Type II and III SMA): 569 Patients," *Journal of the Neurological Sciences* 146 (1997): 67–72.

19. K. Zerres, S. Rudnik-Schoneborn, and M. Rietschel, "Heterogeneity in Proximal Spinal Muscular Atrophy," *Lancet* 336 (1990): 749–750.

20. P. Kaufmann, M. P. McDermott, B. T. Darras, et al., "Prospective Cohort Study of Spinal Muscular Atrophy Types 2 and 3," *Neurology* 79 (2012): 1889–1897.

21. E. Mercuri, R. Finkel, J. Montes, et al., "Patterns of Disease Progression in Type 2 and 3 SMA: Implications for Clinical Trials," *Neuromuscular Disorders* 26 (2016): 126–131.

22. P. Kaufmann, M. P. McDermott, B. T. Darras, et al., "Observational Study of Spinal Muscular Atrophy Type 2 and 3: Functional Outcomes Over 1 Year," *Archives of Neurology* 68 (2011): 779–786.

23. G. Coratti, F. Bovis, M. C. Pera, et al., "Long-Term Natural History in Type II and III Spinal Muscular Atrophy: A 4-Year International Study on the Hammersmith Functional Motor Scale Expanded," *European Journal of Neurology* 31 (2024): e16517.

24. R. S. Finkel, D. Castro, M. A. Farrar, et al., "Interim Report on the Safety and Efficacy of Longer-Term Treatment With Nusinersen in Infantile-Onset Spinal Muscular Atrophy (SMA): Updated Results From the SHINE Study (S25.004)," *Neurology* 92 (2019): S25.004.

25. R. Finkel, "Final Safety and Efficacy Data From the SHINE Study in Participants With Infantile-Onset and Later-Onset SMA. Cure SMA Annual Conference," (2024).

Supporting Information

Additional supporting information can be found online in the Supporting Information section.