Nusinersen in type 1 Spinal Muscular Atrophy: 12-month real world data

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ABSTRACT

Objective: The aim of the study was to report 12-month changes after treatment with Nusinersen in a cohort of 85 type I Spinal Muscular Atrophy patients of age ranging from 2 months to 15 years and 11 months.

Methods: All patients were assessed using the Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP INTEND) and the Hammersmith Infant Neurological Examination-Section 2 (HINE-2).

Results: Two of the 85 had 1 *SMN2* copy, 61 had 2 copies, 18 had 3 copies. In 4 patients the *SMN2* copy number was not available. At baseline the mean CHOP INTEND scores ranged between 0 and 52 (mean: 15.66, SD: $\pm$13.48), and the mean HINE-2 score between 0 and 5 (mean: 0.69, SD:$\pm$1.23). There was a difference between baseline and the 12-month scores on both the CHOP INTEND and the HINE-2 for the whole group ($p<0.001$) as well as for the subgroups with 2 *SMN2* copies ($p<0.001$), and for those with 3 *SMN2* copies ($p<0.001$). The difference was found not only in patients younger than 210 days at baseline ($p<0.001$) but also in those younger than 5 years on the CHOP INTEND and younger than 2 years on the HINE-2.

Interpretation: Our results, expanding the age range and the severity of type I patients treated with Nusinersen over one year, provide additional data on the range of efficacy of the drug that will be helpful to make an informed decision on whether to start treatment in patients of different age and severity.

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Introduction

Spinal muscular atrophy (SMA) is a rare neuromuscular disorder caused by mutations in the *survival motor neuron 1* (*SMN1*) gene. In the last few years there has been an increasing number of therapeutic approaches in clinical trials. Following two pivotal sham controlled double blind studies in the infantile and late onset form of SMA, Nusinersen was recently approved in several countries worldwide [1, 2] and was subsequently made available for all type I patients as part of an Expanded Access Programme (EAP) [3]. This has been an opportunity to assess the efficacy of the drug in a real world setting in a large number of type I patients with a much wider spectrum of age [3] than those enrolled in the ENDEAR study, that only included patients below the age of 7 months, also excluding the patients with severe neonatal onset and with 3 survival motor neuron 2 *(SMN2)* copies. There has been concern that the drug may not show similar efficacy in older patients or in those with a more severe phenotype who were not included in the pivotal study.

The recently published preliminary data in an Italian cohort and in other European countries [4-8] showed promising results even in older infants and children. This was however mostly limited to the initial response in the first 6 months of treatment. A number of questions such as the long term efficacy in relation to the severity of the phenotype or to the *SMN2* copy number have not been fully addressed.

The aim of our study is to report the 12-month follow up, therefore with a duration similar to the ENDEAR study, in a cohort of type I patients with a much wider range of age, severity and *SMN2* copy numbers than those selected for the ENDEAR study.

More specifically, we wished i) to establish if the data in the infants assessed below the age of 7

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months (210 days) were consistent with the ENDEAR study; ii) to assess whether there was any improvement in older children and, if present, if this was related to age, severity, or number of *SMN2* copies; iii) if a longer follow up will help to better define the magnitude of changes. Finally, we also aimed to establish, using dedicated patient reported questionnaires, whether possible improvements on the functional scales were related to the carers' perception of clinically meaningful improvements.

Methods

Patients were initially included in the study following a nationwide search of patients with type I SMA. The details of the EAP approach in Italy have already been reported [3]. As part of the EAP, before Nusinersen became commercially available, the patients had access to the treatment only in the 5 Italian centers previously involved in Nusinersen trials. The results of the first 6 months have recently been published [3].

Of the 104 SMA I patients followed up in the first 6 months of EAP, 100 agreed to continue to be treated while the other 4 decided to interrupt the treatment. In 2 of the 4 because the results did not meet their expectations, in 1 because of the burden related to the procedure and of traveling and, in the other 1, because of the onset of a concomitant disease unrelated to SMA or to the treatment. Of the remaining 100, 4 died, 1 failed a recent scheduled appointment because of intercurrent illness and 26 moved to other centers, as in the last year Nusinersen has become commercially available and could be administered also in other centers. Twelve of the 26 moved to 3 centers (Turin, Trieste, Milan) where the physiotherapist had been fully trained by the original

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participating centers as part of a new prospective study. Data from these centers were considered to be reliable and were therefore included in our follow up study. Data from the remaining 14 patients followed in other centers were not included.

This resulted in 81 patients with full 12-month follow up. Four additional patients who were not reported at 6 months, because too young at that time, had also completed the 12-month assessments and were also included, this resulting in a final cohort of 85 patients.

The study was approved by the Institutional Review Board (Ethics committee) in each center. Written informed consent was obtained from all participants (or guardians of participants) in the study (consent for research).

All patients were assessed using both the Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP INTEND)[9, 10], that includes 16 items with a total score between 0 and 64, and the developmental section of the Hammersmith Infant Neurological Examination (HINE-2), that includes 8 selected motor items scored accordingly to the gradient of normal maturation[11]. Measures were performed by clinical evaluators after training and reliability sessions[6].

The assessments were always performed after 12 months from the first dose of Nusinersen, between the 6th dose of Nusinersen and before the 7th dose, generally performed at 14 months.

Data on nutritional and respiratory support were also noted.

Parent reported questionnaires

A questionnaire was developed and administered to parents/caregiver in order to identify their perception on whether there had been any change that was clinically meaningful to them after 1

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year of treatment. At the end of the questionnaire that included details of specific activities with open and closed questions (analysis in progress) there was a final section with 3 questions asking the parent/caregiver to report:

1) if there had been any change in general function

(a) stable;

(b) improved;

(c) deteriorated.

2) if there was an improvement, to clarify whether this was mainly related to

a) respiratory;

b) motor function;

c) swallowing and speech.

3) if there was a deterioration, to clarify whether this was mainly related to

a) respiratory;

b) motor function;

c) swallowing and speech.

The survey was carried out in all the centers. At the time of filling the questionnaire the families were not informed of the results of the functional assessments.

Statistical analysis

The cohort was stratified according to the criteria used in the 6 months follow up study[6]. This included subdividing them according to the copy number, to the age when they started treatment

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and to the severity of the disease. The cohort was subdivided using the Dubowitz decimal classification[12] , classifying as 1.1 the infants at the more severe end of the spectrum, with severely reduced mobility at birth and early respiratory and bulbar difficulties; as 1.5 as those with the most common phenotype in type I SMA, with inability to raise the legs against gravity or maintain the head posture but having, at diagnosis, no difficulty with feeding and swallowing, and no obvious respiratory distress; and as 1.9 the mildest phenotypes, often diagnosed after the first few months, who achieve some head control and have less respiratory compromise.

Variables were described by mean and standard deviation. Paired sample T-Test or repeated measures analysis of variance (ANOVA) were used to compare mean score of both CHOP INTEND and HINE-2, between baseline and 12-month follow-up. Spearman test to measures the correlation between non parametric variables.

Two separate multiple linear regression model were performed to predict the response to treatment. There are two response variables: the first one is the difference between the CHOP INTEND score at 12 months and the CHOP INTEND score at baseline, the second one the difference between HINE-2 score at 12 months and HINE-2 score at baseline.

The following variables were included in both regression models as predictors: age at start of treatment, score at baseline, severity of diseases type, *SMN2* copy number.

Version 23 of the SPSS software (SPSS, Inc.) was used for all statistical analyses, setting the significance at $p < 0.05$.

Results

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The final cohort includes 85 patients with full 12-month assessments. Their age ranged from 2 months to 15 years and 11 month (mean: 4,70; SD: 4,21).

Two patients had 1 *SMN2* copy, 61 had 2 copies and 18 had 3 copies. In 4 patients the *SMN2* copy number was not available.

In agreement with the Dubowitz decimal classification[12], in our cohort 8 of the 85 were classified as 1.1, 48 as 1.5 and 29 as 1.9.

CHOP INTEND

At baseline the CHOP INTEND scores ranged between 0 and 52 (mean: 15.66, SD: ±13.48).

After 12 months the scores ranged between 0 and 64 (mean: 21.14, SD: ±18.23) (Table 1).

The 12-month changes ranged between -6 and 32 (mean: 5.48, SD: ±7.62). Twelve patients (14.11%) showed negative changes between -1 and -6 (mean: -2, SD: ±1.47). Another 20 (23.52%) remained stable or had one-point improvement. Of the remaining 53 (62.35%), 9 improved 2 points, 44 more than 2 points and 38 more than or equal 4 points. (Figure 1)

HINE-2

At baseline the HINE-2 scores ranged between 0 and 5 (mean: 0.69, SD: ±1.23).

At 12 months the scores ranged between 0 and 16 (mean: 2.16, SD: 3.58) (Table 1).

The 12-month changes ranged between -3 and 12 (mean: 1.34, SD: ±2.90). Four patients (4.70%) showed negative changes. Fifty-three (62.35%) remained stable, 7 had one-point (8.23%), 5 (5.88%) improved 2 points and 16 (18.82%) more than 2 points. Improvements were observed in

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the items assessing head control and sitting with 16 patients achieving sitting independently. An additional patient, classified as 1.9 and treated at 8 months, achieved standing.

Of the 16 infants who achieved a full score for sitting on the HINE-2, 8 were classified as 1.5 and 8 as 1.9. Seven of the 8 classified as 1.5 were treated within 7 months of age and 1 between 6 and 24 months (10 months). Three of the 8 classified as 1.9 were treated within 7 months and the other 5 between 6 and 24 months (11, 9, 8 respectively).

Statistical analysis

Using a paired T-test, there was a significant difference between baseline and 12-month scores on both the CHOP INTEND and the HINE-2 for the whole group ($p<0.001$).

The multiple regression models show that the indipendent variables included in the model (age at baseline, baseline score, *SMN2* copy numbers and severity of disease) predict CHOP INTEND changes ($p<0.0005$) and HINE-2 scores changes ($p<0.0005$) after 12 months of treatment.

On the CHOP INTEND, the multiple correlation coefficient is $R=0.938$; R^2 for overall model was 93.8%, with an adjusted $R^2=87.9\%$ (87.9% of the dependent variable variance is explainable by the independent variables). Copy numbers and CHOP INTEND baseline score did not give a statistically significant contribution to the dependent variable. Age at baseline had an higher impact than other variables by comparing the standardized coefficients.

On the HINE-2, the multiple correlation coefficient is $R=0.733$; R^2 for overall model was 53.7%, with an adjusted $R^2=50.2\%$ (50.2% of the dependent variable variance is explainable by the

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independent variables). Copy numbers and HINE-2 baseline score did not give a statistically significant contribution to the dependent variable. Age at baseline had an higher impact than other variables by comparing the standardized coefficients. Table 2 shows details of the multiple correlations.

Nutritional support

At baseline, 42/85 infants had a gastrostomy inserted or planned in the near future, 7 additional children required gastrostomy between baseline and 12 months. More accurate details on nasogastric tube or swallowing abnormalities are being collected and will be described separately.

Respiratory support

At baseline 50 were on spontaneous breathing, 8 used noninvasive ventilation less than 10 hours, 19 used noninvasive ventilation more than 10 hours and 8 had a tracheostomy. At the last follow up, 20 required longer use of noninvasive ventilation, and tracheostomy was needed in another 2. Two patients showed a reduction in the number of hours on non-invasive ventilation. A more detailed analysis of the respiratory findings is in progress and will be reported separately.

12-month changes and severity

Using a paired T-test, there was a significant difference between baseline and 12-month scores on both the CHOP INTEND and the HINE-2 for the 1.5 ($p<0.001$) and 1.9 ($p<0.001$) subgroups but not for the 1.1 ($p>0.05$). Table 1 and figure 2 report data from the CHOP INTED and the HINE-2 at

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different time points.

On the ANOVA test there was a difference between the 3 severity subgroups on both CHOP INTEND and HINE-2 ($p<0.001$). There was a progressive number of patients improving more than 2 points with decreasing severity. Figure 2A and C show details of the changes in CHOP INTEND scores in the 3 subgroups. Figure 2B and D show details of the changes in HINE-2 scores in the 3 subgroups.

12-month changes and SMN2 copy number

Figure 3 shows CHOP INTEND and HINE-2 progression from baseline to 12 months for SMA patients subdivided by *SMN2* copy number. There was a significant difference between baseline and 12 months on the CHOP INTEND for both the patients with 2 *SMN2* copies ($p<0.001$), and for those with 3 *SMN2* copies ($p<0.001$). Significant difference was also found between baseline and 12 months also on HINE-2 for both the patients with 2 *SMN2* copies ($p=0.002$), and for those with 3 *SMN2* copies ($p=0.012$).

12-month changes and age at start of treatment

Of the 85 patients 6 were younger than 210 days, 23 younger than 2 years, 20 were between 2 years and 4 years 11 months, 29 were between 5 and 11 years 11 months, 5 aged above 12 years. Fig 3 shows details of the changes in scores in different age subgroups.

There was a difference between baseline and 12 months on the CHOP INTEND in patients younger than 5 years (younger than 210 days $p=0.006$; younger than 2 years $p= 0.001$; between 2 and 4

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years p=0.001) but not in those older than 5 (between 5-12 years p=0.576; older than 12 years p=0.763). There was a significant difference between baseline and 12 months on the HINE-2 in patients younger than 2 years (younger than 210 days p=0.011; younger than 2 years p= 0.001;) but not in those older than 2 (between 2 and 4 years p=0.226; between 5-12 years p=0.791; older than 10 years p=1).

Parent/Patient reported questionnaires

On the 72 questionnaires filled by carers of patients followed in the 5 centers who participated to the survey, no one reported a decrease in function, eleven reported an overall stability, and sixty-one a general increase in function. Fifty-three out of 61 reported that the overall perception of improvement was mainly related to an improvement in motor function (85.24%) while the remaining 8 (13.11%) reported that the improvement was due to a combination of factors including motor, respiratory and swallowing.

The mean changes on the CHOP INTEND were 1.45 (SD $\pm$2.84) in the 11 patients in whom an overall stability was reported, and 6.49 (SD $\pm$8.28) in the 53 in whom an overall improvement was reported.Spearman test evidenced a correlation between CHOP INTEND score changes and answers of parents questionnaires (p=0.024).

Discussion

The regulatory approval of Nusinersen after two pivotal trials in infants and young children has been followed by different modalities of access to the drug in different countries. While some

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have granted access to the drug only in type I infants with the same criteria used in the ENDEAR clinical trial, others have granted a much wider label, including all types of SMA at all ages. This has raised some concern, especially in type I SMA, that older children may have lost the therapeutic window and show little or no response to treatment[5].

Furthermore, the possibility to administer the drug in a large number of centers in each country increases the difficulties in getting reliable assessments by trained physical therapists and to share data. Our experience of EAP in Italy provided the opportunity to collect prospective longitudinal data in a large cohort of patients with a much wider range of age and severity than those included in the ENDEAR study, while retaining a structured 'trial-like' approach sharing measures and training and reliability sessions among the centers involved in the data collection. Our results show that despite the wide age and severity range, after 12 months of treatment (6 infusions), there was a mean improvement on the CHOP INTEND of 5.46 points.

It is of note that less than 15% of the 85 patients had negative changes and half of them had lost 1 point only. This is different from what reported in untreated infants and children in natural history studies[13-16]. In contrast, over 60% of the 84 patients improved 2 points and over 50% more than 2 points. The improvements on their CHOP INTEND were more obvious after 6 months, but a further smaller increase was also found between 6 and 12 months.

The magnitude of changes were smaller on the HINE-2, with 25% improving at least 2 points but with a steady mild increase also between 6 and 12 months, suggesting that achieving even partial milestones may be a more complex task, especially in older patients, and may take longer than showing an improvement on the items of the functional scale.

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As expected, on both measures the changes were more obvious in the infants below 7 months of age. The overall results in age group show a clear trend of improvement on both CHOP INTEND and HINE-2 that is different from the negative changes observed in the untreated group in the ENDEAR study [1]and in the recent natural history studies[15-16].

An improvement was however also found in the infants treated between 7 and 24 months on both scales and some improvement, could also be found in the older children on the CHOP INTEND.

Other factors also appeared to be important in determining the possibility of an improvement. The level of increase partly reflected the severity of the phenotype.

In our study we did not have any patients with the most severe phenotype with prenatal onset, labelled as type 0 [17] but we included infants with the very severe phenotype with early onset, classified as 1.1, [12, 15] or I A[18]. These were excluded by the ENDEAR study because thought to be too severe and with too small residual motoneuron activity to be compatible with possible functional improvements. The overall difference between baseline and 12 months was not significant for this subgroup even though some improvement (1, 3 and 5 points respectively) could be noted in 3 of the 5 severely affected infants treated below the age of 2 years. In contrast, the overall difference between baseline and 12 months was significant for the more common (1.5) and the mildest phenotypes (1.9).

The 12-month results in patients with 3 *SMN2* copies expand the findings reported in the ENDEAR study[1], that only included infants with 2 *SMN2* copy numbers. Both subgroups with 2 and 3 copies showed a difference between baseline and 12 months ($p<0.001$).

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Our results therefore confirm the improvement observed after 6 months of treatment, and provide evidence, that, as observed in the ENDEAR study, further improvement can be observed between 6 and 12 months on both measures.

The longer duration of the follow up also allowed to reduce the risk that the initial response may be due to a placebo effect that is more frequent in the first months after treatment.

Previous studies have reported electrophysiological evidence of extremely reduced motoneuron activity after the first months in type I SMA[19] and have also suggested that in type I SMA, the therapeutic window can be hypothesized to be, optimally, within 1 month of age in order to have a motor function response within the first 3 to 6 months of age[20]. As also recently suggested by other studies[4-8], our results provide further evidence that the therapeutic window may be larger than what expected. Our results, expanding the age range and the severity of type I patients treated with Nusinersen, provide additional data that will be helpful for other physicians and families to make an informed decision on whether to start treatment depending on the clinical and genetic features.

At the time the drug became commercially available for all SMA patients in our country, it has been difficult to counsel the families of patients who were older of more severe than those reported in the ENDEAR study, as we could only report data from the pivotal study. Our attitude was to be very cautious on not creating high expectations. In our experience, with the exceptions of the few patients with severe phenotype treated after the first 2 years, some improvement, even if limited to very few points, was often observed in the older patients with the non-severe phenotype at onset. These results are promising and are consistent with what the families have

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reported to be clinically meaningful [21] but should be integrated with other measures assessing care burden and other aspects of activities of daily living.

Unfortunately at the time the EAP started, because of limited funding and for the large number of patients followed in each center, it was not feasible to consistently perform CMAP or MUNE to have objective measurements of the residual motoneuron activity at baseline and of possible changes over time, or to use recent biomarkers like Phosphorylated Neurofilament Heavy Chain [22] that may have helped to better understand the mechanisms underlying the changes observed in our cohort.

Following the commercial availability of Nusinersen there has been discussion on whether such small changes observed in some patients justify the cost of the drug or the effort for the patient and the family to undergo repeated intrathecal procedures and frequent multiple visits to the hospital[23, 24].

In our experience less than 7.5% of the initial cohort left the program, 4.1% immediately after they started and only 3.3% in the follow up. Using a patient reported questionnaire to investigate if they felt there was a functional improvement that was clinically meaningful to them, 73.61% of the responders who filled the questionnaire felt that there had been an improvement that was mainly related to motor function. Interestingly there was a good concordance between the findings reported by the family and those measured by the functional scales. Further studies, using more structured tools, will help to further investigate the impact of the new therapy on patients and carers' quality of life.

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Author Contribution:

MP, GC, VAS, SM, CB, MC, MS, EA, MP, ADA, BB, GB, PT, FS, RDS, MP, OG, EB, GV, FDT, EM contributed to the conception and design of the study; MP, GC, VAS, SM, CB, MC, MS, EA, MP, ADA, PT, FS, RDS, SL, EB, GV, EM, contributed to the acquisition and analysis of data; MP, GC, VAS, SM, CB, MC, MS, EA, MP, ADA, CB, BB, GB, PT, FS, RDS, SL, MP, OG, EB, GV, FDT, EM contributed to drafting the text and preparing the figures.

The full list of the Italian EAP working group is provided in the supplementary table.

Potential conflicts of interest:

MS, MP, BB, GB, PT, FS, SL, MP, OG, CB have nothing to report.

MP, GC, VAS, SM, CB, MC, EA, ADA, RDS, FDT, GV have received funding from Biogen as speakers in sponsored symposia.

ADA, CB, EM, EB, SM are principal investigator for ongoing Ionis Pharmaceuticals, Inc./Biogen clinical trials.

EB, EM received funding as members of advisory boards and received grants for SMA studies from Biogen, Ionis Pharmaceuticals, Inc. .

Biogen, Ionis Pharmaceuticals, Inc. owns patent rights to Nusinersen, a drug used in this study.

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References:

1. Finkel RS, Mercuri E, Darras BT, et al. Nusinersen versus Sham Control in Infantile-Onset Spinal Muscular Atrophy. N Engl J Med. 2017 Nov 2;377(18):1723-32.

2. Mercuri E, Darras BT, Chiriboga CA, et al. Nusinersen versus Sham Control in Later-Onset Spinal Muscular Atrophy. N Engl J Med. 2018 Feb 15;378(7):625-35.

3. Messina S, Pane M, Sansone V, et al. Expanded access program with Nusinersen in SMA type I in Italy: Strengths and pitfalls of a successful experience. Neuromuscular disorders : NMD. 2017 Dec;27(12):1084-6.

4. Aragon-Gawinska K, Seferian AM, Daron A, et al. Nusinersen in spinal muscular atrophy type 1 patients older than 7 months: A cohort study. Neurology. 2018 Aug 29.

5. Gidaro T, Servais L. Nusinersen treatment of spinal muscular atrophy: current knowledge and existing gaps. Developmental medicine and child neurology. 2018 Sep 17.

6. Pane M, Palermo C, Messina S, et al. Nusinersen in type 1 SMA infants, children and young adults: Preliminary results on motor function. Neuromuscul Disord. 2018 Jul;28(7):582-5.

7. Pechmann A, Langer T, Schorling D, et al. Evaluation of Children with SMA Type 1 Under Treatment with Nusinersen within the Expanded Access Program in Germany. J Neuromuscul Dis. 2018;5(2):135-43.

8. Pechmann A, Langer T, Wider S, Kirschner J. Single-center experience with intrathecal administration of Nusinersen in children with spinal muscular atrophy type 1. European journal of paediatric neurology : EJPN : official journal of the European Paediatric Neurology Society. 2018 Jan;22(1):122-7.

9. Glanzman AM, Mazzone E, Main M, et al. The Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP INTEND): test development and reliability. Neuromuscular disorders : NMD. 2010 Mar;20(3):155-61.

Accepted Article

This article is protected by copyright. All rights reserved.

10. Glanzman AM, McDermott MP, Montes J, et al. Validation of the Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP INTEND). Pediatric physical therapy : the official publication of the Section on Pediatrics of the American Physical Therapy Association. 2011 Winter;23(4):322-6.

11. Haataja L, Mercuri E, Regev R, et al. Optimality score for the neurologic examination of the infant at 12 and 18 months of age. The Journal of pediatrics. 1999 Aug;135(2 Pt 1):153-61.

12. Dubowitz V. Chaos in classification of the spinal muscular atrophies of childhood. Neuromuscular disorders : NMD. 1991;1(2):77-80.

13. Kolb SJ, Coffey CS, Yankey JW, et al. Natural history of infantile-onset spinal muscular atrophy. Annals of neurology. 2017 Dec;82(6):883-91.

14. Finkel RS, McDermott MP, Kaufmann P, et al. Observational study of spinal muscular atrophy type I and implications for clinical trials. Neurology. 2014 Aug 26;83(9):810-7.

15. De Sanctis R, Pane M, Coratti G, et al. Clinical phenotypes and trajectories of disease progression in type 1 spinal muscular atrophy. Neuromuscul Disord. 2018 Jan;28(1):24-8.

16. De Sanctis R, Coratti G, Pasternak A, et al. Developmental milestones in type I spinal muscular atrophy. Neuromuscular disorders : NMD. 2016 Nov;26(11):754-9.

17. MacLeod MJ, Taylor JE, Lunt PW, et al. Prenatal onset spinal muscular atrophy. European journal of paediatric neurology : EJPN : official journal of the European Paediatric Neurology Society. 1999;3(2):65-72.

18. Finkel R, Bertini E, Muntoni F, et al. 209th ENMC International Workshop: Outcome Measures and Clinical Trial Readiness in Spinal Muscular Atrophy 7-9 November 2014, Heemskerk, The Netherlands. Neuromuscular disorders : NMD. 2015 Jul;25(7):593-602.

19. Swoboda KJ, Prior TW, Scott CB, et al. Natural history of denervation in SMA: relation to age, *SMN2* copy number, and function. Annals of neurology. 2005 May;57(5):704-12.

Accepted Article

This article is protected by copyright. All rights reserved.

20. Govoni A, Gagliardi D, Comi GP, Corti S. Time Is Motor Neuron: Therapeutic Window and Its Correlation with Pathogenetic Mechanisms in Spinal Muscular Atrophy. Mol Neurobiol. 2018 Aug;55(8):6307-18.

21. Kayadjanian N, Burghes A, Finkel RS, et al. SMA-EUROPE workshop report: Opportunities and challenges in developing clinical trials for spinal muscular atrophy in Europe. Orphanet journal of rare diseases. 2013 Mar 20;8:44.

22. Darras BT, Finkel R , Mercuri, E et al. Neurofilament as a Potential Biomarker for Spinal Muscular Atrophy. Ann Clin Trans Neurol, in press.

23. Lee M, Jr., Franca UL, Graham RJ, McManus ML. Pre-Nusinersen Hospitalization Costs of Children With Spinal Muscular Atrophy. Pediatr Neurol. 2018 Nov 22.

24. Vukovic S, McAdam L, Zlotnik-Shaul R, Amin R. Putting our best foot forward: Clinical, treatment-based and ethical considerations of nusinersen therapy in Canada for spinal muscular atrophy. J Paediatr Child Health. 2019 Jan;55(1):18-24.

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Figure legends:

Figure 1: Individual details of the CHOP INTEND changes according to severity, graded according to the Dubowitz classification, age (1a) and baseline (1b) values. Key to figures: ▲: 1.1(n:8), ●: 1.5(n:48), □: 1.9(n:29).

Figure 2: (A & B) CHOP INTEND and HINE-2 longitudinal data on the cohort subdivided by decimal classification. Error bars represents 95% confidence intervals. Key to figures: —: SMA type 1.1 (n:8) ; ---: SMA type 1.5 (n:48) ⋯⋯: SMA type 1.9 (n:29). (C & D) CHOP INTEND and HINE-2 score changes description on the cohort subdivided by decimal classification. Key to figures: ■ Score >2; ■ Score 2; ■ Score 1; ■ Score 0; ■ Score <0.

Figure 3: (A & B) CHOP INTEND and HINE-2 longitudinal data on the cohort subdivided by age bands. Error bars represents 95% confidence intervals. Key to figures: —: <210days (n:6) ; —: <2 years (n:25); -·-: 2-4 years (n:20); ---: 5-12 years (n:29) ⋯⋯: ≥12 years (n:5) (C & D) CHOP INTEND and HINE-2 score changes description on the cohort subdivided by age bands. Key to figures: ■ Score >2; ■ Score 2; ■ Score 1; ■ Score 0; ■ Score <0.

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Tables:

Table 1: Longitudinal data on CHOP INTEND and HINE-2

Longitudinal data on CHOP INTEND and HINE-2			Age at baseline (years, months)	BASELINE		6 months		9 months		12 months	
				CHOP INTEND	HINE-2	CHOP INTEND	HINE-2	CHOP INTEND	HINE-2	CHOP INTEND	HINE-2
All (n:85)	**All copies (n:85)**	*Mean*	4,75	15,48	0,68	19,50	1,50	20,46	1,96	20,92	2,16
		SD	4,21	13,40	1,23	16,19	2,26	17,40	3,19	18,16	3,58
		range	0,22-15,94	0-52	0-5	0-56	0-11	0-64	0-15	0-64	0-16
	1 copy (n:2)	*Mean*	3,97	3,5	0,00	6,50	0,00	5,00	0,00	2,50	0,00
		SD	4,37	4,95	0,00	9,19	0,00	7,07	0,00	3,54	0,00
		range	0,88-7,06	0-7	0,00	0-13	0-0	0-10	0,00	0-5	0,00
	2 copies (n:61)	*Mean*	5,09	11,98	0,41	16,00	1,11	16,83	1,42	17,10	1,58
		SD	4,40	11,22	0,92	14,95	1,97	16,32	2,69	16,88	2,97
		range	0,27-15,94	0-39	0-4	0-48	0-7	0-55	0-12	0-55	0-12
	3 copies (n:19)	*Mean*	3,30	28,42	1,58	32,68	3,00	35,00	4,11	36,47	4,71
		SD	3,12	12,90	1,68	13,90	2,75	14,06	4,10	14,54	4,78
		range	0,22-10,56	3-52	0-5	5-56	0-11	3-64	0-15	3-64	0-16
	Nd (n:3)	*Mean*	8,52	12,67	1,00	13,67	1,00	11,33	1,00	12,33	0,33
		SD	4,06	11,72	1,73	15,89	1,73	11,85	1,73	14,47	0,58
		range	3,88-11,37	4-26	0-3	4-32	0-3	4-25	0-3	3-29	0-1
1,1 (n:8)	**All copies (n:8)**	*Mean*	2,60	4,88	0,00	6,13	0,00	5,50	0,00	5,88	0,00
		SD	2,23	4,52	0,00	5,51	0,00	5,35	0,00	5,00	0,00
		range	0,62-5,92	0-13	0-0	0-16	0-0	0-15	0-0	0-12	0-0
	1 Copy (n:1)	*Mean*	0,88	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
		SD	-	-	-	-	-	-	-	-	-
		range	-	-	-	-	-	-	-	-	-
	2 copies (n:7)	*Mean*	2,85	5,57	0,00	7,00	0,00	6,29	0,00	6,71	0,00
		SD	2,29	4,39	0,00	5,32	0,00	5,25	0,00	4,75	0,00
		range	0,62-5,92	0-13	0-0	0-16	0-0	0-15	0-0	0-12	0-0
1,5 (n:48)	**All copies (n:48)**	*Mean*	5,55	10,48	0,10	14,78	0,92	15,85	1,06	15,94	1,25
		SD	4,71	10,24	0,47	14,59	1,88	15,78	2,29	16,60	2,62
		range	0,22-15,94	0-42	0-3	0-54	0-7	0-50	0-8	0-56	0-10
	1 Copy (n:1)	*Mean*	7,06	7,00	0,00	13,00	0,00	10,00	0,00	5,00	0,00
		SD	-	-	-	-	-	-	-	-	-
		range	-	-	-	-	-	-	-	-	-
	2 copies (n:39)	*Mean*	5,99	9,26	0,13	12,81	0,79	13,95	1,00	13,90	1,14
		SD	4,80	9,30	0,52	13,42	1,79	15,18	2,36	15,43	2,64
		range	0,27-15,94	0-37	0-3	0-43	0-7	0-50	0-8	0-51	0-10
	3	*Mean*	3,68	19,86	0,00	27,00	1,86	28,71	1,83	30,71	2,60

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	copies (n:7)	*SD*	4,58	12,35	0,00	17,29	2,41	15,61	2,23	17,92	2,79
		range	0,22-10,56	3-42	0-0	5-54	0-5	3-47	0-5	3-56	0-6
	Nd (n:1)	*Mean*	3,88	4,00	0,00	4,00	0,00	4,00	0,00	3,00	0,00
		SD	-	-	-	-	-	-	-	-	-
		range	-	-	-	-	-	-	-	-	-
1,9 (n:29)	**All copies (n:29)**	*Mean*	4,03	26,41	1,83	30,69	2,90	32,07	3,90	33,31	4,10
		SD	3,45	12,72	1,47	14,17	2,50	15,57	3,86	15,97	4,41
		range	0,44-12,19	1-52	0-5	1-56	0-11	1-64	0-15	2-64	0-16
	2 copies (n:15)	*Mean*	3,86	22,07	1,33	28,07	2,47	29,07	3,13	30,27	3,40
		SD	3,49	12,09	1,29	15,10	2,23	16,22	3,34	17,24	3,60
		range	0,44-12,19	1-39	0-4	1-48	0-6	1-55	0-12	2-55	0-12
	3 copies (n:12)	*Mean*	3,10	33,42	2,50	36,00	3,67	38,67	5,25	39,83	5,58
		SD	2,32	10,74	1,45	10,99	2,81	12,28	4,41	11,72	5,25
		range	0,71-8,55	19-52	0-5	21-56	1-11	24-64	1-15	26-64	1-16
	Nd (n:2)	*Mean*	10,85	17,00	1,50	18,50	1,50	15,00	1,50	17,00	0,50
		SD	0,73	12,73	2,12	19,09	2,12	14,14	2,12	16,97	0,71
		range	10,34-11,37	8-26	0-3	5-32	0-3	5-25	0-3	5-29	0-1

Table 1: Baseline and follow-up scores details up to 12 months on the CHOP INTEND and HINE-2 subdivided by decimal classification and *SMN2* copy numbers.

Table 2: Summary of Regression Analyses for Variables Predicting CHOP INTEND AND HINE-2

change

	CHOP INTEND				**HINE-2**			
	B	**p-value**	**Standard error**	**Standardized B Coefficients**	**B**	**p-value**	**Standard error**	**Standardized B Coefficients**
Constant	8,241	0,001	2,222		2,556	0,000	0,593	
Severity of disease	-5,719	**0,045**	2,842	-0,215	-1,697	**0,049**	1,070	-0,166
Copy numbers	-1,375	0,512	2,087	-0,076	-0,103	0,894	0,773	-0,015
Age at baseline	-0,766	**0,002**	0,240	-0,400	-0,301	**0,000**	0,077	-0,409
Baseline score	0,145	0,123	0,093	0,256	0,629	0,060	0,329	0,267
	R2=0,879; F(5,71)=6.609; p<0.0005				R2=0,537; F(5,71)=6.120; p<0.0005			

Table 2: Results of the two multiple linear regression model. Significant p-value are in bold and show the variables that predict the response variable.

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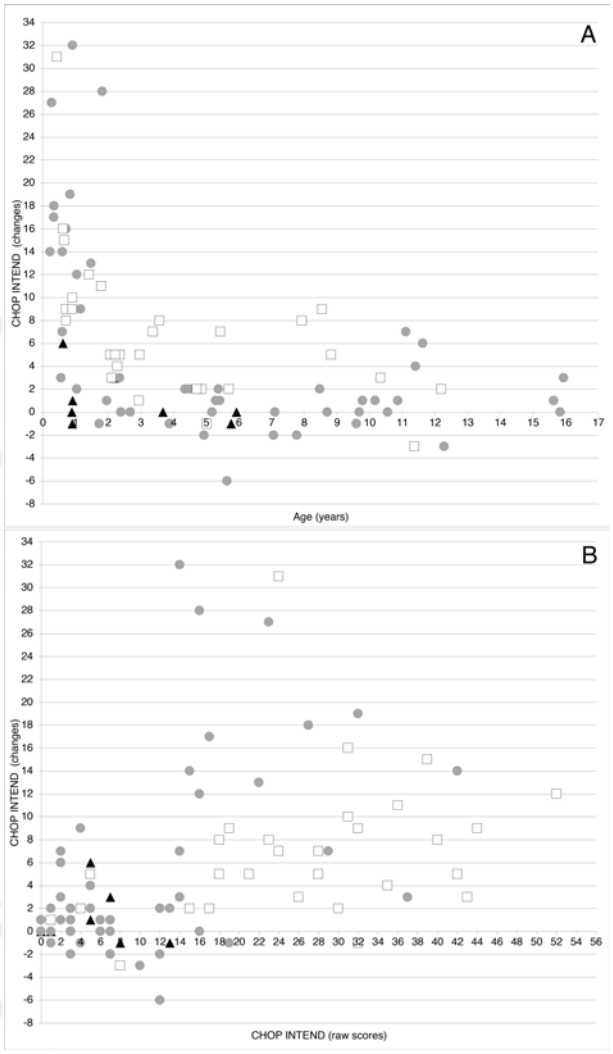

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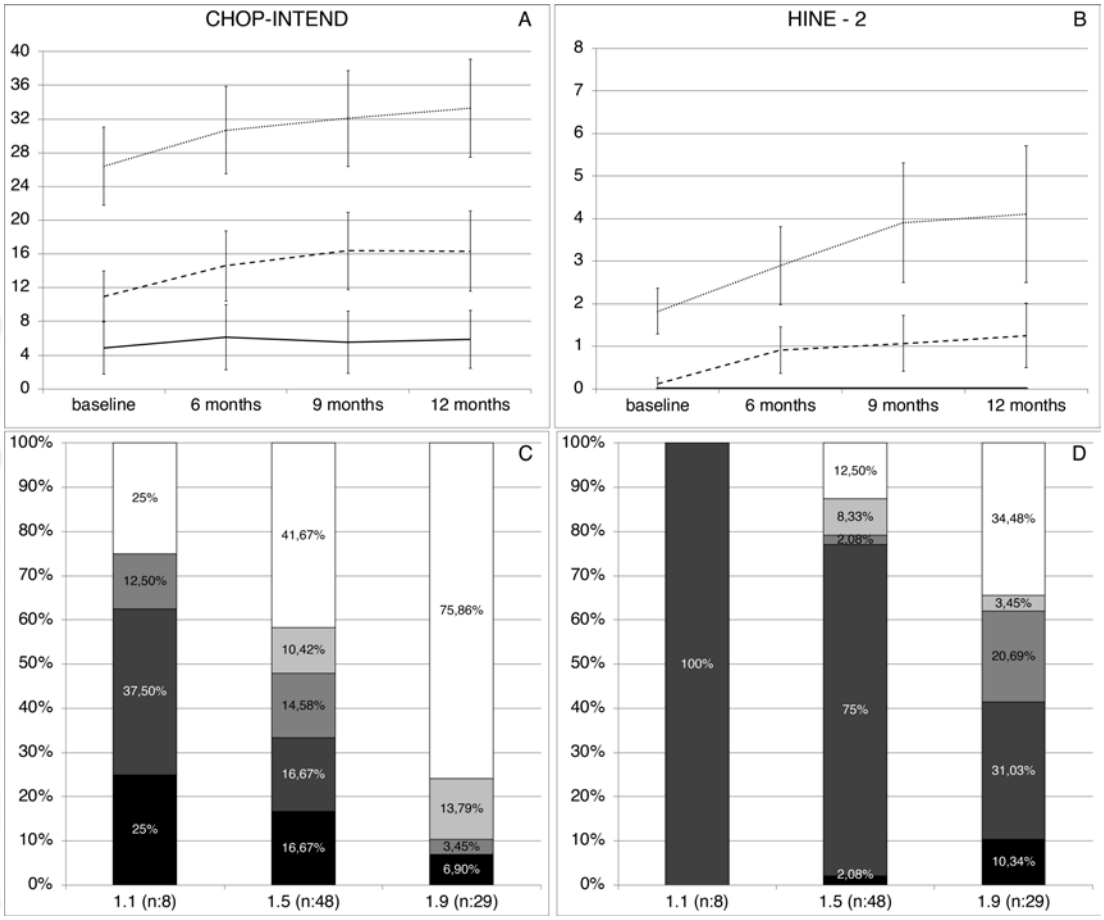

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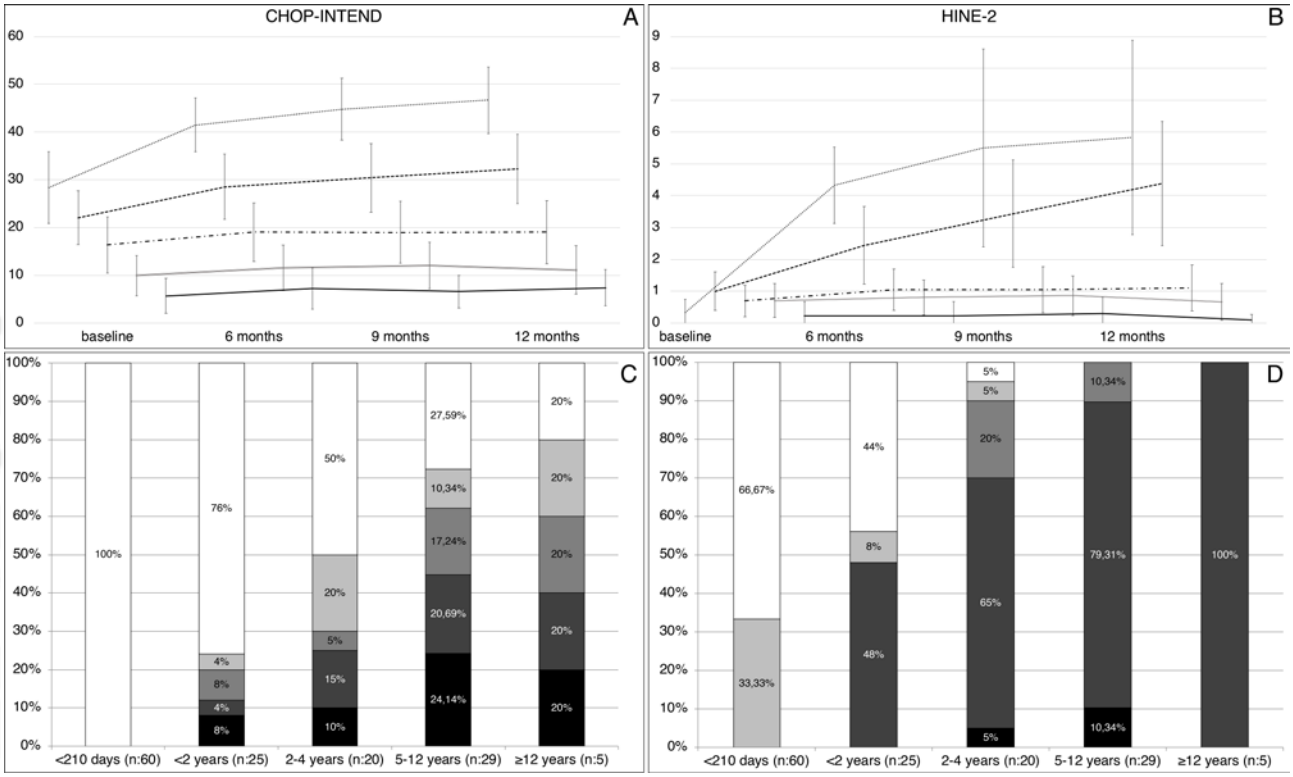

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