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TESI DI LAUREA

Nusinersen treatment in adult Spinal Muscular Atrophy

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1) ABSTRACT

1.1 BACKGROUND

SMA is a genetically determined neuromuscular disease characterized by involvement of the lower motor neuron. SMA presents with muscle weakness and atrophy, mainly proximal, and possible bulbar and respiratory involvement. Since 2017, the first disease modifying drug, the antisense oligonucleotide (ASO) Nusinersen, has been authorized.

1.2 OBJECTIVE

The primary goal of this multicenter retrospective longitudinal study was to assess the long-term functional changes in clinical outcome indicators in a cohort of Nusinersen-treated adult patients with type 2 and 3 Spinal Muscular Atrophy (SMA).

1.3 MATERIALS AND METHODS

Nusinersen, an antisense oligonucleotide that modulates pre-mRNA splicing of the Survival Motor Neuron 2 gene (1), was used to treat 140 ambulant ("*walker*") and non-ambulant ("*sitter*") SMA patients, ranging in age from 18 to 74 years (median age 35). Nusinersen 12 mg was administered by intrathecal route to the patients on days 1 (T0), 14 (T0+14), 28 (T0+28), 63 (T0+63) (loading doses) and later approximatively every 4 months (maintenance doses: T6, T10, T14, etc). Clinical evaluations on patients were performed at T0 and then every four months using three assessment scales: Hammersmith Functional Motor Scale-Expanded (HFMSE), Revised Upper Limb Module (RULM), and Six-Minute Walking Test (6MWT).

1.4 RESULTS

In patients with SMA type 3, compared to the baseline score, a significant increase at HFMSE score was observed up to T38 (median change + 1 at T6, T10, T22, T30; +2 at T14, T18, T34, T38). The positive results were detected consistently in SMA 3 “sitter” patients, while, in “walker” patients, significance was lost at time point T22, after two years of treatment. Considering the RULM score, there were no significant median changes in SMA 3 “walker” patients, owing to a strong ceiling effect. The distance walked at the 6MWT significantly improved in SMA 3 “walker” patients from the baseline to T22 (maximum median change +20.5 m at T10, $p<0.0001$) and, from T26 to T34, a trend toward a non-significant positive change was noted (median change +17.5 m at T26, +12 m at T30 and +20 m at T34). Patients with SMA type 2 had no significant changes in median HFMSE and RULM scores between T0 and the subsequent time periods, even if there was a temporary trend toward positive increases in RULM at T6-T14.

Overall, the rate of responders, meaning patients who have improved their motor function by a clinically significant value, ranged from 24% to 43% considering HFMSE, from 19% to 34% considering RULM and from 28 to 38% considering 6MWT. These data are referred to the entire cohort of SMA patients and are statistically significant up to T34.

Moreover, the rate of patients who have not significantly improved or worsened in the same timeframe ranged from 52% to 73% considering HFMSE, from 52% to 71% considering RULM and from 51% to 65% considering 6MWT. This data is clinically relevant, considering that SMA natural history predicts a progressive decline of the motor function over time.

1.5 CONCLUSIONS

Our findings add to the growing body of “real-world” evidence for long-term Nusinersen effectiveness in adult SMA type 3 patients. At 22-26 months after starting treatment, the motor function in SMA type 3 “walker” subgroup appears to be stabilized, presumably indicating the maximum functional improvement possible. Due to the small number of adult SMA type 2 patients in our sample, definitive conclusions in this category of individuals are not possible.

2) RIASSUNTO

2.1 BACKGROUND O PRESUPPOSTI DELLO STUDIO

La SMA è una malattia neuromuscolare geneticamente determinata caratterizzata da interessamento del secondo motoneurone, che si presenta con debolezza muscolare, atrofia muscolare prevalentemente prossimale e possibile interessamento bulbare e respiratorio. Dal 2017 è stato autorizzato il primo farmaco in grado di cambiare la storia naturale della malattia, l'oligonucleotide antisenso (ASO) Nusinersen.

2.2 SCOPO DELLO STUDIO

L'obiettivo primario di questo studio retrospettivo longitudinale multicentrico è stato quello di valutare i cambiamenti funzionali a lungo termine negli indicatori di esito clinico in una coorte di pazienti adulti trattati con Nusinersen con atrofia muscolare spinale di tipo 2 e 3 (SMA).

2.3 MATERIALI E METODI

Nusinersen, un oligonucleotide antisenso che modula lo splicing pre-mRNA del gene Survival Motor Neuron 2 (1), è stato usato per trattare 140 pazienti ambulanti ("walker") e non-ambulanti ("sitter"), con età compresa tra 18 e 74 anni (età mediana 35). Nusinersen, con un dosaggio di 12 mg, è stato somministrato per via intratecale ai pazienti nei giorni 1 (T0), 14 (T0+14), 28 (T0+28), 63 (T0+63) (dosi di carico) e successivamente approssimativamente ogni 4 mesi (dosi di mantenimento: T6, T10, T14, ecc.). Le valutazioni cliniche sui pazienti sono state eseguite a T0 e poi ogni quattro mesi utilizzando tre scale di valutazione: Hammersmith Functional Motor Scale-Expanded (HFMSE), Revised Upper Limb Module (RULM) e Six-Minute Walking Test (6MWT).

2.4 RISULTATI

Nei pazienti con SMA tipo 3, rispetto al punteggio di base, è stato osservato un aumento significativo del punteggio HFMSE fino a T38 (variazione mediana + 1 a T6, T10, T22, T30; +2 a T14, T18, T34, T38). I risultati positivi sono stati rilevati costantemente nei pazienti con SMA tipo 3 "sitter", mentre, nei pazienti "walker", la significatività è stata persa al punto temporale T22, dopo due anni di trattamento. Per quanto riguarda il punteggio RULM, non ci sono stati cambiamenti mediani significativi nei pazienti "walker" con SMA tipo 3, a causa di un forte “ceiling effect”. La distanza percorsa al 6MWT è notevolmente migliorata nei pazienti con SMA tipo 3 "walker" dalla determinazione basale fino a T22 (variazione mediana massima +20,5 m a T10, $p<0.0001$) e, da T26 a T34, è stata osservata una tendenza positiva non significativa (variazione mediana +17,5 m a T26, +12 m a T30 e +20 m a T34). I pazienti con SMA di tipo 2 non hanno avuto cambiamenti significativi nei punteggi mediani di HFMSE e RULM tra T0 e i periodi di tempo successivi anche se c'è stata una tendenza verso un aumento del punteggio RULM da T6 a T14. Nel complesso, considerando l'intera coorte di pazienti con SMA, il tasso di pazienti che hanno migliorato la loro funzione motoria di un valore clinicamente significativo va dal 24% al 43% alla scala HFMSE, dal 19% al 34% al RULM e dal 28 al 38% al 6MWT. Tali valori sono statisticamente significativi fino a T38. Nello stesso periodo di tempo, il tasso di pazienti che non sono significativamente migliorati né peggiorati nella funzione motoria varia dal 52% al 73% per l’HFMSE, dal 52% al 71% per il RULM e dal 51% al 65% per il 6MWT. Questo dato è clinicamente rilevante considerando che la storia naturale della malattia prevede un progressivo declino della funzione motoria nel tempo.

2.5 CONCLUSIONI

I nostri risultati si aggiungono al crescente numero di prove dell'efficacia “real-world” a lungo termine di Nusinersen nei pazienti adulti con SMA di tipo 3. A 22-26 mesi dall'inizio del trattamento, la funzione motoria del sottogruppo "walker" sembra stabilizzarsi, indicando presumibilmente il massimo miglioramento funzionale possibile. A causa del ridotto numero di pazienti adulti con SMA di tipo 2 nel nostro campione, non sono state possibili conclusioni definitive in questa categoria di individui.

3) INTRODUCTION

3.1 SPINAL MUSCULAR ATROPHY

3.1.1 Overview and epidemiology

Spinal muscular atrophy (SMA) comprises a group of neuromuscular disorders, mostly inherited with an autosomal recessive pattern, causing degeneration of the anterior horn cells in the spinal cord with irreversible loss of lower motor neurons. SMA is clinically characterized by progressive muscle weakness and atrophy, predominating in proximal limb muscles, with possible concomitant bulbar and respiratory muscle involvement (2). The estimated incidence is 1/6000 to 1/10000 live births with carrier frequency of 1/40 to 1/60 (3).

Most phenotypes of SMA are characterized by an affection of the "survival motor neuron" gene, also known as *SMN1*, coding for the SMN protein, which is essential for motor neuron homeostasis.

SMA manifestations can range from a severe infantile to a mild chronic adult disease, fitting into five main phenotypes based on age of onset and motor milestones achieved:

- Type 0 (congenital SMA) is the most severe form, associated with an extreme lack of the SMN protein. It is congenital with neonatal onset, and it is clinically characterized by a "floppy infant" syndrome, with a profound muscle weakness and limpness of the child. The mothers can experience a reduction or total lack of fetal movements during the pregnancy. Newborns experience hypotonia, areflexia and possibly arthrogryposis at the ankles and wrists or dislocation of the hips. Respiratory failure develops in the first hours of life, leading to a fatal outcome;
- Type 1 (severe SMA, or Werdnig-Hoffmann disease) is the severe infantile form, with onset before the age of 6 months. It is the most common type, responsible for about 50% of SMA patients. After cystic fibrosis, it is the second most frequent cause of death from a recessively inherited disease, occurring once in every 20,000 live births. 3 clinical subgroups can be defined according to the severity of clinical signs. Overall, it is

characterized by profound hypotonia, symmetrical flaccid paresis, lack of head control, resulting n in a “floppy infant” syndrome. Spontaneous mobility is poor and antigravity movements are not possible. However, if the effect of gravity is removed, the muscles can have some grade of contraction. These patients never acquire the ability to sit unsupported. Muscle volume is decreased and fasciculations are occasionally visible in the tongue. Newborns tend to assume the so-called “frog position”, with their arms abducted and flexed at the elbow, and their legs rotated externally, abducted at hips and flexed at the knees. Some patients develop seemingly normally for several months before the weakness finally becomes apparent. In these, proximal muscles, such as those of the trunk, pelvis, and shoulder-girdle are at first disproportionately affected, while distal muscles of head, hands and feet retain mobility. These patients seem to have a less rapid decline than those affected in utero or at birth. Eventually, as the months pass, the disease progresses gradually, spreading to all skeletal muscles except for the ocular ones. Even respiratory muscles are affected, leading to intercostal paralysis with a degree of chest collapse. Consequently, the breathing becomes paradoxical, with chest retraction and abdominal protrusion during the inspiration, and the opposite in expiration. The disease results in respiratory insufficiency, which usually develops within the first year of life. Average survival is not more than two years (4);

- Type 2 (intermediate SMA, or Dubowitz disease) is the intermediate form with onset during infancy. The symptoms occur between the ages of 7-18 months; the patients are able to sit and they may stand up, but can’t walk independently. They later develop orthopedical conditions such as severe scoliosis and joint contractures. Fine tremors are common, especially in reach tasks, and deep tendon reflexes are absent. There is a spectrum of severity: the weakest patients present bulbar impairment, which affects their ability to chew and swallow. The majority of SMA type 2 patients survives into adulthood, but may develop respiratory failure requiring mechanical ventilation, which is a major determinant of prognosis;
- Type 3 (mild SMA, or Kugelberg-Welander disease) is a chronic form with onset in childhood, after 18 months of life. It includes heterogenous patients who typically reach all major motor milestones, including independent

walking, but develop proximal muscle weakness. In some cases it might cause loss of ambulation with a need for wheelchair assistance, whereas in other cases it is possible to lead a productive adult life with only minor muscular weakness. In this regard, a significant difference is observed between patients with an onset of weakness before and after 3 years of age, defining the types 3a and 3b. Patients who lose ambulation may later develop medical issues related to poor mobility, such as scoliosis and osteoporosis;

- Type 4 (adult SMA) is the mildest form, with adult onset and mild course. This group includes patients who are able to walk in adulthood. It is the rarest form, responsible for less than 5% of all SMA patients. The onset is typically in the third decade. It is clinically characterized by mild weakness of proximal muscles, mainly quadriceps, without respiratory and nutritional problems. It can be classified into three subtypes depending on age of onset; an earlier onset is associated with a more severe course (3,5–7).

SMA is overall characterized by progressive proximal muscle atrophy and weakness, as well as loss of deep tendon reflexes. The cognition is usually spared, and often patients excel and tend to have higher than average intelligence (7). The patients can also be classified as “sitters”, “non-sitters” and “walkers” to better acknowledge their functional status and responses to available therapies.

Table I. Spinal Muscular Atrophy classification. Adapted from Kolb et al., 2015; Arnold et al., 2018.

Type	Age of onset	Highest function	Natural age of death
0	Prenatal	Respiratory support	<1 mo
1	0-6 mo	Never sit	<2 y
2	<18 mo	Never walk	>2 y
3	>18 mo	Stand and walk	Adult
3a	18 mo – 3 y	Stand and walk	Adult
3b	>3 y	Stand and walk	Adult
4	>21 y	Stand and walk	Adult

3.1.2 Etiopathogenesis

The more frequent SMA is an autosomal-recessive disorder caused by deletion or mutation of Survival Motor Neuron 1 gene (*SMN1*), discovered in 1995. The gene, which codes for SMN protein, is located in chromosome 5 at q13 coding region (2). Because of this localization, the largest group of the disease is known as "SMA 5q". The most frequent defects are deletions of *SMN1* exons 7 and 8 or exon 7 only, whereas a minority of cases presents a compound heterozygosity with a mutation on one chromosome and a deletion or gene conversion on the other chromosome (8). Missense mutations that can lead to disease have also been recently studied in various SMN protein domains (9). Less often, different patterns of inheritance can be associated with the disease (not 5q SMA). The X-linked SMA group is related to mutations in gene *UBA1*, which codes for a protein involved in ubiquitination, or in the androgen receptor (AR) gene in Xq12 (10). The autosomal dominant group, finally, includes an adult form caused by mutations in *VABP* (vesicle-associated membrane protein) gene and another form that only affects the lower limbs, associated with mutations in gene *DYNC1H1* (dynein cytoplasmic 1 heavy chain 1). Even though affected siblings usually have very similar clinical patterns of disease, the same mutation may lead to different phenotypes in different families, so additional modifying post-transcriptional or non-genetic attributes must be playing a role (4).

SMN is a 38 kilodalton (kD), 294 aminoacids protein found in the cytoplasm and nucleus of all cells and it is crucial for motor neuron activity. It is demonstrated to play a critical role in spliceosome assembly and many other tasks, such as cellular trafficking, mRNA processing and transport, regulation of translation and stress granule formation. Moreover, key roles have also been reported for SMN in DNA recombination and repair, signal transduction, endocytosis, autophagy, mitochondrial homeostasis and bioenergetic pathways (5,11,12). However, the main complex implicated in the pathogenesis of the disease is the SMN complex involved in small nuclear ribonucleoprotein (snRNP) assembly; snRNPs are crucial for the recognition of splicing sites and the removal of introns from pre-mRNA, therefore an altered snRNP assembly leads to an impaired mRNA splicing (13). The proteins affected by the incorrect splicing include Stasimon, which is required for motor circuit function as it was demonstrated that its upregulation rescued deficient neuromuscular junction transmission in SMN deficient animal models.

Other genes abnormally spliced are chondrolectin, agrin and neurexin2, and they could also play a role in the disease, as they are proteins expressed at neuronal level (12).

Five major SMN domains have been identified as linked to SMA, based on the position of intragenic mutations:

1. Gemin2 binding domain (Ge2BD), in the N-terminal, encoded by exon 2a;
2. A central Tudor domain, encoded by exon 3;
3. A proline rich domain encoded by exons 5 and 6;
4. A carboxyl-terminal tyrosine-glycine box encoded by exons 6 and 7, essential for SMN self-oligomerization;
5. The last 16 aminoacids encoded by exon 7, critical for protein stability.

The first three of them are the most conserved domains, where most missense mutations occur (5).

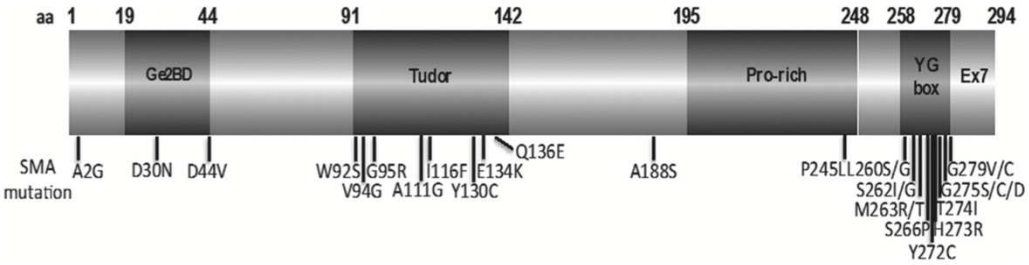

***Figure 1**. Organization of the SMN protein functional domains. From Lefebvre et al., 2020.*

SMA clinical presentation correlates with the expression of a gene nearly identical to *SMN1*, called *SMN2*, which is also located within the SMN locus and produces a truncated, partially functional SMN protein, that alone is insufficient for normal motor neuron function (5). The general population has 0 to 8 *SMN2* copies.

In more than 95% of cases, SMA results from the loss of *SMN1* but retention of *SMN2*. The two genes have a 99% homology, as they differ for only five nucleotides. A C>T substitution disrupts an exonic splicing enhancer and creates an Exonic Splice Silencer (ESS) causing the exclusion of exon 7 during transcription. As a result, most of *SMN2* mRNAs lack exon 7 (SMNΔ7) and produce a protein that is less functional, unstable and degraded rapidly. Only 10-

20% of the *SMN2* gene product is fully functional, as it is able to evade the improper splicing (14,15).

It is demonstrated that the complete absence of SMN protein is lethal both in patients and in model systems; it therefore follows that all SMA patients retain a variable number of copies of SMN2. An increased number of genomic copies of *SMN2* correlates with a milder disease, and there is a genotype-phenotype association related to the copy number (8). Therefore, there is prognostic value in accurate measurement of *SMN2* copy number from patients being evaluated for SMA (15).

Table II. Number of copies of *SMN2* per phenotypic subtype. Adapted from Butchbach et al., 2016.

Type	Predicted number of *SMN2* copies
0	1
1	2
2	3
3	3-4
4	>4

As for the cellular pathogenesis, there are several hypotheses that aim to explain how the absence of SMN results in SMA disease. Firstly, biochemical and histological studies have demonstrated that SMN protein is ubiquitously expressed and is especially required during the gestational and neonatal stages of development. After birth SMN expression levels decline, however motor neurons continue to express high levels of it and thereby are highly susceptible to SMN depletion in SMA disease. The various possible damage mechanisms studied so far include many hypotheses:

- The defective snRNP's formation may affect the splicing of genes crucial for motor neuron circuitry, as previously stated in this paragraph;
- Since SMN is located in motor neuron axons, there could be an axonal disruption;

- As showed by studies performed on animal models, there is an early vulnerability of the neuromuscular junction (NMJ), and it is still unclear if it is primarily related to the lack of SMN protein or if it is secondary to a motor axon failure;
- Different neurons are differently affected during various stages of the disease depending on their size and NMJ maturation pathway (7).

So, as it happens in other neuromuscular diseases, the neuromuscular junctions are pathological targets, and this is also reflected in the anatomical pattern. Proximal upper and lower limb muscles are affected before the distal ones, and there is also a loss of abdominal and thoracic muscles, while oculomotor muscles are preserved. Although muscle atrophy in SMA is secondary to motor neuron degeneration, some abnormalities are also evident at the muscular level, such as altered differentiation, histological abnormalities and impaired progenitor cells (5).

3.1.3 Diagnosis

Electromyography and muscle biopsy features of denervation were once the basis for diagnosis. Nowadays they have been overtaken by molecular testing for homozygous deletion or mutation of the *SMN1* gene. Given the efficiency of molecular testing and high frequency of SMA carrier condition, it should be an early consideration in any infant with "floppy infant" features, weakness or hypotonia (11). The absence of *SMN1* exon 7 (regardless of the presence of exon 8) confirms the diagnosis. The test reaches 95% sensitivity and nearly 100% specificity (3).

Molecular diagnosis of SMA has historically been made with polymerase chain reaction (PCR)-based assay followed by digestion of the product with specific restriction endonucleases. Numerous assays have since been developed to quantify *SMN2* copy number in DNA samples from SMA patients. The technique has been improved by digital PCR (dPCR) to overcome some limitations associated with the PCR-based assays (15).

Ancillary tests available are:

- Creatinine kinase (CK) dosage on blood sample, which is usually normal or very mildly elevated;
- Nerve conduction studies (NCS), as motor nerves may show diminished motor action potentials;
- Needle electromyography (EMG). SMA type I presents denervation; types II and III show neurogenic patterns;
- Muscle biopsy is mostly obsolete as a diagnostic tool, but in SMA patients it shows a neurogenic pattern (2).

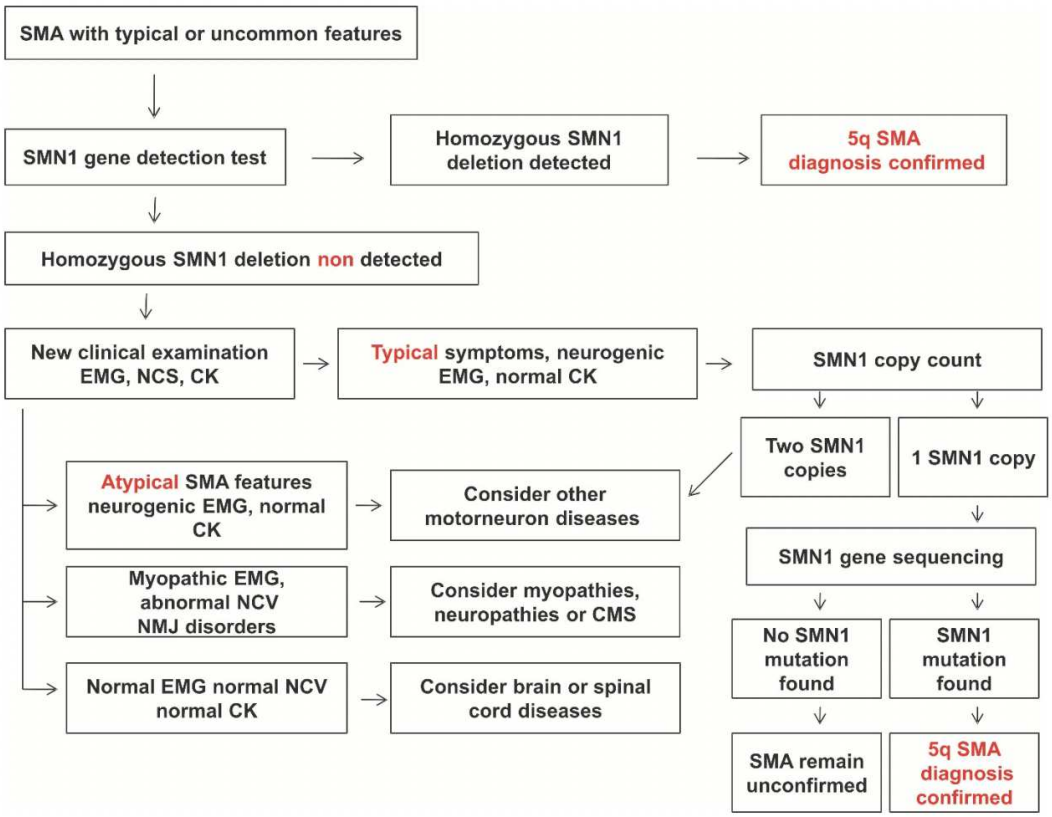

***Figure 2**. Diagnostic algorithm for Spinal Muscular Atrophy. From D'Amico et al., 2011.*

3.1.4 Differential diagnosis

In the infants, who have the most severe forms of SMA, the differential diagnosis shall include all other causes of hypotonia and delayed motor development, which consist in a large part of the differential diagnosis of the “floppy infant” syndrome, including:

- Congenital myopathies;
- Glicogenoses;
- Neonatal myasthenia gravis;
- Prader-Willi syndrome;
- Disorders of fatty acid metabolism.

The preservation of deep tendon reflexes and relative lack of progression of muscle weakness are characteristics that point towards the latter disorders.

Developmental delay is another major category of disease that must be studied, considering syndromes such as:

- Down syndrome;
- Cretinism;
- Prader-Willi syndrome;
- Achondrodysplasia.

Moreover, certain forms of muscular dystrophy, notably myotonic dystrophy, may have neonatal onset and interfere with motor development, for example, showing difficulty in sucking. In that case, however, the weakness is not as severe or diffuse as in infantile SMA. In addition, the mother may display myotonia, which can be used as an element of differential diagnosis.

Other pathologies that should be considered in early childhood are polyneuropathies, polymyositis, nemaline and central core myopathy, which can all manifest with weakness. Moreover, children with untreated chronic diseases, like celiac disease and cystic fibrosis, may be hypotonic to the point of simulating a neuromuscular disease. In these cases, usually, tendon reflexes are preserved and strength returns when the medical problem is corrected. Finally, some polio-encephalopathies and leukodystrophies may simulate SMA symptoms, but they also show evidence of cerebral involvement.

There remains a group of patients with motor underdevelopment and hypotonia that cannot be classified, once gathered in a group called “amyotonia congenita”. It is likely that this group comprehends cases of congenital myopathy that in the future will be characterized by application of modern histochemical, ultrastructural, and genetic techniques (4).

In patients with SMA type 3, which has an intermediate onset, the differential diagnosis should consider myopathies, neuropathies, neuromuscular junction disorders and other motor neuron disorders. In SMA type 4, with an adult onset, the differential overlaps with the intermediate forms, but also includes later onset disorders, such as amyotrophic lateral sclerosis and Kennedy disease (11).

3.1.5 Pathologic findings

In muscle biopsies performed in patients with SMA type 1 at 1 month of age abnormalities may be found, such as a typical picture of group atrophy. Other defects can be identified in nerve cells in the spinal cord and motor nuclei in the lower brainstem, including a reduction in number of cells, various stages of degeneration, chromatolysis and cytoplasmatic inclusions, as well as neuronophagia. In addition, there is gliosis as a replacement process and secondary degeneration in nerves and roots. All the other systems of neurons are unaffected, including the corticospinal and corticobulbar (4).

3.1.6 Management

The diagnosis of SMA implies a complexity of medical problems which have to be managed by a multidisciplinary team. The follow-up coordination should be handled by an expert in neuromuscular disorders, generally the neurologist or pediatric neurologist, who is able to manage the multiple respiratory, nutritional, orthopedic, rehabilitative, emotional and social problems that develop in most of SMA patients. This management will allow to monitor the various aspects that are known to be part of the disease progression and, when possible, to provide anticipatory care. (3).

The actual consensus statement on SMA standard of care has been updated in 2018 by Mercuri, Finkel et al. and it defines topics such as physical therapy and rehabilitation, orthopedic care, nutrition, pulmonary care, acute care in the hospital setting ethics and palliative care. Mainly, SMA patients' follow-up recommendations include:

1. **Neuromuscular and musculoskeletal evaluation.**

   SMA patients should have a clinical assessment every six months, including a physical examination focused on the musculoskeletal system and related functional impairment. These should also include means of assessments of strength and range of joint motion, as well as motor functional scales and timed tests meant to reflect activities of daily living.

2. **Rehabilitation.**

   Physiatrist involvement is important to prescribe frames, orthotics and wheelchairs to improve quality of life and mobility. Moreover, SMA patients should undergo regular sessions of physiotherapy, as it is demonstrated to influence trajectories of disease progression. The key points in rehabilitation are postural control, stretching, mobility and exercise.

3. **Orthopedic management.**

   Patients suffer from orthopedic complications such as scoliosis, hip subluxation, contractures and susceptibility to fractures. Particularly, surgical monitoring is required for scoliosis with periodic radiographies and consideration for spinal fusion and bracing. The decision to perform surgery is predicated mainly on curve magnitude (i.e. major curve Cobb angle ≥50°) and rate of progression (≥10° per year).

4. **Nutritional management.**

   The main gastrointestinal issues in SMA patients include swallowing dysfunction, dysphagia, weight control and gastrointestinal dysfunction. Regular assessments of growth are fundamental, and a nutritionist should be involved to promote an appropriate diet. It is important to monitor the weight, as well as fluid, macronutrient and micronutrient intake, especially calcium and vitamin D intake for bone health. In SMA type I and II feeding

tubes are commonly used for total or supplementary nutrition, whereas GI surgical recommendations depend on the individual situation. In milder SMA phenotypes the main nutritional concern is the risk of obesity as the disease can reduce mobility and may increase risk of obesity-related comorbidities.

5. **Pulmonary management.**

   Pulmonary disease is the major cause of morbidity and mortality in SMA, especially in types I and II. Respiratory failure is mainly caused by a restrictive lung disease due to expiratory and intercostal muscles involvement. Other important issues are swallowing dysfunction and reflux. The complications include recurrent chest infections, nocturnal oxygen desaturation, nocturnal hypoventilation and daytime hypercapnia. The clinical assessment shall always include a physical evaluation and spirometry, eventually complemented by pulse oximetry, capnography, sleep study with CO_2 recording. Airway clearance, ventilation and specific medication are the fundamental interventions which must be considered according to the individual case (16,17).

3.1.7 Treatment

Ever since the discovery of SMA molecular pathogenesis, the main focus for treatment has been to increase SMN levels. The various therapeutic strategies are based fundamentally on replacing *SMN1* or increasing *SMN2* gene expression. The development of animal models as well as targeted approaches is what ultimately lead to therapeutic development for SMA.

The different approaches can be summarized in the below list:

- Early efforts used histone deacetylase (HDAC) inhibitors to increase the expression of *SMN2*, with molecules such as suberoylanilide hydroxamic acid (vorinostat), hydroxyurea, trichostatin A, sodium butyrate, phenylbutyrate, and valproic acid. Their mechanism of action was to activate the *SMN2* promoter resulting in increased full-length SMN protein. The HDAC inhibition caused by these drugs is not specific to SMN, leading to potential adverse reactions. In the end, their apparent benefit was not

confirmed in subsequent randomized, placebo-controlled trials, leading to a dismission of their use for the treatment of SMA (7,18);

- Later, the extensive characterization of the molecular elements governing *SMN2* exon 7 splicing and inclusion led to the development of SMA specific antisense oligonucleotides (ASO). ASOs are therapeutic RNA molecules specifically designed to bind to their complementary sequences within a targeted intron or exon. This interaction can either enhance or disrupt the targeted splicing event; in this case the target is to increase exon 7 inclusion in *SMN2*-derived mRNA (14). Nusinersen (Spinraza®, Biogen), targeted to the ISS-N1 element in an Intronic Splicing Silencer (ISS) of intron 7, is the only antisense oligonucleotide approved by both Food and Drug Administration (FDA) in 2016 and European Medicines Agency (EMA) in 2017 for intrathecal treatment of all patients suffering from SMA (19), following the evidence of efficacy in a double blind controlled clinical trial on 121 SMA type 1 patients diagnosed before 7 months of age (20);
- In 2020 FDA authorized a small molecule with a similar mechanism, called Risdiplam (Evrysdi®, Roche), with oral administration and systemic distribution. It was followed by EMA in 2021. It has been deducted from preclinical studies that Risdiplam binds selectively to two SMN2 pre-mRNA sites, a 5' splice site (5' ss) of intron 7 where U1 snRNA is bound and an Exonic splicing enhancer 2 (ESE2) in exon 7, leading to an increased exon 7 inclusion and expression of functional SMN protein (21). It is currently indicated for patients 2 months of age and older, with a clinical diagnosis of SMA Type 1, 2 or 3 with one to four *SMN2* copies (22). The oral administration brings significant benefits: not only it is more bearable for patients, but also it lets the drug reach the other systemic tissues affected by the disease. Moreover, the systemic distribution to date has not proven to cause significant side effects: in the JEWELFISH trial, carried on 174 patients, Risdiplam was overall well tolerated. The most notable AEs include constipation, diarrhea, rash, fever and vomiting (23);
- Another strategy is gene replacement therapy, which consists in viral mediated therapies that aim to replace the entire *SMN1* gene. The therapy currently in use is Onasemnogene Abeparvovec (Zolgensma®, Novartis), a nonreplicating adeno-associated virus 9 (AAV9) that is capable of crossing

the blood–brain barrier and target cells in the CNS exporting the missing gene. It was approved by FDA in 2019 and by EMA in 2020 and it is indicated for patients with a bi-allelic mutation in the SMN1 gene and a clinical diagnosis of SMA Type 1, or patients with a bi-allelic mutation in the *SMN1* gene and up to 3 copies of the *SMN2* gene. It is delivered as a single intravenous injection and it is currently the most expensive drug in the world, with a price that has reached two million Euros for one single infusion (24,25). The cost is justified by the drug's effectiveness, as a phase 1 study on 15 infants affected by SMA type 1 showed incredible results: at two years following a single Zolgensma injection, all patients were alive, 92% could sit unassisted and 17% could even walk unassisted. Also, the respiratory function was increased and the patients were also able to feed themselves. The earlier the drug was administered, the better was the outcome (23).

3.2 NUSINERSEN

3.2.1 Overview

Nusinersen (Spinraza®, Biogen) is the first disease modifying drug approved to treat both pediatric and adult patients with SMA. It was discovered by Adrian Krainer and team in 2010, following the 2006 Singh et al. study on *SMN2* introns splicing. After a number of clinical studies, it was approved by FDA on December 23, 2016 and by EMA on May 30, 2017. Market authorization has been approved in more than 40 countries throughout the world. Nusinersen has orphan drug designation in the United States and Europe (19,26,27).

3.2.2 Antisense Oligonucleotides (ASOs)

Antisense oligonucleotides (ASOs) are short chemically synthesized strings of nucleotides, generally about 12-30 nucleotides long, specifically designed to bind to a target RNA. They follow Watson-Crick base pairing rules, and they are created complementary to the mRNA which encodes a target gene. In this way, they can selectively modulate the expression of genes involved in the pathogenesis of diseases. The short length of ASOs contributes to their specificity, as it makes them capable of uniquely binding to only one target RNA. ASOs have been tested in many disorders, such as cancer, metabolic, viral diseases, inflammatory, and neurological disorders, and nowadays they represent an important basis of therapy (28,29).

As for the molecular mechanism of ASOs, they generally promote RNA cleavage and degradation or use occupancy-only mechanisms, sometimes referred to as steric blocking. The drug chemistry and design determine its whole action on RNA.

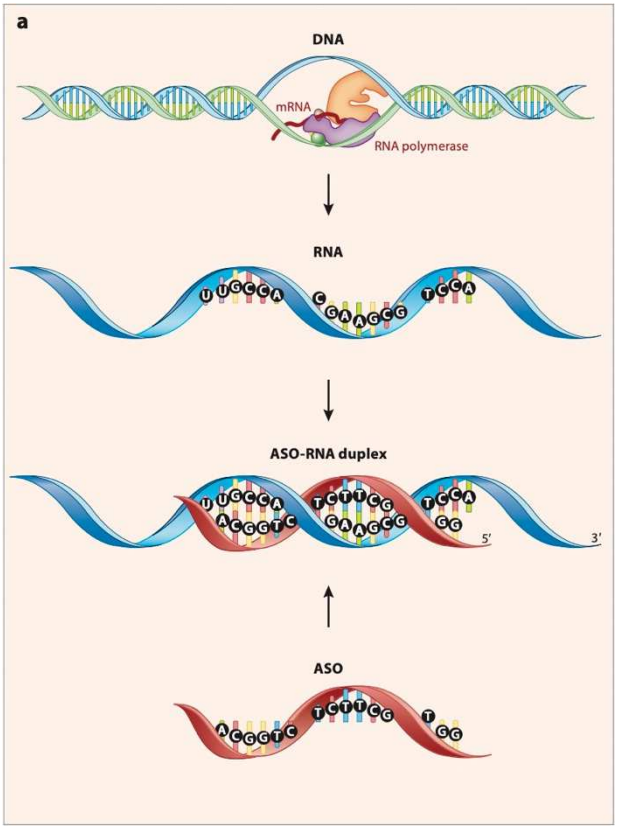

Figure 3. *ASO binding to target RNA. From Bennett et al., 2019.*

Since nucleic acid chains are very rapidly degraded in the human body, chemical modifications are required to enhance the drug-like properties of ASOs improving their pharmacokinetics. The molecular conformation as single-strand or double-strand chains, the electrical polarity and the incorporation into particular formulations are all elements that can determine the drug stability, for example allowing the binding with circulating proteins such as albumin (28). Intravenous and subcutaneous administrations both permit the distribution to most peripheral tissues, with the greatest tropism towards liver, kidney, bone marrow, adipocytes and lymph nodes. Moreover, intrathecal administration allows to distribute within the cerebrospinal fluid (CSF) and in nervous system tissues, as ASOs are not generally capable of crossing the blood brain barrier (30). The excretion is mostly renal and urinary, as unchanged drugs or metabolized in the form of cleaved fragments. Depending on the charge, polarity and hydrophilicity, ASOs can be also be eliminated with feces (31).

3.2.3 Pharmacodynamics

Nusinersen is a modified antisense oligonucleotide that binds to *SMN2* increasing the expression of full-length SMN protein. It specifically binds to an intron splicing N1 silencer sequence, called ISS-N1, located in intron 7 of *SMN2* gene; the bond promotes exon 7 inclusion, therefore increasing the percentage of efficient mRNA produced by *SMN2*. The active molecule has a molecular weight of 7501 Dalton and consists of single strand 18-mer 2'-O-(2-methoxyethyl) (MOE) phosphono-thioate oligodeoxyribonucleotides, also known as ASO-10-27 5'-TCACTTTCATAATGCTGG-3'. The molecular formula is $C_{234}H_{323}N_{61}O_{128}P_{17}S_{17}Na_{17}$ (1,32–34).

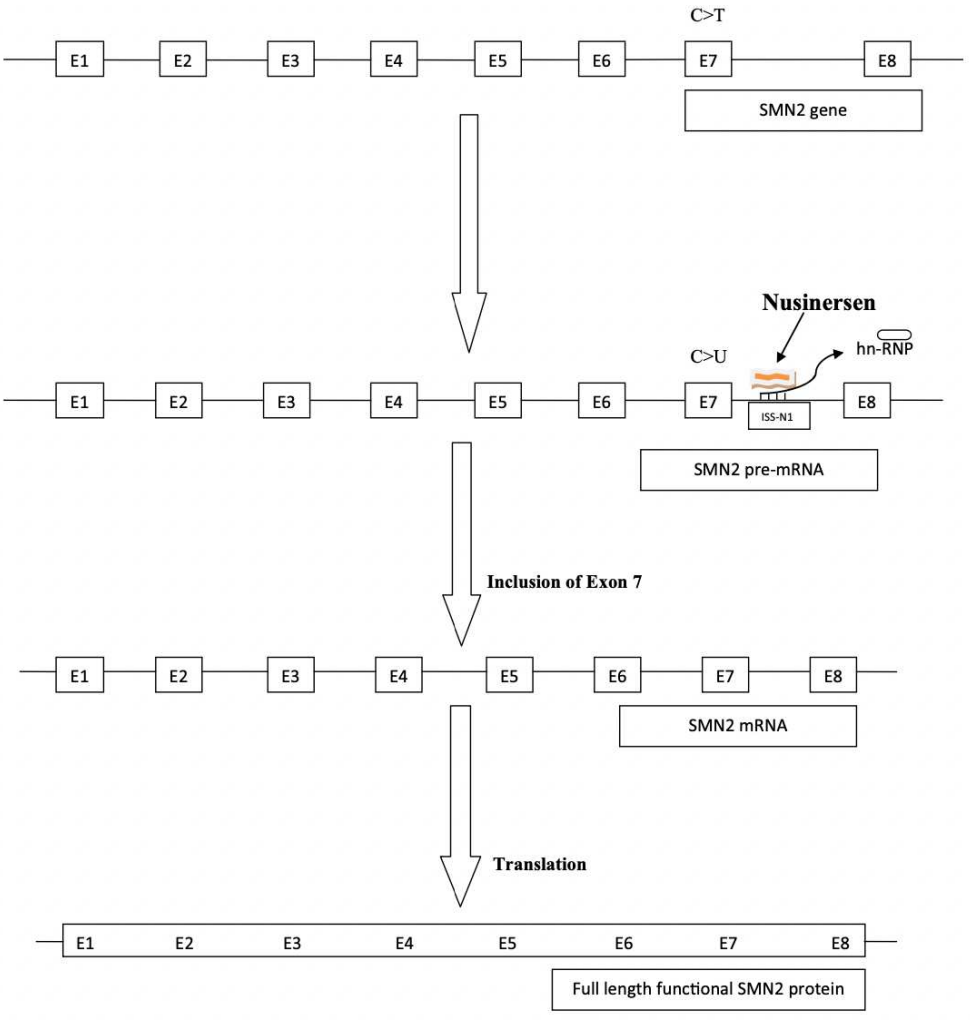

***Figure 4**. Nusinersen increases the inclusion of exon 7 during translation, thanks to the modulation of splicing of SMN2 pre-mRNA. From Maharshi et al., 2017.*

3.2.4 Pharmacokinetics

Nusinersen is intended for intrathecal administration to specifically target the central nervous system (CNS). It does not cross the blood-brain barrier if administered otherwise. Thus, peak plasma concentrations are lower than that in the CSF. The mean half-life is of 63-87 days in plasma, and 135-177 days in CNF, which accounts for triannual frequency of administration of its maintenance dose. Plasma volume of distribution is 29L, whereas cerebrospinal fluid (CSF) volume of distribution is 0.4 L, indicating much higher levels in CSF than in plasma. Nusinersen is neither a substrate nor an inducer/inhibitor of cytochrome P450, indicating relatively fewer drug interactions. It is metabolized with a hydrolysis process mediated by 3' and 5' exonuclease. Elimination is via kidney (1,33).

3.2.5 Clinical trial results

The road to the first approved ASO therapy for SMA required years of preclinical and clinical work. Nusinersen safety, pharmacokinetics and efficacy have been examined in a number of phase I, II, and III studies. Clinical outcomes have been evaluated with assessment scales such as:

- Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP-INTEND), a 16-item motor function test designed for SMA type 1. Each item is valued with a score from 0 to 4, and the total score ranges from 0 to 64;
- Compound muscle action potentials (CMAPs), the summation of all motor unit potentials registered with a supramaximal electrical stimulus of a specific nerve;
- Hammersmith Functional Motor Scale–Expanded (HFMSE), a 33-item motor scale that assesses activities related to daily living. Each item is valued with a score from 0 to 2, and the total score ranges from 0 to 66;
- Hammersmith Infant Neurological Examination, section 2 (HINE-2), a test which documents the achievement of motor milestones in infants. It consists of 26 items, each one valued with a score from 0 to 3, and the total score ranges from 0 to 78;

- Pediatric Quality of Life Inventory (PedsQL), a group of scales that assess physical, emotional, social and school functioning;
- Revised upper limb scale (RULM), a 20-item instrument that targets activities of daily living (e.g. bringing hands from lap to table, picking up small items, pushing buttons, bringing hands above shoulders). Each item is variably valued with a score from 0 to 2, and the total score ranges from 0 to 37;
- Six Minute Walking test (6MWT), an objective evaluation which measures the distance a person can walk quickly in six minutes;

(29,32,33)

The main stages of clinical development consist of ten studies as shown in Fig. 5. The studies were not conducted in healthy volunteers because of the intrathecal route of administration of the drug.

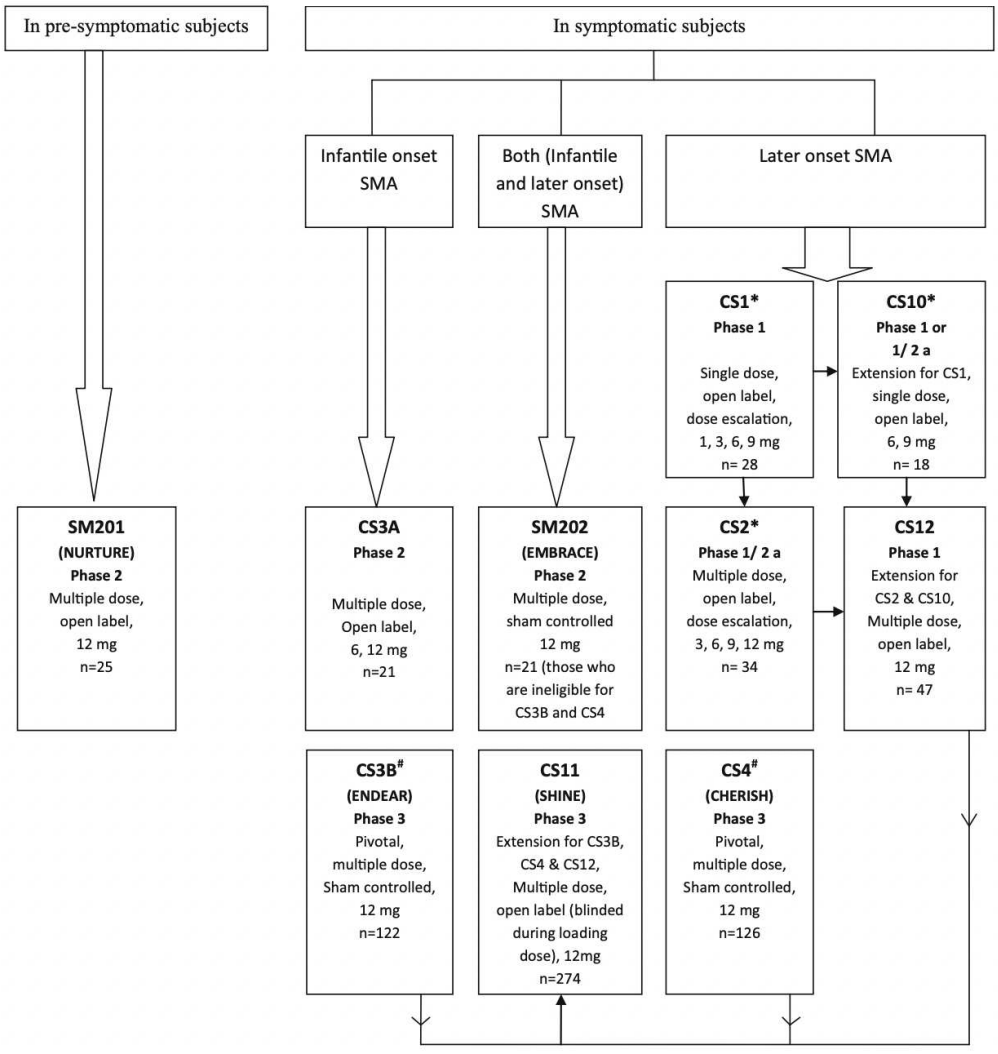

***Figure 5**. Flow chart representing clinical development of Nusinersen. From Maharshi et al., 2017.*

The initial phase 1 clinical study was an open-label, single-ascending dose study to assess safety, tolerability, pharmacokinetics and clinical effect in pediatric patients with later onset type 2 and type 3 SMA. The results showed that Nusinersen was overall well-tolerated with a single intrathecal injection. Moreover, it showed encouraging signs of activity and there were no clinically significant changes in safety assessments. No serious adverse events (AEs) occurred (26,35).

The phase 1 study was then followed by open-label phase 2 studies that made use of a loading regime to quickly reach steady-state drug concentrations in tissues. The results again demonstrated no safety or tolerability concerns with multiple doses. The pharmacokinetics were consistent and the initial observations of improvement in motor function were replicated. In one infantile onset study, intrathecal administration was well tolerated and improvement in motor function, motor milestone achievements, muscle electrophysiology and ventilation-free survival data were promising in comparison with natural history cohort. NURTURE study demonstrated usefulness in starting treatment even before the onset of symptoms (26).

Phase 3 clinical studies were performed both in infantile onset and in childhood onset SMA and they were carried out with sham procedure controls, as placebo injections would have represented an unfavorable risk/benefit in the patient population. Two pivotal trials, CS3B (ENDEAR) and CS4 (CHERISH), demonstrated significant and clinically meaningful motor improvements as well as a favorable risk-benefit profile respectively in infants with SMA type 1 and in children with SMA type 2 or 3. Both trials were ended early after interim analysis showed a significantly greater benefit in motor milestones and survival benefit in the Nusinersen arm (26,36).

In relation to the striking results from the phase 3 studies, thanks to the overwhelming evidence of a highly favorable benefit-risk profile in different SMA populations, FDA approved Nusinersen for treatment of both pediatric and adult patients with all types of SMA only three months after the filling, in December 23, 2016. Nusinersen trials demonstrated that SMA is treatable and that it is possible not only to slow down progression, but also to improve disease symptoms. The results show greater efficacy if treatment is initiated early and support newborn

screening for this disease (26,27). However, Nusinersen treatment is not curative, and the guidelines still recommend therapy to enhance the patients' quality of life. Supportive care represents the foundation of management for all patients with SMA (33).

3.2.6 Clinical use

The recommended Nusinersen treatment regimen is currently the same for all SMA types. It consists of a fixed-dose scheme of 12 mg administered intrathecally with a bolus injection over 1 to 3 minutes starting with a loading regimen on day 1, 15, 29 and 63, followed by maintenance doses every 4 months (29). Lumbar puncture (LP) with a spinal anesthesia needle is typically the chosen procedure for administration, but in individuals for whom it is not feasible, high cervical or intraventricular delivery could be an alternative. Nusinersen vials are composed of a 5 mL solution containing 12 mg of Nusinersen; therefore, before the administration, each patient should have 5 mL of CSF removed. Vials must be stored in the refrigerator until the time of use, then warmed to room temperature before the use (19,37).

The procedure can be done by different health care specialists, such as neurologists, anesthesiologists, oncologists or neuroradiologists. Ultrasound (US) or fluoroscopy are techniques that may help the process in the setting of severe scoliosis or spinal orthopedic implants. Sedation could be indicated to ease the administration, depending on the patient's clinical status (29,37).

If an involuntary interruption of therapy occurs, the missed dose should be administered as soon as possible, and then continue as prescribed to rapidly restore trough drug concentration (38).

3.2.7 Adverse effects

Adverse effects in Nusinersen treatment are mostly attributable to SMA symptoms or lumbar puncture implications. From the review of available data, there are no safety concerns directly attributed to the drug.

The most common adverse reactions associated with Nusinersen treatment include:

- Headache (50%);
- Upper and lower respiratory tract infections (39-43%);
- Back pain (41%);
- Post LP-syndrome (41%);
- Constipation (30%);
- Atelectasis (14%).

Patients were also found to have higher incidence of paradoxical breathing, respiratory symptoms and increased requirements for respiratory support (33,37).

An integrated analysis of seven completed clinical trials found that overall incidence of serious AEs was lower in the treated group than in the sham procedure group (41% vs 61%). The only higher frequency symptom in the Nusinersen arm is headache, but no patients have had any indication of complications such as communicating hydrocephalus or increased intracranial pressure. The follow up study SHINE is collecting long-term safety and tolerability data (39).

Other antisense oligonucleotides have shown two main complications, thrombocytopenia and renal toxicity, and the regulatory authorities have warned about the possibility of such complications with Nusinersen. For this reason, prior to administration it is recommended to perform blood tests including platelet count, prothrombin time and spot urine protein testing, and to keep them monitored (33).

To prevent headaches, it is preferable to use a small Gauge spinal needle, preferably non-cutting, and to keep the patient mostly recumbent or semi-recumbent on the day following the procedure (29).

4) OBJECTIVE

The main purpose of the following study is to retrospectively assess the long-term efficacy of Nusinersen in a large cohort of adult Italian patients suffering from Spinal Muscular Atrophy type 2 and type 3, through longitudinal clinical evaluation, according to validated functional scales.

5) MATERIALS AND METHODS

5.1 POPULATION SELECTION

In the selection of patients for this retrospective cohort study, to determine the efficacy of Nusinersen, all of the following inclusion criteria were applied:

- Clinical diagnosis of Spinal Muscular Atrophy type 2 and 3, based on age of onset and maximum achieved motor function;
- Molecular confirmation by identification of genetic alterations in the *SMN1* gene;
- Age above 18 years.

A total of 140 patients have been identified from the database analysis of 18 secondary and tertiary Italian centers for the management of SMA patients, largely coinciding with those selected for a recent safety and efficacy study on Nusinersen by Maggi et al. (40), with extension of the follow-up period and recruitment of new patients. The participating centers are the following: Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano; Azienda – Ospedale – Università di Padova, Clinica Neurologica; Fondazione IRCCS Ca' Granda Ospedale Maggiore di Milano Policlinico, Milano; Università di Pisa, Pisa; IRCCS AOU San Martino, Genova; Università degli Studi della Campania Luigi Vanvitelli Scuola di Medicina e Chirurgia, Napoli; Ospedale San Raffaele, Milano; Dipartimento di Neuroscienze, Università degli Studi di Torino; Ospedale Binaghi, Cagliari; UO Neurologia, Azienda Provinciale per i Servizi Sanitari di Trento; Azienda Ospedaliero-Universitaria di Parma; UOC Clinica Neurologica, IRCCS Istituto di Scienze Neurologiche, Bologna; Università degli Studi di Bari; Dipartimento di Scienze Neurologiche, AOU Ospedali Riuniti di Ancona, Ancona; Fondazione Istituto Neurologico Nazionale Mondino IRCCS, Pavia; Unità di Neurologia, Dipartimento di Neuroscienze, Udine; Dipartimento di Neurologia, Bolzano; Azienda Ospedaliera Spedali Civili di Brescia, Brescia. Our Center at the University of Padova participated with the single-center larger cohort of 31 patients.

5.2 STUDY PROTOCOL AND CONSENT

The study protocol was approved by the Ethics Committees of the 18 participating centers (Protocol ID: SMADU). Informed consent has been obtained for all participating patients, as set out in the Helsinki Declaration.

5.3 NUSINERSEN PROCEDURE OF INFUSION

All the patients in this study received standard intrathecal treatment by infusion of Nusinersen 12 mg with initial four doses during the loading phase (baseline or T0, +14 days, +28 days, +63 days) and subsequent single maintenance doses every 4 months. Loading doses are coded by letter L and dose number, hence L1, L2, L3 and L4, while maintenance doses are indicated by letter M and dose number, M1 onwards.

The infusion procedures were completed for a total of: 140 patients at the baseline (T0), 140 patients at 6 months from the baseline (T6), 140 patients at 10 months from the baseline (T10), 140 patients at 14 months from the baseline (T14), 131 patients at 18 months from the baseline (T18), 121 patients 22 months from baseline (T22), 109 patients 26 months from baseline (T26), 84 patients 30 months from baseline (T30), 55 patients 34 months from baseline (T34), 23 patients 38 months from baseline (T38) and 8 patients 42 months from baseline (T42).

The median duration of follow-up in the entire cohort and subgroup of patients with type 3 SMA is 30 months, while for patients with type 2 SMA it is of 22 months.

Intrathecal access was achieved by standard lumbar rachicentesis procedure in 97/140 patients (69.29%), while RX-guided procedures were used in 27/140 (19.29%) and CT-guided in 16/140 patients (11.43%).

5.4 NEUROMUSCULAR CLINICAL EVALUATION

Before every infusion each patient was subjected to an overall functional neuromuscular assessment by suitably trained personnel. The following functional motor scales have been used as primary outcome measures for the evaluation of treatment efficacy:

- Hammersmith Functional Motor Scale Expanded (HFMSE), consists of 33 items. A score from 0 to 2 is assigned to each item, depending on whether the required motor task is performed correctly, with compensation or is not executed at all. The maximum score is 66 points, and a higher score corresponds to better motor performance. Movements evaluated by HFMSE are related to motor functions, such as maintaining a sitting or standing posture, ability to roll, ability to sit up from lying, climbing a staircase, jumping etc. (41);
- Revised Upper Limb Module (RULM), dedicated to the evaluation of motor function of the upper limbs, is composed of 20 items with a maximum score of 37 points. A higher score corresponds to a better functional state (42);
- 6 Minutes Walking Test. The 6-Minute Walk test (6MWT) measures functional endurance during prolonged ambulation. To perform the test, the patient is asked to walk along a 25 meters long corridor for 6 minutes, and the walked distance is recorded as well as the rests or falls that may eventually occur. Initially, the test has been used in cardio-pulmonary disease trials, but recently it acquired relevance in the evaluation of neuromuscular disorders because it is an objective test, it is easy to administer, well tolerated by patients and better related to daily activity performance status than other tests.

For the purposes of this work, patients capable of walking, independently or with aids, without the external support of an operator for at least a few steps have been defined as “walkers”.

Table III. HFMSE items and clinical meaning. Adapted from Pera et al., 2017.

HFMSE item	HFMSE activities	Everyday activities
1	Able to sit on chair with legs off bed with or without support.	Sitting on a normal school chair or public space; sitting on toilets; sitting in cars.
2	Able to sit on floor cross legged or legs stretched in front.	Play on floor with siblings; sit on lounge chairs, deck-chair; picnic; travel with less equipment; inclusion in activities.
3	Able to bring hands to face at eye level.	Wash face; eat; put on glasses; answer telephone; blow nose.
4	Able to bring hands to head.	Scratch head; wash, brush, style hair; put on hat; dress upper body.
5	Roll to side.	Sleep alone; caregiver doesn't have to wake up to turn them; help during dressing lying down.
6-7-8-9	Roll.	Play; sleep well; sunbathe; experience space; reach for something at sides when lying down.
10	Able to lie down from sitting.	Independence: lie down and rest when tired; rest on the back; safety: fall in a controlled way (avoid head trauma).
11	Able to raise head when lying prone.	React to stimulus, surroundings exploration; read a book; not be afraid of choking; watch TV; on beach not get sand in face.
12-13	Able to prop on forearms or extended arms.	Read a book; watch TV; stretch back; sun bathe.
14	Able to sit up from lying.	No need for assistant; independence; sit up to drink at night.
15	Able to four-point kneel.	Play; hide; fit under small spaces.
16	Able to crawl.	Move around; experience space; get objects; play.
17	Lift head from supine.	Change head position; drink at night; read; watch TV; check the clock or alarm.

18	Stand with support.	Use toilet standing (boy); use full-length mirror, perceive body; shower properly; climb in car; cook.
19	Stand without support.	Public spaces: wait for bus, stand in queue; cook; use a sink; dress; reach object from a shelf.
20	Able to walk.	Freedom; get to places; not have to rely on wheelchair batteries.
21-22	Able to flex hip from supine.	Dress (pants, socks); scratch legs, kill mosquitoes, change position.
23-24-25-26	Able to half kneel.	Pick up objects from floors; tie shoe laces; put away object on low surfaces; pet a dog; play; make a proposal; kneel in church; talk with a kid.
27	Able to go from standing to sitting.	Not get hurt when falling; sit on grass or sand; pet a dog; sit beside a friend; pick up something from floor.
28	Able to squat.	Sit when needed; Pick up objects from floor; pee; tie shoes; pull up trousers.
29	Able to jump.	Have fun, play; dance, gymnastics; avoid obstacles; normality.
30-31-32-33	Go up and down stairs.	Absence of barriers; go to friend's home regardless of where they live; live at own home.

Table IV. Items of the RULM.

Description	0	1	2	3	4	5	6
A. entry item	No useful hand function	Can hold pencil, pick up token, drive a powered chair, use a phone key pad	Can raise 1 or 2 hands to mouth	Can raise plastic cup with 200g weight to mouth using two hands	Can raise both hands to shoulder height without compensation. Elbow bent in or in extension	Can raise both hands above the head by flexing the elbow shortening circumference of the movement	Can abduct both arms elbows in extension in a full circle until they touch above the head

Description	0	1	2
B. bring hands to table	Unable to bring 1 hand onto table	Brings one hand completely to table	Brings two hands completely to table
C. complete the written path without stopping or taking pencil off the paper	With pencil in hand unable to hold it or make a mark	Able to complete the path but needs to stop or raise pencil from paper	Able to complete the path without stops or raising hand from paper
D. pick up tokens	Cannot pick one token	Can pick one token	Can pick up and hold 2 tokens
E. place token into cup • On table: horizontal • At shoulder height: vertical	Unable to bring token	Able to bring token to cup lying horizontally	Able to bring token into cup placed at shoulder level
F. Reach to the side and touch the token; bring hand at shoulder height and above	Can't bring hand to shoulder height	Brings hand to shoulder height, elbow can be bent or extended	Brings hand above shoulder height, elbow at least at eye level
G. push button light with one hand	Unable to turn the light on with one hand	Able to turn the light on momentarily with	Able to turn the light on permanently with

		fingers and/or thumb of one hand. Elbow cannot be higher than the wrist	fingers and/or thumb of one hand. Elbow cannot be higher than the wrist
H. tearing paper	Cannot tear folded piece of paper	Tears the sheet of paper folded in 2, beginning from the folded edge	Tears the sheet of paper folded in 4, beginning from the folded edge
I. open Ziploc container	Unable to open	Able to open Ziploc container completely on table or against body	
J. Raise cup with 200g to mouth	Unable to get cup to mouth	Cup with 200g to mouth 2 hands	Cup with 200g to mouth 1 hand
K. lift weight and bring it from one circle to the other without sliding between horizontal circles (midline circle to outer on tested side)	Unable	Slide 200g weight	Lift 200g weight
L. lift weight and bring it from one circle to the other without sliding between horizontal circles (midline circle to outer on tested side)	Unable	Slide 500g weight	Lift 500g weight
M. lift weight and bring it from one circle to the other without sliding between diagonal circles (across midline, inner to outer	Unable	Slide 200g weight	Lift 200g weight

circle on opposite side)			
N. bring 500g sand weight from lap to table or eye level	Unable to bring weight to table using two hands	Brings weight onto table using two hands	Brings weight to eye level using two hands
O. Bring both arms above head – shoulder abduction	Unable	Can raise both arms simultaneously above head only by flexing the elbow (using compensation)	Can abduct both arms simultaneously elbows in extension in a full circle until they touch above the head
P. bring 500g weight above shoulder height – shoulder abduction	Unable to lift 500g weight even with compensation	Able to lift 500g weight with compensation	Able to lift 500g weight without compensation
Q. bring 1k weight above shoulder height – shoulder abduction	Unable to lift 1kg weight even with compensation	Able to lift 1kg weight with compensation	Able to lift 1kg weight without compensation
R. bring hand above shoulder height - shoulder flexion	Unable	Able with compensation	Able without compensation
S. bring 500g weight above shoulder height – shoulder flexion	Unable to lift 500g weight even with compensation	Able to lift 500g weight with compensation	Able to lift 500g weight without compensation
T. bring 1kg weight above shoulder height – shoulder flexion	Unable to lift 1kg weight even with compensation	Able to lift 1kg weight with compensation	Able to lift 1kg weight without compensation

In addition to motor functional data, we also collected data on the specific *SMN1* genotype, number of *SMN2* copies, age of onset, duration of the disease, age at the beginning of treatment with Nusinersen, need for Salbutamol or other adjunctive therapies, physiotherapy, surgical interventions, non-invasive ventilation or cough machine, chronic therapies, adverse events and other significant events.

5.5 STATISTICAL ANALYSIS

The term "improved" identifies patients who, at the end of the study period, presented an improvement over the baseline of 3 points or more on the HFMSE scale, 2 points on the RULM scale and 30 meters distance on 6MWT. These threshold values were previously identified in the literature as “clinically significant” and were used in previous clinical trials (40,41,43,44). The term “worsened” refers to patients who, at the end of the study period, had a worsening of at least 3 points at HFMSE, 2 points at RULM and 30 meters at 6MWT. The patients who did not show a significant improvement or worsening are referred as “stable”.

For the purposes of this study, the term “responders” included both “improved” and “stable” patients, as they oppose to the natural history of the disease, which foresees a progressive worsening.

All variables have been summarized according to median and interquartile range (IQR). The distribution of quantitative and ordinal variables between the various patient groups was compared by the Wilcoxon-Mann-Whitney test.

The statistical significance was set to $p < 0.05$. The statistical analysis was performed with software R version 4.1.2.

6) RESULTS

6.1 DEMOGRAPHIC AND CLINICAL, GENETIC AND FUNCTIONAL FEATURES AT BASELINE

The median age of onset of pathology in the 140 patients recruited was 3.5 years (range 0-17, IQR 2-10), while the median age at the beginning of therapy (T0) was 35 years (range 18-74, IQR 26.75-47.25). The patients recruited were predominantly male (86/140, 61.43%). 17/140 patients were SMA 2 (12.14%), while 123/140 were SMA type 3 (87.86%), of which 57 "*sitters*" and 66 "*walkers*". All patients with SMA type 2 had homozygous deletion of exons 7 and/or 8 of the *SMN1* gene. Three patients with type 3 SMA (2.86% of the total) had a compound heterozygosity for a 7 and/or 8 exonic deletion, variably associated with missense mutations (2 cases, p.Tyr130 and p.Gln157), nonsense (1 patient, p.Trp102) and a small insertion (1 patient, c.110insC, causing a frameshift mutation).

Table V summarizes the key clinical features, the *SMN1* gene mutations, the *SMN*2 haplotype and the motor functional evaluation at the baseline using HFMSE, RULM and 6MWT scales.

Table V. Clinical features and evaluation of motor function at baseline (T0).

	Total SMA		SMA 2		SMA 3 *sitter*		SMA 3 *walker*	
Age of onset (years)	140	3.5 (2-10)	17	0.8 (0.6-1.5)	57	3 (2-6)	66	8 (3-13)
Age at T0 (years)	140	35 (26.75-47.25)	17	24 (23-38)	57	40 (31-53)	66	33 (27-44.5)
Disease duration at T0 (years)	140	29.5 (22.3-41)	17	23,2 (21.75-35)	57	37 (28-47.7)	66	26 (19.625-38.875)
Gender (F/M)	140	54 / 86	17	5 / 12	57	16 / 41	66	33 / 33
***SMN1* genotype** - **Del** - **miss** - **non** - **ins**	140	 136 2 1 1	17	 17 0 0 0	57	 56 1 0 0	66	 63 1 1 1
Number of *SMN2* copies - **1** - **2** - **3** - **4** - **NA †**	140	 1* 5 45 64 25	17	 0 3 10 2 2	57	 0 2 18 23 14	66	 1* 0 17 39 9
Salbutamol (%)	140	31 (22.14%)	17	5 (29.41%)	57	12 (21.05%)	66	14 (21.21%)
FKT (%)	140	89 (63.57%)	17	14 (82.35%)	57	42 (73.68%)	66	33 (50%)
HFMSE score	140	24 (6-49)	17	0 (0-4)	57	9 (3-20)	66	50 (41.25-56)
RULM score	140	29 (17-37)	17	4 (0-13.5)	57	20 (16-26)	66	37 (34-37)
6MWT (m)	66	335 (230.5-435)	0	NA	0	NA	66	335 (230.5-435)

Age and scores at functional scales expressed as median (IQR).

** SMA 3 "walker" patient with single copy of SMN2 but carrying the SMN1 missense mutation c.859G>C.*

† Count of the number of copies of SMN2 not available for 25 patients.

Legend: F, female, HFMSE, Hammersmith Functional Motor Scale Expanded; M, male; 6MWT, 6 minutes walk test; NA, not available; RULM, Revised Upper Limb Module; SMA, Spinal muscular atrophy; SMN2, survival motor neuron 2 gene; del, deletion exons 7 and/or 8; miss, missense mutation in compound heterozygosis with exonic deletion; non, nonsense mutation in compound heterozygosity with exonic deletion; ins, small insertion in compound heterozygosity with exonic deletion; FKT, physiokinesitherapy.

6.2 EFFECT OF NUSINERSEN ON MOTOR FUNCTION

6.2.1 HFMSE

The absolute HFMSE score at the various time points is reported in Figure 6, while Figure 7 reports the variation at each time point of the HFMSE score compared to the baseline. The HFMSE score in patients with SMA type 2 has not changed significantly throughout the T0 to T42 follow-up (Table VI). In the group of patients with type 3 SMA, compared to baseline score, a significant median change of +1 point was observed at T6 (IQR 0, 3; p<0,0001), T10 (IQR 0, 4; p<0,0001), T22 (IQR 0, 3; p<0,0001) and T30 (IQR 0, 4; p=0,0003). In addition, a median improvement over T0 of +2 points was found in group SMA type 3 at T14 (IQR -0, 4; p<0,0001), T18 (IQR 0, 4; p<0,0001), T34 (IQR 0, 5; p=0,0001) and finally T38 (IQR 0, 5; p=0,0013). The analysis of the SMA type 3 "*sitter*" subgroup of patients shows that statistically significant improvements are present up to time point T38, with a median score increase, compared to T0, up to +2.5 points (IQR -1, 6.75; p=0.0002) to T34. On the contrary, in the "*walker*" subgroup, significance is lost at time point T22 (Table VI), about two years after the start of treatment.

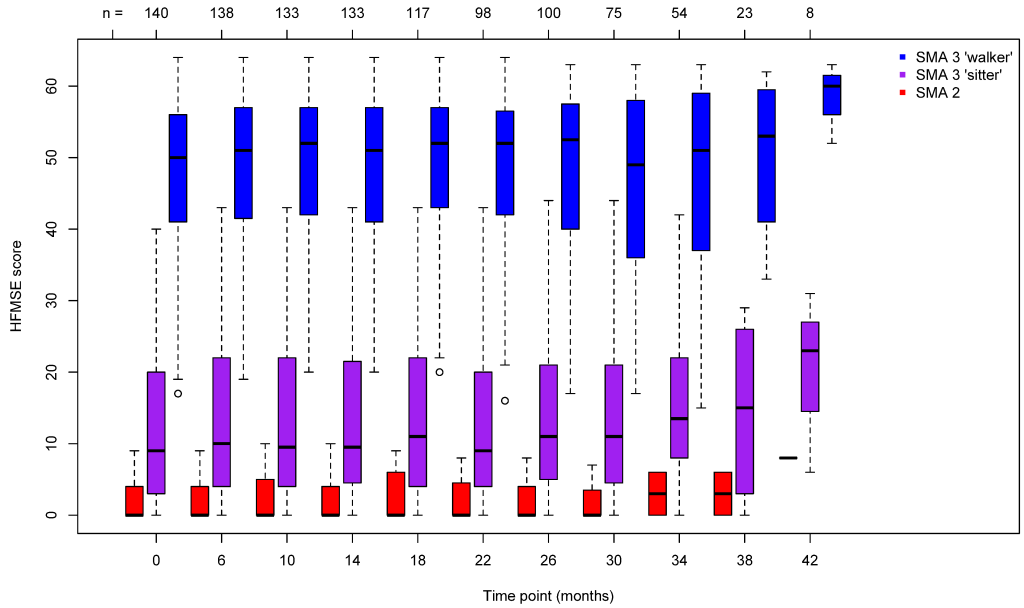

Figure 6. Box-whisker plots of HFMSE absolute scores during the follow-up. *The various colors correspond to the patient subgroups (red: SMA2, purple: SMA3 sitter and blue: SMA3 walker). The boxes identify the interval between the first and third quartiles of the distribution. The horizontal thick lines indicate the median score values while the Whiskers indicate the maximum/minimum values or the first/third quartile ±1.5 x interquartile range (IQR), in the presence of extreme values. Legend: n = number of patients evaluated at each time point; HFMSE, Hammersmith Functional Motor Scale–Expanded.*

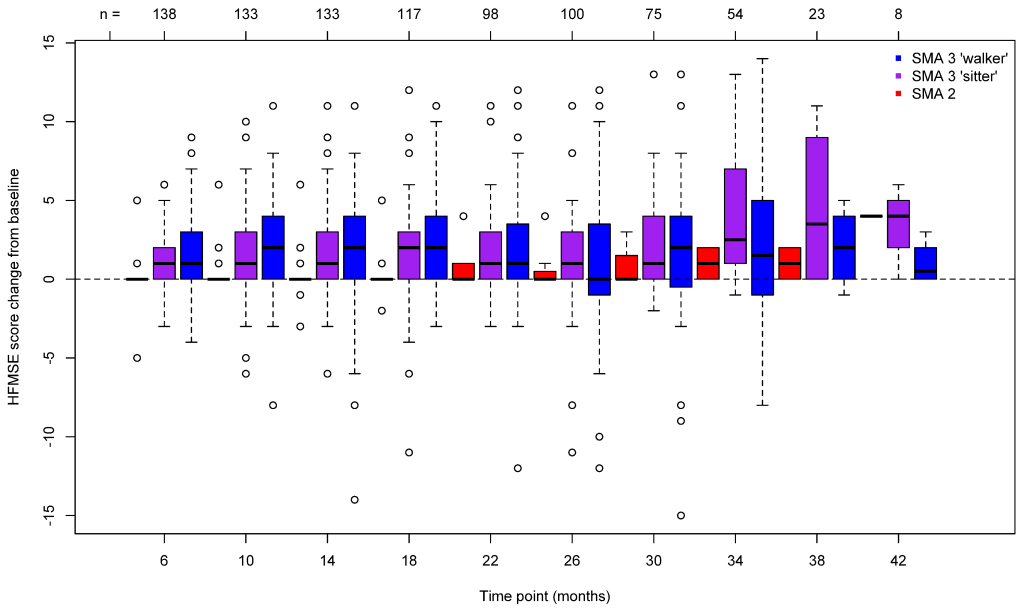

Figure 7. Box-whisker plots of the median score variation at the HFMSE scale versus the baseline during the follow-up. *The various colors correspond to the patient subgroups (red: SMA2, purple: SMA3 sitter and blue: SMA3 walker). The boxes identify the interval between the first and third quartiles of the distribution. The horizontal thick lines indicate the median score values while the Whiskers indicate the maximum/minimum values or the first/third quartile ±1.5 x interquartile range (IQR), in the presence of extreme values. Legend: n = number of patients evaluated at each time point; HFMSE, Hammersmith Functional Motor Scale–Expanded.*

6.2.2 RULM

The changes of the RULM score at the various time points are reported in Table VI; Figure 8 shows the trend of the absolute value of the RULM score in the various SMA subtypes studied, while Figure 9 shows the changes in the score compared to the baseline at the various time points. In the SMA type 3 "*sitter*" subgroup a statistically significant median score change was observed from T10 up to T26. The median score improved by 1 point compared to T0 at time points T10 (IQR 0, 3; p=0,0014), T14 (IQR 0, 3.5; p=0,0006), T18 (IQR 0, 3; p=0,0021) and T26 (IQR 0, 3; p=0,0028). A statistically significant improvement of +1.5 points was found at T22 (IQR 0, 3.25; p<0.0001). In the subset of SMA type 3 "*walkers"* analysis, no changes were observed in the scores obtained on the RULM scale, because of a marked roof effect (Table VI).

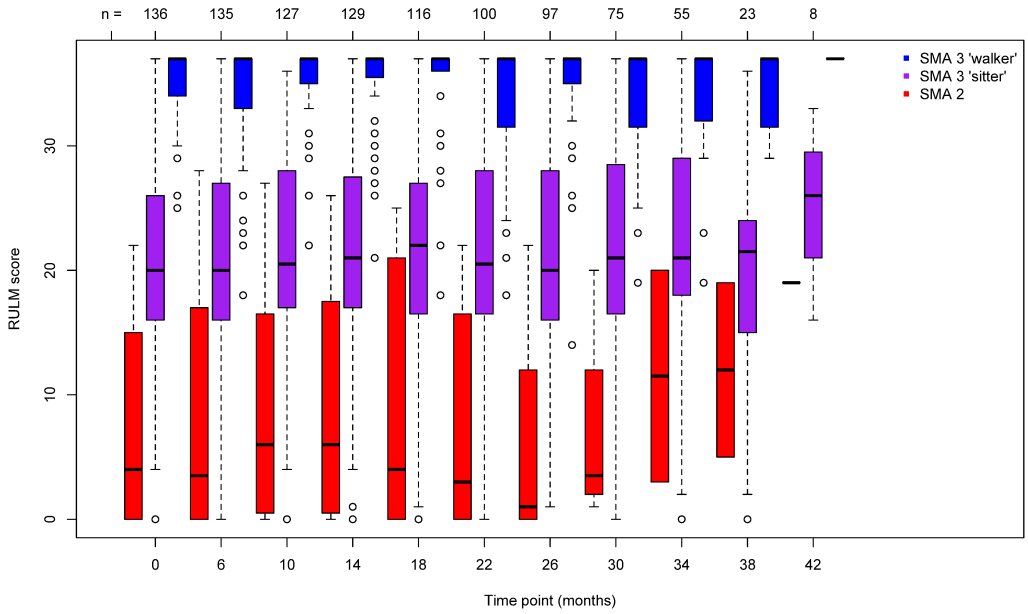

Figure 8. Box-whisker plots of RULM absolute scores during the follow-up. *The various colors correspond to the patient subgroups (red: SMA2, purple: SMA3 sitter and blue: SMA3 walker). The boxes identify the interval between the first and third quartiles of the distribution. The horizontal thick lines indicate the median score values while the Whiskers indicate the maximum/minimum values or the first/third quartile ±1.5 x interquartile range (IQR), in the presence of extreme values. Legend: n = number of patients evaluated at each time point; RULM, Revised Upper Limb Module.*

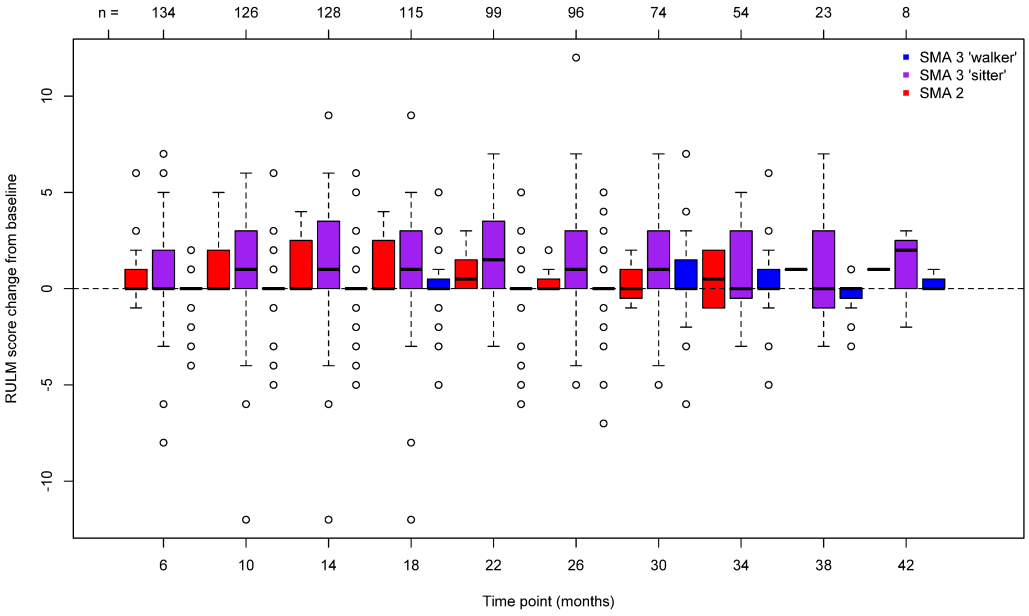

Figure 9. Box-whisker plots of the median score variation on the RULM scale versus the baseline during the follow-up. *The various colors correspond to the patient subgroups (red: SMA2, purple: SMA3 sitter and blue: SMA3 walker). The boxes identify the interval between the first and third quartiles of the distribution. The horizontal thick lines indicate the median score values while the Whiskers indicate the maximum/minimum values or the first/third quartile ±1.5 x interquartile range (IQR), in the presence of extreme values. Legend: n = number of patients evaluated at each time point; RULM, Revised Upper Limb Module.*

6.2.3 6MWT

Figure 10 shows the trend of the absolute value of the distance walked during the follow-up of SMA 3 "*walker*" patients; Figure 11 represents graphically the variation of the distance walked at the various time points compared to baseline. The distance walked at 6MWT was significantly improved during the follow-up at time points T6 (median +11 m, p<0,0001), T10 (median +20.5 m, p=<0.0001), T14 (median +20 m, p=0,0002), T18 (median +19,5 m, p=0,002) and T22 (median +17 m, p=0,001). From the second year of therapy, the statistical significance is lost in the presence of an overall positive trend with a median improvement in the distance travelled of +17.5 m at T26, +12 m at T30 and +20 m at T34 (Table VI).

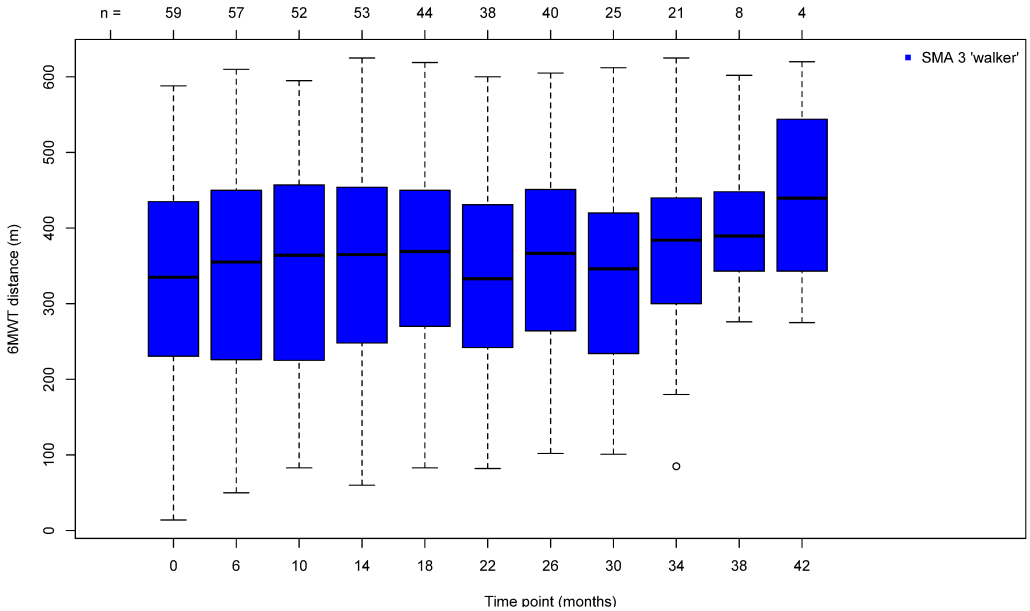

Figure 10. Box-whisker plots of the distance walked at 6MWT by the SMA3 "walker" patients during the follow-up.. *The boxes identify the interval between the first and third quartiles of the distribution. The horizontal thick lines indicate the median score values while the Whiskers indicate the maximum/minimum values or the first/third quartile ±1.5 x interquartile range (IQR), in the presence of extreme values. Legend: n = number of patients evaluated at each time point; 6MWT, 6 min walking test.*

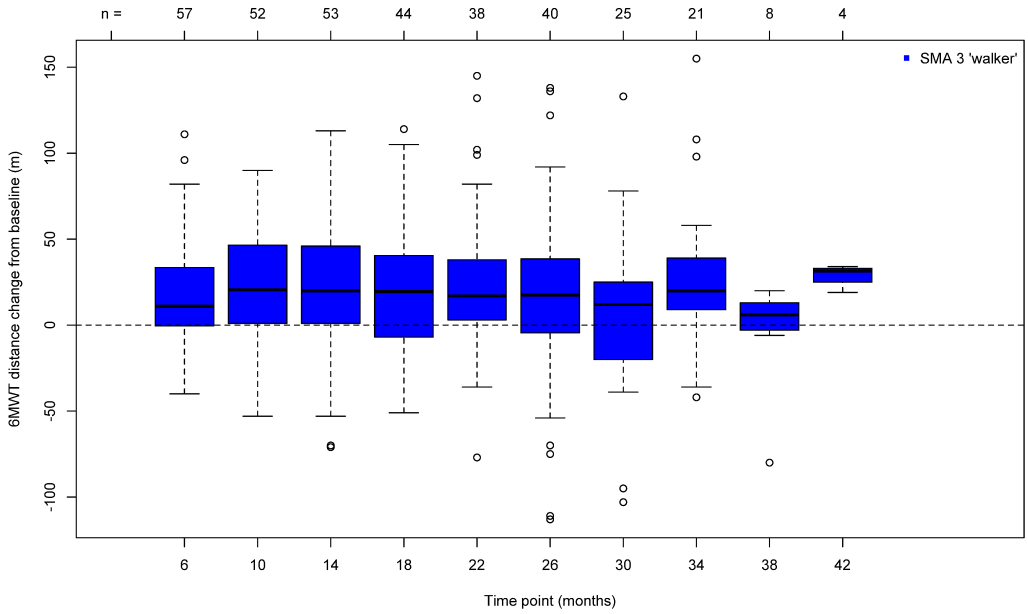

Figure 11. Box-whisker plots of the median variation of distance walked by the SMA3 "walker" patients at 6MWT compared to the baseline during the follow-up.*). The boxes identify the interval between the first and third quartiles of the distribution. The horizontal thick lines indicate the median score values while the Whiskers indicate the maximum/minimum values or the first/third quartile ±1.5 x interquartile range (IQR), in the presence of extreme values. Legend: n = number of patients evaluated at each time point; 6MWT, 6 min walking test.*

	Table VI. Functional changes at various time points (T6-T42) in subgroups of patients with SMA type 2 and SMA type 3 (also subdivided in sitters and walkers).																			
		All SMA				SMA2				SMA3 sitter				SMA3 walker				All SMA3			
Time points	Variable	n	Median	IQR	Wilcoxon p value	n	Median	IQR	Wilcoxon p value	n	Median	IQR	Wilcoxon p value	n	Median	IQR	Wilcoxon p value	n	Median	IQR	Wilcoxon p value
ΔT0-T6	HFMSE	138	0	0 ~ 2	<0.0001	17	0	0 ~ 0	0.71	57	1	0 ~ 2	<0.0001	64	1	0 ~ 3	<0.0001	121	1	0 ~ 3	<0.0001
	RULM	134	0	0 ~ 1	0.037	16	0	0 ~ 0.5	0.1	57	0	0 ~ 2	0.031	61	0	0 ~ 0	0.6	118	0	0 ~ 1	0.11
	6MWT	57	11	-3 ~ 32	<0.0001	NA	NA	NA	NA	NA	NA	NA	NA	57	11	-3 ~ 32	<0.0001	57	11	-3 ~ 32	<0.0001
ΔT0-T10	HFMSE	133	1	0 ~ 3	<0.0001	16	0	0 ~ 0	0.18	56	1	0 ~ 3	0.0003	61	2	0 ~ 4	<0.0001	117	1	0 ~ 4	<0.0001
	RULM	126	0	0 ~ 1	0.0002	15	0	0 ~ 2	0.022	54	1	0 ~ 3	0.0014	57	0	0 ~ 0	0.43	111	0	0 ~ 1	0.0015
	6MWT	52	20.5	1.5 ~ 46.25	<0.0001	NA	NA	NA	NA	NA	NA	NA	NA	52	20.5	1.5 ~ 46.25	<0.0001	52	20.5	1.5 ~ 46.25	<0.0001
ΔT0-T14	HFMSE	133	1	0 ~ 3	<0.0001	16	0	0 ~ 0	0.68	56	1	0 ~ 3	<0.0001	61	2	0 ~ 4	0.0003	117	2	0 ~ 4	<0.0001
	RULM	128	0	0 ~ 2	0.0001	15	0	0 ~ 2.5	0.022	55	1	0 ~ 3.5	0.0006	58	0	0 ~ 0	0.5	113	0	0 ~ 2	0.001
	6MWT	53	20	1 ~ 46	0.0002	0	NA	NA	NA	NA	NA	NA	NA	53	20	1 ~ 46	0.0002	53	20	1 ~ 46	0.0002
ΔT0-T18	HFMSE	117	1	0 ~ 4	<0.0001	13	0	0 ~ 0	0.58	51	2	0 ~ 3	0.0008	53	2	0 ~ 4	0.0001	104	2	0 ~ 4	<0.0001
	RULM	115	0	0 ~ 2	0.0003	12	0	0 ~ 2.25	0.058	51	1	0 ~ 3	0.0021	52	0	0 ~ 0.25	0.3	103	0	0 ~ 2	0.0015
	6MWT	44	19.5	-6.5 ~ 39.25	0.002	0	NA	NA	NA	NA	NA	NA	NA	53	19.5	-6.5 ~ 39.25	0.002	44	19.5	-6.5 ~ 39.25	0.002
ΔT0-T22	HFMSE	98	1	0 ~ 3	<0.0001	8	0	0 ~ 1	0.17	43	1	0 ~ 3	0.001	47	1	0 ~ 3.5	0.12	90	1	0 ~ 3	<0.0001
	RULM	99	0	0 ~ 3	0.0014	8	0.5	0 ~ 1.25	0.098	44	1.5	0 ~ 3.25	<0.0001	47	0	0 ~ 0	0.96	91	0	0 ~ 3	0.0033
	6MWT	38	17	3 ~ 37.75	0.001	0	NA	NA	NA	NA	NA	NA	NA	38	17	3 ~ 37.75	0.001	38	17	3 ~ 37.75	0.001
ΔT0-T26	HFMSE	100	0	-1 ~ 3	0.0043	7	0	0 ~ 0.5	0.37	45	1	0 ~ 3	0.017	48	0	-1 ~ 3.25	0.13	93	1	-1 ~ 3	0.066
	RULM	96	0	0 ~ 2	0.0036	7	0	0 ~ 0.5	0.37	43	1	0 ~ 3	0.0028	46	0	0 ~ 0	0.58	89	0	0 ~ 2	0.0058
	6MWT	40	17.5	-3.75 ~ 37.25	0.053	0	NA	NA	NA	NA	NA	NA	NA	40	17.5	-3.75 ~ 37.25	0.053	40	17.5	-3.75 ~ 37.25	0.053
ΔT0-T30	HFMSE	75	1	0 ~ 4	0.0002	4	0	0 ~ 0.75	1	36	1	0 ~ 4	0.0006	35	2	-0.5 ~ 4	0.065	71	1	0 ~ 4	0.0003
	RULM	74	0	0 ~ 2	0.025	4	0	-0.25 ~ 0.5	1	35	1	0 ~ 3	0.053	35	0	0 ~ 1.5	0.33	70	0	0 ~ 2.75	0.03
	6MWT	25	12	-20 ~ 25	0.42	0	NA	NA	NA	NA	NA	NA	NA	25	12	-20 ~ 25	0.42	25	12	-20 ~ 25	0.42
ΔT0-T34	HFMSE	54	2	0 ~ 5	<0.0001	2	1	0.5 ~ 1.5	1	22	2.5	1-6.75	0.0002	30	1.5	-0.75 ~ 4.75	0.75	52	2	0 ~ 5	0.0001
	RULM	54	0	0 ~ 2	0.038	2	0.5	-0.25 ~ 1.25	1	23	0	-0.5 ~ 3	0.036	29	0	0 ~ 1	0.41	52	0	0 ~ 2	0.044
	6MWT	21	20	9 ~ 39	0.019	0	NA	NA	NA	NA	NA	NA	NA	21	20	9 ~ 39	0.019	21	20	9 ~ 39	0.019
ΔT0-T38	HFMSE	23	2	0 ~ 5	0.0009	1	1	0.5 ~ 1.5	1	10	3.5	0.5-8.25	0.022	11	2	0-4	0.34	21	2	0 ~ 5	0.0013
	RULM	23	0	-0.5 ~ 1	0.72	2	1	1 ~ 1	0.35	10	0	-0.75 ~ 2.75	0.35	11	0	-0.5 ~ 0	0.27	21	0	-1 ~ 0	0.82
	6MWT	8	6	-1.5 ~ 12.5	0.45	0	NA	NA	NA	NA	NA	NA	NA	8	6	-1.5 ~ 12.5	0.45	8	6	-1.5 ~ 12.5	0.45
ΔT0-T42	HFMSE	8	2	0 ~ 4	0.058	1	4	4 ~ 4	1	3	4	44683	0.37	4	0.5	0-1.5	0.37	7	1	0 ~ 3.5	0.1
	RULM	8	0.5	0 ~ 1.25	0.34	1	1	1 ~ 1	1	3	2	0 ~ 2.5	0.59	4	0	0 ~ 0.25	1	7	0	0 ~ 1.5	0.46
	6MWT	4	31.5	28 ~ 32.5	0.13	0	NA	NA	NA	NA	NA	NA	NA	4	31.5	28 ~ 32.5	0.13	4	31.5	28 ~ 32.5	0.13

6.3 RATE OF RESPONDER TO THE NUSINERSEN THERAPY

Table VII shows the number of responders patients to therapy with Nusinersen in a selection of time points. Patients have been divided into 3 subgroups: “improved”, “worsened” or “stable”. In particular, the term “improved” defines an increase of 3 or more points to the HFMSE scale, 2 or more points to the RULM and 30 meters or more to the 6MWT; the term “worsened” refers to a decrease of 3 or more points to the HFMSE scale, 2 or more points to the RULM and 30 meters or more to the 6MWT. Patients who did not significantly increase or decrease their motor function fall under the definition of “stable”.

Considering the entire SMA cohort, the rate of “improved” at the HFMSE is 24% at T6, 32% at T10, 34% at T14, 39% at T18, 37% at T22, 31% at T26, 39% at T30, 43% at T34 and 43% at T38. At the same timepoints, the “stable” patients ranged from 52% to 73%, while the “worsened” were between 0% and 12%.

The rate of “improved” patients at the RULM is 19% at T6, 25% at T10, 28% at T14, 30% at T18, 32% at T22, 26% at T26, 34% at T30 and 33% at T34. The “stable” patients ranged from 52% to 71%, while the “worsened” ranged from 8% to 15% at the considered timepoints.

In the SMA type 3 walkers, the “improved” patients at the 6MWT are 28% at T6, 37% at T10, 38% at T14, 36% at T18 and 37% at T22. At the same timepoints, the “stable” patients ranged from 51% to 65%, while the “worsened” were between 6% and 11%.

Overall, less patients with SMA type 2 “improved” compared to SMA type 3, but, less patients “worsened”.

Table VII. Rate of response to therapy with Nusinersen at various time points (T6-T42) in subgroups of patients with SMA type 2 and SMA type 3 (also subdivided in sitters and walkers).

		All SMA							SMA2							SMA3 "sitter"							SMA3 "walker"							All SMA3						
	time point	n	W	S	I	W%	S%	I%	n	W	S	I	W%	S%	I%	n	W	S	I	W%	S%	I%	n	W	S	I	W%	S%	I%	n	W	S	I	W%	S%	I%
HFMSE	T6	138	4	101	33	3%	73%	24%	17	1	15	1	6%	88%	6%	57	2	41	14	4%	72%	25%	64	1	45	18	2%	70%	28%	121	3	86	32	2%	71%	26%
	T10	133	7	84	42	5%	63%	32%	16	0	15	1	0%	94%	6%	56	4	35	17	7%	63%	30%	61	3	34	24	5%	56%	39%	117	7	69	41	6%	59%	35%
	T14	133	11	77	45	8%	58%	34%	16	1	14	1	6%	88%	6%	56	3	33	20	5%	59%	36%	61	7	30	24	11%	49%	39%	117	10	63	44	9%	54%	38%
	T18	117	10	61	46	9%	52%	39%	13	0	12	1	0%	92%	8%	51	4	25	22	8%	49%	43%	53	6	24	23	11%	45%	43%	104	10	49	45	10%	47%	43%
	T22	98	5	57	36	5%	58%	37%	8	0	7	1	0%	88%	13%	43	2	24	17	5%	56%	40%	47	3	26	18	6%	55%	38%	90	5	50	35	6%	56%	39%
	T26	100	12	57	31	12%	57%	31%	7	0	6	1	0%	86%	14%	45	4	27	14	9%	60%	31%	48	8	24	16	17%	50%	33%	93	12	51	30	13%	55%	32%
	T30	75	6	40	29	8%	53%	39%	4	0	3	1	0%	75%	25%	36	0	25	11	0%	69%	31%	35	6	12	17	17%	34%	49%	71	6	37	28	8%	52%	39%
	T34	54	3	28	23	6%	52%	43%	2	0	2	0	0%	100%	0%	22	0	11	11	0%	50%	50%	30	3	15	12	10%	50%	40%	52	3	26	23	6%	50%	44%
	T38	23	0	13	10	0%	57%	43%	2	0	2	0	0%	100%	0%	10	0	5	5	0%	50%	50%	11	0	6	5	0%	55%	45%	21	0	11	10	0%	52%	48%
	T42	8	0	4	4	0%	50%	50%	1	0	0	1	0%	0%	100%	3	0	1	2	0%	33%	67%	4	0	3	1	0%	75%	25%	7	0	4	3	0%	57%	43%
RULM	T6	134	13	95	26	10%	71%	19%	16	0	12	4	0%	75%	25%	57	7	33	17	12%	58%	30%	61	6	50	5	10%	82%	8%	118	13	83	22	11%	70%	19%
	T10	126	10	85	31	8%	67%	25%	15	0	10	5	0%	67%	33%	54	6	26	22	11%	48%	41%	57	4	49	4	7%	86%	7%	111	10	75	26	9%	68%	23%
	T14	128	13	79	36	10%	62%	28%	15	0	10	5	0%	67%	33%	55	7	23	25	13%	42%	45%	58	6	46	6	10%	79%	10%	113	13	69	31	12%	61%	27%
	T18	115	12	68	35	10%	59%	30%	12	0	8	4	0%	67%	33%	51	7	21	23	14%	41%	45%	52	5	39	8	10%	75%	15%	103	12	60	31	12%	58%	30%
	T22	99	11	56	32	11%	57%	32%	8	0	6	2	0%	75%	25%	44	5	17	22	11%	39%	50%	47	6	33	8	13%	70%	17%	91	11	50	30	12%	55%	33%
	T26	96	8	63	25	8%	66%	26%	7	0	6	1	0%	86%	14%	43	4	22	17	9%	51%	40%	46	4	35	7	9%	76%	15%	89	8	57	24	9%	64%	27%
	T30	74	9	40	25	12%	54%	34%	4	0	3	1	0%	75%	25%	35	5	15	15	14%	43%	43%	35	4	22	9	11%	63%	26%	70	9	37	24	13%	53%	34%
	T34	54	8	28	18	15%	52%	33%	2	0	1	1	0%	50%	50%	23	5	8	10	22%	35%	43%	29	3	19	7	10%	66%	24%	52	8	27	17	15%	52%	33%
	T38	23	4	15	4	17%	65%	17%	2	0	2	0	0%	100%	0%	10	2	4	4	20%	40%	40%	11	2	9	0	18%	82%	0%	21	4	13	4	19%	62%	19%
	T42	8	1	5	2	13%	63%	25%	1	0	1	0	0%	100%	0%	3	1	0	2	33%	0%	67%	4	0	4	0	0%	100%	0%	7	1	4	2	14%	57%	29%
6MWT	T6	57	4	37	16	7%	65%	28%	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	57	4	37	16	7%	65%	28%	57	4	37	16	7%	65%	28%
	T10	52	3	30	19	6%	58%	37%	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	52	3	30	19	6%	58%	37%	52	3	30	19	6%	58%	37%
	T14	53	6	27	20	11%	51%	38%	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	53	6	27	20	11%	51%	38%	53	6	27	20	11%	51%	38%
	T18	44	4	24	16	9%	55%	36%	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	53	6	27	20	11%	51%	38%	44	4	24	16	9%	55%	36%
	T22	38	3	21	14	8%	55%	37%	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	38	3	21	14	8%	55%	37%	38	3	21	14	8%	55%	37%
	T26	40	7	19	14	18%	48%	35%	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	40	7	19	14	18%	48%	35%	40	7	19	14	18%	48%	35%
	T30	25	4	15	6	16%	60%	24%	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	25	4	15	6	16%	60%	24%	25	4	15	6	16%	60%	24%
	T34	21	2	12	7	10%	57%	33%	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	21	2	12	7	10%	57%	33%	21	2	12	7	10%	57%	33%
	T38	8	1	7	0	13%	88%	0%	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	8	1	7	0	13%	88%	0%	8	1	7	0	13%	88%	0%
	T42	4	0	1	3	0%	25%	75%	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	4	0	1	3	0%	25%	75%	4	0	1	3	0%	25%	75%

W: Worsened (≤ -3 HFMSE, ≤ -2 RULM, ≤ -30 m 6MWT). S: Stable (HFMSE > -3 and < +3; RULM > -2 and < +2; 6MWT between > -30 m and < +30 m. I: Improved (≥ +3 HFMSE, ≥ +2 RULM, ≥ +30 m 6MWT)

7) CONCLUSIONS

A growing interest in defining the clinical effect of disease-modifying therapies (DMT) in SMA adult patients is emerging. An increasing number of papers in the literature support the safety and efficacy of Nusinersen in individuals with SMA, even in the adult patients initially excluded from the early registration studies [Pane et al, 2019; Aragon-Gawinska et al., 2018; Audic et al., 2020; Hagenacker et al., 2020; Maggi et al., 2020; Coratti et al., 2021]. The need to define the clinical effect of the DMT in adult is outstanding since between 80 to 90 percent of adult SMA patients in Europe remains untreated with DMT, although interested in the treatment [Gusset et al., 2021], The main limitation to obtain exhaustive information on the efficacy of these new treatments is driven by the lack of natural history data for untreated adult SMA type 2 and 3 patients and the relative heterogeneity of the functional scales used in the various studies [Piepers et al., 2008, Mercuri et al., 2015; Wadman et al., 2018; Wijngaarde et al., 2020; Annoussamy et al., 2020; Coratti et al., 2020].

This retrospective longitudinal study conducted in adult patients with SMA type 2 and type 3 aimed to assess, in the "real-world" setting, the effect of the Nusinersen treatment in an extended time frame compared to the previous report by Maggi et al. in 2020 (40) attempts to fill this gap of knowledge

Statistically significant changes in the HFMSE and RULM were observed in the entire cohort of SMA 3 patients up to 34 months of follow-up. The functional improvement on HFMSE was larger in the "*sitter*" subgroup of patients that maintain a positive trend of clinical improvement up to 38 months after the start of therapy (median score improvement from baseline between +1 point and +3.5 points from T6 to T38). In SMA 3 "*walker*" patients, on the other hand, scores on the HFMSE and distance walked at 6MWT significantly improve up to two years after the initiation of therapy (T22) and then maintain a positive trend at subsequent follow-up time points.

It is possible that this trend reflects the attainment of the maximum motor and functional improvement possible for SMA 3 walker patients under Nusinersen therapy. Longer-term follow-up of such patients, and possibly, of patients who discontinue Nusinersen therapy, will be necessary to test this hypothesis will be necessary to test this hypothesis.

Our study confirmed the relative poor sensitivity of RULM in detecting changes in highly functional SMA type 3 "*walker*" patients because of a marked "ceiling effect". In "*sitters*" subgroup, on the contrary, RULM allowed the detection of statistically significant positive changes up to time point T26 (median +1 point), with subsequent relative apparent stabilization.

The analysis of the SMA2 subgroup of patients is limited by the relatively small sample size, especially for advanced time points (the median follow-up of 22 months). The usefulness of the HFMSE scale in the evaluation of this subclass of patients appears to be strongly limited by a major "floor effect", while the RULM underwent modest variations during the follow-up.

The efficacy of Nusinersen is confirmed for the HFMSE in almost all subgroups of patients and overall in the entire cohort, considering that the rate of patients who did not significantly worsen ranges from 88% to 100% in the different time points up to T38. The frequency of patients who did not significantly worsen considering the RULM scale, analyzing the entire cohort, tends to stabilize between 85% and 92% up to T34. For the 6MWT, the frequency of not significantly worsened "*walker*" patients peaked at 94% after 10 months of therapy, and it tends to decrease from the second year, when the sample of patients is reduced and significance is lost.

Major limitations of this study include the small number of patients with SMA type 2 and the retrospective nature of the study, with the loss of a limited amount of data for some of the variables studied during the follow-up. Another limitation is the failure to use a long-term self-reported subjective efficacy scale although a subset of the patients covered by the present work has been evaluated, 14 months after the start of therapy, through the questionnaire Individualized Neuromuscular Quality of Life (INQoL) (45).

In conclusion, this study confirms the efficacy of Nusinersen in the treatment of adult patients with type 3 SMA at more than 34 months of follow-up. As the observation period was lengthened compared to the Maggi et al report, an apparent relative stabilization emerged, approximately two years after the initiation of therapy, in the scores on the HFMSE and 6MWT in the subgroup of SMA 3 walkers and, to a lesser extent, in the RULM score in the subgroup of SMA 3 sitter patients. The relative paucity of the sample of patients with SMA type 2, in particular at the

most advanced time points, does not allow definitive conclusions about the efficacy of Nusinersen in this subgroup of patients.

SMA is a progressive disease also in adults, with progressive disability accumulation over time, without stabilization. Considering the progression rates reported in the literature in all SMA subtypes using different functional scales, this study demonstrates the long-term effectiveness of Nusinersen in stabilizing/improving a disease considered incurable and progressive until 2016.

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