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25 years of the SMN genes: the Copernican revolution of spinal muscular atrophy

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The new era of advanced therapies has influenced and changed the views and perspectives of a neuromuscular disease such as spinal muscular atrophy (SMA). Being an autosomal recessive motor neuron disorder, characterized by different degrees of muscle weakness, after 25 years of the discovery of the determinant and modifier genes (*SMN1* and *SMN2*, respectively) three SMN-dependent specific therapies are already approved by FDA (two by EMA), so that worldwide patients are currently under clinical investigation and treatment. This success was the combined effort mainly of patients and families, physician and researchers, advocacy groups and several Institutions together with the support of pharmaceutical companies. Progression trajectories, phenotypes, follow-up and care of the patients are continously evolving. Clinical investigations are currently demonstrating that early diagnosis and intervention are essential for better and more effective response to treatment, consistently improving prognosis. This scenario has created the need for awareness, early diagnosis and even implementation of of newborn screening programs. New views and perspectives of patient and family expectations, genetic counselling and multidisciplinary care: a truly Copernican revolution in neuromuscular and genetic diseases.

Key words: spinal muscular atrophy, early diagnosis and intervention, advanced therapies, genetic counselling, antisense oligonucleotides, gene therapy

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FDT has received grant support to conduct NBS on SMA from Biogen and serves as a consultant to Biogen, AveXis and Roche.
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Introduction/overview

Spinal muscular atrophy (SMA) linked to 5q is an autosomal recessive neuromuscular disorder caused by the degeneration of alpha motor neurons of the spinal cord anterior horns. The main manifestation of the disease is muscle weakness by denervation followed by respiratory failure and infant death in the most severe cases. However, the experience of patients is dominated by the downstream complications such as compromised respiration, impaired nutrition, deformities (i.e. scoliosis and contractures) and limited functional ability. SMA is one of the commonest severe hereditary disorders of infancy and early childhood, with an incidence estimated of 1/6000 to 1/10000 births and a carrier frequency of 1/35 to 1/50 [1]. Originally described by Guido Werdnig and Johann Hoffmann in the XIX century [2], after several decades in the XX century of clinical descriptions and eponymous classifications, the interest of SMA started to increase in 1995, when the causative *SMN1* gene was discovered by the group of Judith Melki [3] (Fig. 1). With the advent of animal models, preclinical studies contributed to test therapeutic alternatives (the translational research decade between 2000 and 2010). In 2011, clinical trials (CT) in humans where initiated.

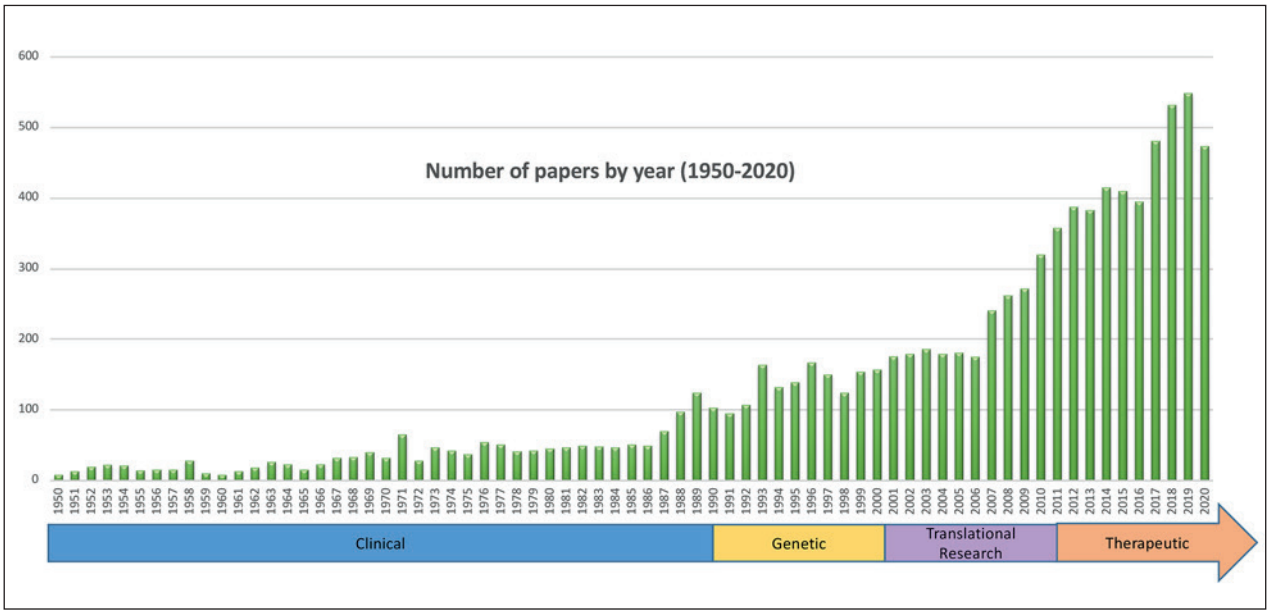

Figure 1. Stages of SMA progress in the last 70 years. Several decades considering 1950 onwards to the nineties were purely clinic. The genetic decade starts with the discovery of the SMN1 gene in 1995 doubling the number of publications. A translational research decade from 2000 to 2010 is following defined by the availability of animal models to test therapies and preclinical studies that also increased the number of publications. The last decade started in 2011, and includes the different clinical programs and the growing interest in SMA during the last years with more than 500 publications in 2019 and a higher number (n = 578) in the current unfinished year 2020 (Source: PubMED last entry December 2, 2020).

In less than ten years, three advanced therapies in SMA have been already approved by FDA. An antisense oligonucleotide (ASO) that affects splicing of the pre-mRNA (*nusinersen*, Spinraza®) in 2016 [4], a self-complementary adeno associated virus serotype 9 (AAV9) gene therapy (*Onasemnogene Abeparvovec*, ZolgenSMA®) in 2019 [5] and an oral compound that acts as splicing modifier (*risdiplam*, Evrysdi®) in 2020 [6].

SMA clinical picture is viewed as a continuous spectrum of manifestations ranging from serious congenital forms to minimal manifestations in adulthood. To better follow-up and categorize SMA patients, a classification into three main types based on age at onset and maximum milestones achieved have been reported in 1992[7] and several subtypes have been also defined [8].Type I, the most severe form, manifests early in the first weeks or months of life, with generalized hypotonia. Patients are so weak that never achieve the sitting position. Natural history studies indicate that more than 90% of these cases will have died by 2 years of age due to complications of respiratory problems [9,10]. Type I is known as the severe form given that the patients are so weak that never achieve the sitting position. Three subtypes can be identified: type Ia that starts very early after birth and also may overlap in some cases with the congenital (type 0) severely extreme form; type Ib corresponds to the typical form that start before the three months and usually patients never have head control; type Ic is detected after three months and patients may have some head control but never sit independently. In the type II form, patients manifest the disease after the 6 months of life and are able to sit but never walk independently and are permanently confined to a wheelchair (type IIa). Some stronger patients are able to stand up and even perform few steps with support (type IIb). In the type III form, patients can walk, but depending on the age of onset (less or more than 3 years), patients may lose the walking ability sooner in childhood (type IIIa) or later in adult life (type IIIb) respectively [8]. All these SMA types are the result of insufficient amounts of SMN protein which is encoded by two genes: Survival motor neuron 1 (*SMN1*) and Survival motor neuron 2 (*SMN2*) both located in a complex region of chromosome 5 (5q13) [3]. Although the SMN protein is ubiquitously expressed in all cells to guarantee living and survival, lower levels, as seen in SMA, are insufficient to protect motor neurons and the neuromuscular system [11,12].

SMA genetics and SMN protein function

The SMN genes are located in a region of chromosome 5q13 harbouring a segmental duplication [3]. *SMN1*

and *SMN2* share 99% homology, with few nucleotide changes in the coding region. Nonetheless, *SMN2* is an hypomorphic allele of *SMN1*, due to the alternative splicing of the 8th exon (exon 7), mediated by a C→T transition in position +6 [13-19]. This substitution disrupts interactions of the pre-mRNA with splicing enhancer and silencer proteins such that *SMN2* transcripts predominantly exclude exon 7 [13,19-24]. *SMN2* genes do not produce sufficient full length SMN protein to prevent the onset of the disease but, on the other hand, because each *SMN2* gene can still produce full-length SMN transcripts, no patient is devoid of SMN protein. Likely due to ancestral unequal crossing-over events, *SMN2* copy number is variable in patients and inversely related with the severity. Although *SMN2* is considered a good predictor of disease evolution, the correlation is not absolute and discordances may exist that need further investigations. (See Calucho et al. 2018, for a meta-analysis) [15,25].

Since the identification of the *SMN1* gene, a number of functions have been attributed to the SMN protein. So far, we know that SMN is ubiquitous, highly conserved across species, highly expressed during early development, and that SMN levels are higher in spinal cord and brain, but significantly down-regulated after birth [26-28]. SMN protein is member of a large, highly stable macromolecular complex that localizes in both the nuclear and cytoplasmic compartments of the cell [29]. While we know that SMN protein produced by the *SMN1* gene is fully functional, several lines of experimental evidence suggest that SMNΔ7 protein is rapidly degraded [30,31]. The SMN C-terminal domain is highly conserved and responsible for oligomerization, a process that is indispensable for its inclusion into the SMN complex. It has been hypothesized that the inability of SMNΔ7 protein to oligomerize, coupled with the resulting reduction in interactions with its own partners, might be responsible for the instability of this isoform [32].

The best-characterized function of the SMN complex is in the assembly of small nuclear ribonucleoproteins (snRNPs) which are involved in several aspects of RNA metabolism (see ref. 33 for a review). However, the link between SMN-snRNP biogenesis and SMA pathology remains unclear.

Several studies have evaluated the role of SMN protein in the two cell types which are more likely the specific targets of the disease: motor neurons and skeletal muscle. In motor neurons, SMN is localized in growth cones, along the axon and in the pre- and post-synaptic sides of the neuromuscular junctions (NMJ) [34-39]. SMN is subject to cytoskeletal-based, bidirectional transport between the soma and growth cones suggesting that SMN may have a cytoplasmic function related to neuronal transport of proteins and mRNA required at the distal tips of axons [38,40-42]. SMN protein deficiency could lead to the disruption of axonal transport and localization of several mRNAs, and/or of the assembly of specific snRNPs involved in transport and translation of a subset of axonal mRNAs: these defects would be responsible for the pathogenesis of SMA (see ref. 42 for a review). However, there is still debate why motor neurons are so sensitive to lower amounts of SMN in comparison with other neuronal cells.

Biomarkers in SMA

The landscape of SMA has been revolutionized over the last few years by the availability of effective treatments. The usual view of SMA type I-III needs to be updated for several reasons: firstly, the treatment of patients has revealed novel emerging phenotypes that do not fell in any of the classical forms [17]; secondly, the spreading of newborn screening programs is changing the diagnosis of SMA into that of subjects with a genetic defect who might or not develop early signs of the condition [43]. Additionally, the available outcome measures are not enough sensitive to detect tiny improvements that may still be clinically relevant, as in the case of the treatment of patients with a long story of disease. All these items have made mandatory the identification of prognostic, response and predictive biomarkers.

Even though some modifier genes have been reported (very recently reviewed by Kariyawasam et al., 2019) [44], so far the only genetic biomarker with clinical relevance is the determination of *SMN2* copy number, alongside with two alternative splicing-modulating variants (rs121909192 and rs1454173648, also known as NM_017411.3:c.859G > C and NM_017411.3:c.835-44A > G respectively [14,15]. Among *SMN2* gene products, full length transcript levels in peripheral blood correlate with the phenotype better than SMN protein levels [45,46]. For both, few longitudinal data are available [47]. Besides that, a number of efforts have been done to identify SMN-independent molecular markers, such as the SMA-MAP, neurofilament dosage, and few miRNAs [49,50]. Regarding the SMA-MAP, to our knowledge, beside the original cross-sectional study, no longitudinal data have been published so far. Among the other biomarkers, the most promising are thought to be the dosage in plasma of the phosphorylated neurofilament heavy chain (pNF-H) that allowed to differentiate SMA individuals from healthy controls. pNF-H levels were longitudinally dosed in patients treated with Nusinersen, showing a rapid decline and raising levels comparable to those of controls [50]. Albeit promising, the clinical impact of these data is limited by the insufficient number of healthy controls analysed; moreover we notice that the slope of pNF-H levels decay in patients is similar to that observed in controls with the highest levels of neurofilaments. Other biomark-

ers are also under study such as creatinine (Crn) in blood. A recent study showed that decreased Crn levels reflect progressive denervation and disease severity, suggesting that Crn is a candidate biomarker for SMA progression [51].

Beside molecular markers, some instrumental markers have also been evaluated: the majority of data available regards Compound Motor Action Potential (CMAP) and Motor Units Estimation Number (MUNE), the latter being the most reliable.

Newborn screening

The debate on the opportunity to perform newborn screening (NBS) for SMA has been issue of lively debate in the SMA community over the last years ahead of treatment availability [52,53]. At the time, the lack of effective therapies prevented the general consensus on this matter. The excellent results obtained with the pre-symptomatic treatment of SMA children in the NURTURE study [54], has changed the perspective and has made NBS a compelling need for both family associations and scientific community. Guidelines and operating workflows have been discussed and developed [43,55,56], pilot studies are ongoing or ready to start [43,57-61]. The results we are rapidly gaining are enlightening some crucial aspects and the pros and cons of the approach. Firstly, the advantage of the early treatment of expected severe patients is undoubtful, both in terms of health gain for children and of social, familial and economic burden [62]. Secondly, the scenario of SMA nosology is moving from the conventional classification based on the onset of clinical signs to the identification of oligo-asymptomatic subjects with an early molecular diagnosis. On the other side, some points remain open: 1) the different studies are providing quite variable incidence figures for SMA, ranging from inexplicably low levels (1/28137 in New York State) [57], to 1 in 11,545 in Australia [59], 1 in 7096 in Germany [58], 1 in 8398 in Belgium [60], 1 in 17,181 in Taiwan [63]. The preliminary data of our pilot study in two Italian Regions, indicate an incidence of 1 in 4861 (over the first 53477 neonates, updated at Dec 7th, unpublished data); 2) the stop-or-go for treatment starting remains *SMN2* copy number assessment, that still requires cross-validation and standardization across the different laboratories [15,16]; 3) the gold standard for treatment and follow-up of patients with 4 or more *SMN2* copies is still debated [15]; 4) the prevalence of asymptomatic subjects bearing *SMN1* homozygous deletion in the general population is unknown. The next few years will be of key relevance to discern these points and to get the widest spreading of NBS programs worldwide. The prevention programs of SMA are thus evolving from the treatment of symptomatic patients (tertiary prevention) to that of pre-symptomatic newborns (secondary prevention). Universal carrier screening programs (primary prevention) are also to be taken into account: these could constitute a complementary approach to allow couples to perform informed reproductive choices and eventually reduce the burden of the disease in general [43,64]. Once again, the availability of genomic biomarkers to predict the phenotypic severity is crucial.

The present therapeutic advances

After development of suitable animal models during the translational research decade (Fig. 1), the investigation of preclinical therapies has been successful to open the way to initiate clinical trials in patients [65-67]. A summary of the three approved SMN dependent therapies, including mechanisms of actions, administration and main trials involved is outlined in Table I. The earliest of the three programs was the nusinersen clinical program that started in 2011. *Nusinersen* (Spinraza®), an antisense oligonucleotide, can modulate *SMN2* splicing facilitating the inclusion of exon 7 to produce higher amounts of full-length SMN protein. Results of two pivotal clinical trials (ENDEAR and CHERISH) with loading doses and sustained intrathecal injection in type I SMA infants and late onset non-ambulant SMA patients led to wide label approval of this first tailored treatment in 2016 by FDA and in 2017 by EMA [4,68]. Expanded access programs as well as real world data confirmed safety and efficacy in more than 11.000 patients worldwide [69]. However, as mentioned above, the most impressive results have been obtained in pre-symptomatic patients with two and three *SMN2* copies detected because of previous family history of type I or type II disease (NURTURE clinical trial) [70]. These neonates started treatment up to 6 weeks of age and the majority of patients involved in this study were able to stand alone and walk independently.

A second clinical successful program started in 2013 with a single intravenous injection for a systemic-delivery of AAV9 with the coding part of *SMN1* as a gene transfer approach (AVXS-101) to replace *SMN1* in infants with SMA type I [5,72,73]. *Onasemnogene Abeparvovec*, (ZolgenSMA®) was approved in 2019 by FDA and in 2020 by the EMA becoming the most expensive drug in the market [73]. Ongoing studies and treatment access programs, targeting diverse population of patients, cover at present more than 400 infantile patients and also a number of pre-symptomatic cases. A third program refers to the oral compound RG7916 or Risdiplam, (Evrysdi®) which is a splicing modifier which also increase the inclusion of exon 7 and the amount of complete SMN protein. The results of their pivotal clinical trials in type I patients (FIREFISH) and type II-III patients (SUNFISH) led to the approval by FDA in 2020 [6].

The exclusive targeting of the central nervous system rather than the systemic approach is still an evolv-

Table I. Approved SMN dependent therapies for SMA (based and adapted from references 4,5,6,70,74,75,76 and www.clinicaltrials.gov).

	Nusinersen (Spinraza)	AVXS-101 (ZolgenSMA)	Risdiplam (Evrydi)
Type of therapy	18 mer antisense oligonucleotide specific to ISSN1	Self-complementary adeno associated virus 9 with human coding SMN1	Pyridazine derivative, binds to ESE2 on the 5'-ss site on exon 7
Mechanism of action	Increase amount of complete SMN protein from SMN2	Production of SMN protein from SMN1	Increase amount of complete SMN protein from SMN2
Administration route	Intrathecally (loading doses and sustained dose every 4 months)	Intravenously (one shot)	Oral (daily)
Pivotal Clinical trials	ENDEAR, CHERISH, NURTURE	AVXS 101, SPRINT, STRIVE	FIREFISH, SUNFISH, RAINBOWFISH
Number of patients treated (Clinical trials, Access programs and Real world data)	> 11,000	> 600	> 500
Approval	All SMA types (FDA 2016- EMA2017)	Age < 2 years: FDA (2019); type I up to 3 SMN2 copies: EMA, 2020	Age > 2 month: FDA 2020; EMA pending

ing issue [17]. Indeed, even though motor neurons appear the more sensitive cells to reduced levels of SMN, the protein is ubiquitously expressed and a number of extra neuromuscular findings has been reported, particularly in the most severe patients, including autonomic nervous system involvement, congenital heart defects, vascular defects, liver, pancreas, intestine and metabolic deficiencies [74].

Finally, although the three medications showed a therapeutic benefit when administered alone in most treated patients [4,5,75-77], they cannot be considered the cure of SMA, thus the investigation of combinatorial treatment is envisaged [17].

A number of other medications with a SMN-independent mode of action are under active investigation, and ergo might be transversally useful also in other neuromuscular disorders. These include for example neuroprotectors, neuromuscular junction stabilizers, muscle function activators or myostatin inhibitors. A summary can be found at www.clinicaltrials.gov and an updated pipeline in www.curesma.org. It is possible that in a near future, after their effectivity is demonstrated, these therapies may be incorporated into the protocols of SMA treatment. In this point, more preclinical studies and clinical investigation in patients should be performed to demonstrate their possible synergistic or additive effects.

What has changed during the last years in SMA and where are we going

We are witnessing an era of changes due to the live transforming therapies in SMA (Tab. II). There is an increasing interest in the disease that is reflected in the growing number of studies and publications (Fig. 1). More investigators and clinicians are discovering and becoming devoted to this fascinating disease and the possibility to apply advanced treatments [77]. This is also influencing other fields of rare genetic disorders in general and neuromuscular diseases in particular. SMA is an example of success that may encourage and give hope to patients, families, clinicians and researchers that an integrative collaboration could be successful to the main objective of stop the disease progression, rescue the phenotype or even an envisaged cure when therapy is applied as early as possible in some patients [78]. Research must go on: the awareness of the disease is now evaluating early manifestations for advancing the clinical detection, updates for wider genetic diagnosis programs (to give the patients the possibility to confirm disease and the option of treatment), and moving towards a better characterization of modifiers beyond the *SMN2* copies [15]. Other crucial issues are study and validation of biomarkers of disease evolution and response to treatments. Giving the rapid progression of severe SMA, a delay in treatment may impact the evolution with irreversible loss of function and reduced motor response. Therefore, the successful results in pre-symptomatic therapies support the inclusion of SMA in the newborn screening programs. A new SMA scenario of classification and progression trajectories is envisaged considering the increasing number of patients that will start the therapy during the neonatal period [43,64,77]. The impact of therapies in patients and families will modify the

Table II. What has changed in SMA over the last years and where are we going. More explanation in the text (Uppercase numbers show representative references of the text).

Increasing interest in the disease and record of scientific publications [77] (Fig. 1)
Defining of manifestations and awareness for early clinical detection [75-77]
Updating in genetic diagnosis, characterization of modifiers and validation of biomarkers [15,16,48-50]
Definition of new standards of care: from reactive to proactive [77,80]
Following up: the arrival of the multidisciplinary team [79,80]
Evolving of the SMA phenotypes and trajectories [17]
Changing perspectives in genetic counselling [64]
Managing expectations and sharing decision making for therapy [17,43,58,69,75,76]
Towards new SMA classifications [9,17,64,77]

burden of the disease and health policies.

We are also defining new standards of care moving from the traditional reactive approach to a more proactive and preventive approach that is also demonstrated by the expanding number of professionals and specialities that are involved in the follow-up of these patients [79,80]. These "new" patients under treatment present evolving phenotypes and trajectories that should be carefully defined in each case [17].

There is also a change in the genetic counselling of the disease [64]. A perception that SMA is no longer an untreatable disease is achieving consensus based on the promising results of therapies and the growing battery of available treatments. The perspective of families and reproductive decisions may evolve consequently. Although medications have demonstrated efficacy, patients with severe SMA are fragile and complications and death may happen to some patients even under therapy. For all these reasons, it is important to manage the expectations of the families with an adequate communication to establish a sharing decision making for therapy and psychological support. A further challenge that stands out is to accomplish the principle of wide access and equity for these expensive therapies to those in need. This requires the combined efforts of physicians, biomedical scientists, health-care economists, public-health experts, companies, funders and governments [78]. We all have to find a way to ensure that the costs in this Copernican revolution are not assumed by families that have already suffered SMA for too long.

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