Title: GENETIC THERAPIES FOR INHERITED NEUROMUSCULAR DISORDERS

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MS has participated in scientific advisory board activities for Roche.

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Summary:

Inherited neuromuscular disorders encompass a broad group of genetic conditions caused by a variety of genes whose discovery has expanded greatly in the past three decades. The discovery of such genes has allowed for more precise diagnosis and the development of specific therapeutic approaches based upon the genetic basis and pathways that contribute to the pathophysiology of the disease. Research is starting to show concrete results, and the outcome of recent translational research work treatments have begun to deliver the first approved therapies, using genetic therapy. Two diseases for which the development of personalized genetic therapy is already advanced are Duchenne muscular dystrophy (DMD) and spinal muscular atrophy (SMA). In this article we aim to review recent trials and therapies emerging in DMD, SMA and other less common childhood neuromuscular disorders.

Introduction

Duchenne muscular dystrophy (DMD) and spinal muscular atrophy (SMA), are the most frequent debilitating neuromuscular disorders affecting children. The understanding of the genetic basis and the knowledge gathered on the disease specific complications has led in the last 2 decades to dramatic improvement of the anticipatory care and survival for affected children [1-4] and, more recently, to the advent of experimental therapeutic approaches.

Two broad strategies are being followed: the first one, initiated a decade ago, relates to the correction of the mutant RNA processing. This strategy utilises either antisense oligonucleotides (AONs) or small molecules that can modify mutant RNA splicing. [5-16] A conceptually separate but related approach exploits drugs that alter the translation of mutant mRNA by inducing a partial read-through of nonsense mutations. [17] More recently the advances in adeno-associated virus (AAV) development have taken viral gene therapy forward in SMA, in DMD and other rare neuromuscular diseases.

These novel therapies are rapidly changing the way these neuromuscular diseases are being managed. While it will be many years before we have a fuller picture on the impact of these

interventions on the long term disease course, there is optimism that several of these therapies are having clinically meaningful positive effects upon the natural history of these diseases. It is timely for professionals to have a general review on the most significant therapeutic advances for these disorders.

1. **Duchenne muscular dystrophy: from molecular genetics to genetic therapies**.

DMD is due to mutations in the *DMD* gene, located on the short arm of chromosome X, encoding dystrophin, a protein located under the plasma membrane (sarcolemma) of muscle fibres where it mainly serves a function of preserving structural integrity of muscle fibres.

The most common mutations affecting DMD boys are out-of-frame deletions removing one or more exons, found in approximately 65% of DMD boys. Out-of-frame duplications and single nucleotide (non-sense) mutations are found in a proportion of patients between 10 and 15%, respectively, while the remaining mutations are splice site mutations or other small insertions/deletions mutations. A milder allelic variant named Becker muscular dystrophy (BMD), is also due to *DMD* mutations, often deletions that however are in-frame, i.e. allow the production of an internally partially deleted protein which determines a milder disease course.

1.1 DMD: Antisense oligonucleotides (AONs)

In the last decade experimental efforts have focused on the technology of splice switching AON targeting DMD boys with out-of-frame deletions so that a BMD-like in frame deletion is achieved [Figures 1 and 2]. AON are modified stretches of nucleic acids complementary to the mRNA; two different chemical modifications have been used, the 2'-methoxyethyl (2'OMe) modification, and the morpholino (PMO) chemistries. The AON strategy is facilitated by the fact that there are common deletions in the DMD gene that could be "re-framed" by skipping a single exon. As an example, skipping exon 51 restores the reading frame of ~ 15% of all the boys with deletions. It has been suggested that by having 10 AONs to skip 10 different exons it would be possible to deal with more than 70% of all DMD boys with deletions. [18] Both AON chemistries have been used in DMD clinical trials, from proof of concept single dose, local administration (intramuscular) studies, to larger repeated

doses studies in which both safety, clinical efficacy and efficiency in restoring dystrophin protein production were measured. One drawback of the AON approach is the requirement for regular administrations, weekly using a systemic route, as they only modify the way in which pre-mRNA splicing occurs.

Both the 2'OMe and PMO chemistries demonstrated in early clinical trials the ability to restore dystrophin production following a single intramuscular injection in DMD boys. [19, 20] Both chemistries progressed their developmental pathways with increasing larger studies, aimed at demonstrating the safety, feasibility and efficacy of the chronic systemic administration.

The largest studies have been performed using the 2'OMe chemistry originally developed by Prosensa, subsequently in partnership with GSK and with Biomarin, using a drug capable of skipping exon 51 (drisapersen). The outcome of these studies was variable, with randomised placebo controlled medium size studies demonstrating both clinical efficacy in the first year of the administration, and the production of low levels of dystrophin in 59-72% of patients [21], followed by a larger phase III study that failed to meet the clinical endpoint (NCT02636686). [22] There were also difficulties to demonstrate dystrophin restoration on muscle biopsies in this latter study, mainly related to challenges in collecting and properly storing muscle biopsies in the large multicentre study that highlighted the need to develop robust standard operative procedures for collecting and shipping biopsies.

While the outcome of the phase III study was a significant disappointment, important lessons were learned related to the optimal way to handle the muscle biopsies, and on optimal inclusion criteria for the clinical trials. Indeed, the different outcomes of the studies appears to be at least partly related to important differences between the inclusion criteria of the successful phase II studies, and the unsuccessful phase III study, with more children recruited into the latter trial being very close to the time of loss of ambulation and presenting an overall more advanced stage of disease. As DMD is characterised by progressive loss of muscle mass, and as AON can only induce the production of limited dystrophin in the residual muscle fibres, the recruitment of children at very advanced stage of disease in whom muscle has been replaced by fibro-adipose tissue, blunts the possibility to detect a clinical response. Eventually the limited clinical efficacy but also concerns related to the adverse event profile of the chronic s.c. administration of the 2'OMe AONs (renal and

coagulation adverse events, and severe local skin reactions), led FDA to reject a filing application of Biomarin in 2016 on drisapersen. [23] This was followed shortly after by the cessation of the entire 2'OMe AON DMD program including AONs to skip exon 45, 44 and 53.

In a parallel effort, a PMO AON designed to skip exon 51 was developed in collaborative efforts between the UK MDEX consortium led by one of the authors (FM) and the company Sarepta Therapeutics (originally named AVI Biopharma). Similarly to what demonstrated for the 2'OME drisapersen, the PMO also induced dystrophin restoration after a single intramuscular injection and the production of dystrophin in a follow-on i.v. phase IIa study lasting 3 months (NCT00159250, NCT00844597). [24, 25] Subsequent longer duration studies performed in the US by Dr Jerry Mendell demonstrated the production of dystrophin in most of the patients receiving the morpholino AON (named Eteplirsen)[26], and a divergence of the clinical course between the small number of children treated (12 children) and a concomitant natural history study population (NCT01396239) [27], in which patients had identical inclusion criteria used in the clinical trial. These encouraging data led the FDA to conditionally approve Eteplirsen in the US, while the evaluation in the EU is underway. This approval was warmly welcomed by the patient community but also raised internal criticism within the FDA due to the limited size of the treated patient population, the low amount of dystrophin produced in the trial [27] and the lack of a placebo controlled arm. Nevertheless the agency concluded that there was plausibility of efficacy, and given the benign safety profile of Eteplirsen (commercial name EXONDYS 51), recommended conditional approval, pending the outcome of larger confirmatory studies. A larger randomised placebo controlled study using morpholino AONs to target either exon 45 or exon 53 is currently underway (NCT02500381). FM and other investigators from Europe (including EM and investigators from Paris and Newcastle) have recently reported on the successful restoration of dystrophin, the primary biochemical outcome, in a study funded by the European Community and Sarepta, following the administration of a novel PMO AON targeting exon 53 (NCT02310906) [28]. In parallel efforts, Sarepta has initiated a study to assess the efficacy of Eteplirsen in young children (aged 6 months- 4 years, when there is much better preserved skeletal muscle mass). (NCT03218995)

Following the conditional approval of Eteplirsen, efforts from a number of different players, including Sarepta, and Wave Therapeutics, are now focused on the identification of next generation AONs capable of inducing the production of higher levels of dystrophin, ideally also in the heart as neither the 2'OMe nor the PMO AONs induce meaningful protein expression in this organ. At the time of writing preliminary safety data were presented by Sarepta regarding a next generation peptide conjugated morpholino (PPMO) AON [29]; and Wave Therapeutics has announced the initiation of a clinical developmental programme of AON to skip exon 51 using a novel stereochemical modification of AON with a 2'OMe backbone. Both chemistries appear to have increased potency compared to the first generation AON used so far. This next generation AON will start clinical trials in 2018.

1.2 Approaches targeting nonsense mutations

A different approach of mutation-specific therapies was used by PTC Therapeutics with a drug (ataluren) targeting DMD nonsense mutations. These are found in approximately 10–15% of patients with DMD and are responsible for creating a premature stop codon into the dystrophin mRNA with subsequent inability to produce a functional protein. Ataluren should allow the readthrough of the nonsense mutations and the partial restoration of full-length functional dystrophin production. A phase IIa, open-label, dose-ranging (NCT00264888) study in DMD patients with nonsense mutation, demonstrated a modest increase in dystrophin expression after 28 days of treatment. Both a phase IIb, randomised, double-blind, placebo-controlled trial (NCT00592553) and a subsequent confirmatory phase III trial (ACT DMD) failed to achieve their primary endpoint after 48 weeks in patients receiving ataluren (40 mg/kg/day) versus placebo but showed a slowing of disease progression measured by 6-minute walk distance changes. This was most obvious in the subgroup of patients (prespecified in the ACT DMD study) who were in the intermediate stages of ambulatory decline, i.e. those with baseline 6MWD between 300 and 400 m. These results were supported by the secondary outcome measures, including timed function tests and functional scales. [30] These studies also helped the DMD community to better appreciate inclusion and stratification criteria in designing clinical trials for DMD. As ataluren was generally well tolerated, the overall efficacy and safety profile led to a conditional approval by EMA, while the drug is being considered by FDA.

1.3 DMD: AAV gene therapy

The sheer size of the DMD cDNA (14Kb) makes it very challenging to package its full coding sequence into a viral vector such as the adeno-associated viral (AAV) vectors, many serotypes of which target skeletal and cardiac muscle with high efficiency. The capacity for an AAV transgene is ~4.5 kb, less than half of the *DMD* cDNA. Thus, shortened transgenes, coding for partially functional minidystrophins containing essential domains of the dystrophin protein have been generated. The principle of these transgenes is derived from rare BMD deletions that remove large portion (~ 50%) of the protein and yet result in a partially functional dystrophin and a relatively mild phenotype. Viral delivery to muscle is associated with an immune response against the viral vector which precludes the possibility to perform repeated administrations of the vector. It is therefore essential to develop efficient strategies with a realistic perspective of producing a therapeutic benefit for the affected individuals who would otherwise be vaccinated against the AAV for life without realistic possibilities to receive subsequent AAV administrations. One of the challenges of the field over the last few years has therefore been that of optimising the transgene for the highly internally deleted dystrophin protein; to identify an optimal promoter that directs dystrophin protein expression in muscle and heart; and to search for optimal AAV vectors which can efficiently target skeletal and cardiac muscles following systemic administration. Several studies have shown the potential of AAV8 and AAV9 to induce production of an internally deleted dystrophin protein in most of the muscles of the dystrophic *mdx* mice and of the dystrophic dog, with improvement of the histological parameters and of the clinical symptoms after systemic delivery. [31-37] Based on these encouraging results, several academic groups and industrial partners (Jerry Mendell at Nationwide Children Hospital in collaboration with Sarepta; Pfizer; Solid Bioscience; Genethon) are at the advanced planning stage for phase I clinical trials in which escalating doses of either AAV8 or 9 will be administered systemically to DMD boys. The primary outcome will be safety, but dystrophin protein production and exploratory clinical efficacy will also be essential outcomes of these studies. The first study to recruit a DMD boy for the AAV delivery was at Nationwide Children hospital on the 4th of January 2018.

1.4 CRISPR technology

Amongst the novel strategies to correct dystrophin gene mutations a special mention is for the RNA-guided, nuclease-mediated genome editing systems based on type 2 clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated proteins (Cas). Several recent studies have applied this technology for editing the mutation in the *mdx* dystrophic mice in vivo by use of adeno-associated viral vectors carrying CRISPR–Cas9 to edit specific regions of the dystrophin gene. These preclinical studies showed a widespread expression of dystrophin after both local and systemic delivery, suggesting that this approach has potential for the development of future therapies for DMD.[38] . Nevertheless further studies aimed at containing the long term enzymatic activity and potential for off-target effects are required before this technology could be considered for safe following *in-vivo* therapy for DMD.

2. Spinal muscular atrophy: from molecular genetics to genetic therapies.

Spinal muscular atrophy (SMA) is a motor-neuron disease characterized by generalised muscle atrophy and weakness. SMA is caused by the dysfunction and eventually death of a-motor neurons in the spinal cord ventral horn secondary to deletions (95%) or other rare mutations of the Survival Motor Neuron 1 gene (*SMN1*) on chromosome 5q13 which encodes the survival of motor neuron (SMN) protein. [39-41] The estimated incidence is 1 in 10,000 live births, with a carrier frequency of 1/40-1/60 in Caucasian but lower in the African population. The classification of SMA is based on the age of onset and maximum motor abilities achieved, with a broad range of phenotypes from very weak infants unable to sit (type 1), non-ambulant children able to sit (type 2), to ambulant children (type 3) [42, 43]

Due to a large inverted duplication region located at chromosome 5q, 2 variants of the *SMN* gene exist on each allele in humans: a telomeric (*SMN1*) and a centromeric variant (*SMN2*). The coding sequence of *SMN2* differs from that of *SMN1* by a crucial exonic nucleotide variation (840C > T), which does not alter the aminoacid sequence but results in alternative splicing of exon 7. Due to this alternative splicing, *SMN2* genes produce a reduced amount (~10%) of full length transcripts (SMN-fl) and protein, while the majority of the mRNA lacks exon 7 which gives rise to a truncated and unstable protein. [44] The small proportion of full length transcript derived from *SMN2* which includes exon 7 results in the production of low

levels of full length functional SMN that is sufficient to prevent lethality yet not enough to compensate for the loss of *SMN1*, resulting in motor neuron disease. All patients indeed retain at least one copy of *SMN2*, generally 2-4. Less than 10% of SMA Type 1 patients retain only one copy of the *SMN2* gene and almost invariably have a congenital onset and very severe, early lethal course. The complete absence of MN protein is considered to be lethal in the human. In general, the *SMN2* copy number—and thus the total amount of full-length SMN—is inversely correlated with the severity of the disease. [45, 46]

Antisense Oligonucleotides for the Treatment of Spinal Muscular Atrophy:

A major focus of therapeutic developments has been on increasing the full-length SMN protein. In particular, AONs designed to enhance exon 7 inclusion of *SMN2*, leading to increased production of full-length SMN protein. (Figure3) These AONs are not capable of crossing the blood-brain barrier, requiring repeated intrathecal administrations. [47, 48]

Early open label clinical trials of the AON Spinraza (also known as Isis 396443, SMN_{Rx} and nusinersen), demonstrated a good safety profile and encouraging efficacy data both in type I and type 2 SMA individuals. A phase II, open-label, dose escalation study of nusinersen in 20 infants with infantile-onset SMA showed progressive improvements in motor function and prolonged survival when compared to natural-history data. [49]

A subsequent large randomised double blind controlled clinical trial (ENDEAR) in which infants under 7 months of age with type I SMA received either Spinraza or sham procedure, had two primary efficacy end points. The first was a motor-milestone response, assessed using the Hammersmith Infant Neurological Examination (HINE-2). The second end point was event-free survival, which was defined as the time to death or the use of permanent assisted ventilation (tracheostomy or ventilatory support for ≥16 hours per day for >21 continuous days in the absence of an acute reversible event). In the interim analysis, a significantly higher percentage of infants in the nusinersen group than in the control group achieved new milestones.

Following the positive results of the interim analysis, the study was prematurely interrupted, allowing to all participants to be rolled over into an open label study (SHINE, NCT02594124), designed to assess the effects of longer treatment duration.

The positive interim results were confirmed at the final analysis, with 51% of the infants in the nusinersen group and none in the sham group achieving new milestones. [50] The risk of death or the use of permanent assisted ventilation was 47% lower in the nusinersen group than in the sham group.

Approximately half the infants in the nusinersen group who received permanent assisted ventilation did so within 13 weeks after they received the first dose; this result indicates that a minimum treatment time is required to see the full benefits of nusinersen. Infants with disease duration at screening longer than the median duration of 13.1 weeks were more likely to need permanent assisted ventilation than those with shorter disease duration suggesting that early initiation of treatment may maximize its efficacy.

Similar results have been obtained in a parallel large randomised double blind controlled clinical trial (CHERISH, NCT02292537) including children with late onset SMA (mainly type 2 SMA). The positive results from this study prompted a fast FDA and EMA approval. While the drug is being evaluated for licensing, an Expanded Access Program (EAP) has been initiated by Biogen, to allow patients with type I SMA to receive the drug until it becomes available through commercial means.

Finally, an ongoing Phase II, open-label, single-arm study, Nurture (NCT02386553) is evaluating the effect of nusinersen in 20 pre-symptomatic infants (most likely to develop SMA Type I or II). At the interim analysis conducted in October 2017 out of the nine infants who completed the day 365 assessment, none died or required respiratory intervention; all achieved sitting, 5/9 crawling, 5/9 walking with assistance, 3/9 standing alone and 2/9 walking alone. These results confirm that early initiation of nusinersen provides larger clinical benefits than in the more advanced symptomatic stage of SMA.

Other *SMN2* splicing modifiers for the Treatment of Spinal Muscular Atrophy:

Orally available small molecules have proved to be able to modify exon 7 splicing and promote inclusion of exon 7 into *SMN2* mRNA, by interacting with proteins that are themselves involved in the regulation of SMN2 exon 7 splicing. Administration of these compounds to mice models of severe SMA led to an increase in SMN protein levels, improvement of motor function, and substantial prolongation of life span. [51, 52]

Ongoing studies using these small molecules are currently in phase I/II clinical trials for SMA. (NCT02908685; NCT02913482; NCT03032172; NCT02268552)

Viral Gene therapy for SMA

As a monogenic disease affecting motorneurons, which are not mitotically active cells, SMA is a good target for vector-based gene replacement therapy aimed at delivering a functional copy of a human *SMN1* gene to the patient. AAV 9 expressing SMN showed efficient transduction of motor neurons as well as nearly complete correction of the SMA phenotype in mice; due to its ability to cross the blood–brain barrier after systemic (intravenous) delivery in mouse models, AAV type 9 is an excellent contender to deliver the SMN1 gene . [53-55]

The first gene therapy phase I clinical trial to assess the safety of intravenous delivery of scAAV9-SMN in type 1 SMA infants was conducted at a single site in US by Jerry Mendell. (NCT02122952) This open-label, dose-escalation clinical trial of AVXS-101 has completed enrolment and is active. A total of 15 infants were enrolled in this study; participants were allocated in 2 cohorts receiving 6.7e13 vg/kg of AVXS-101 (n=3) and 2.0e14 vg/kg of AVXS-101 (n=12) delivered as a single intravenous administration.

The primary outcome in the study was safety and tolerability. The secondary outcome measure is an efficacy measure as defined by the time from birth to an “event,” defined as death or at least 16 hours per day of required ventilation support for 14 consecutive days in the absence of acute reversible illness.

Encouraging preliminary data were recently published [56]; as of the data cut-off on August 2017, all 15 patients were alive at 20 months of age and did not require permanent mechanical ventilation. In contrast, only 8% of the patients in a historical cohort did not require permanent mechanical ventilation at 20 months of age. [57]

Of the 12 patients who received the high dose, 11 sat unassisted, 9 rolled over and 2 walked independently. Elevated serum aminotransferase levels occurred in all patients but were attenuated by prednisolone and there were no other abnormalities on liver –function testing.

A larger multicentre Phase III open-label single-dose, by intravenous infusion, gene replacement therapy clinical trial for patients with SMA type 1 is currently active in the US and soon in Europe. A phase I study with intrathecal delivery of AVXS-101 is also now actively recruiting SMA type 2 patients in the US.

Other genetic therapies for neuromuscular disorders

Follistatin Gene Therapy for Becker muscular dystrophy.

In parallel efforts focused on BMD, an AAV vector has been designed to deliver follistatin (FS), a potent myostatin antagonist, a protein that inhibits muscle growth and differentiation. Preclinical studies in dystrophic animals resulted in increased muscle mass and strength [58, 59]. In this proof-of-principle clinical trial (NCT01519349), adult patients with BMD have received an intramuscular injection of the follistatin AAV gene directly into thigh muscle on one (first cohort) or both legs (2nd and 3rd cohort). A muscle biopsy was performed 180 days after the injection to evaluate the muscle fibers size. The primary endpoint was safety while the secondary endpoints were related to muscle function and strength, quadriceps muscles imaging (MRI), muscle biopsies and thigh circumference measurement. Preliminary results showed encouraging safety data together with a degree of improved walking distance at the high dose. Histological changes showed reduced fibrosis and central nucleation with more normal fiber size distribution especially at high dose. [60, 61]

AAV gene therapy programme for Limb girdle muscular dystrophy due to mutations in the sarcoglycans genes.

The limb-girdle muscular dystrophies (LGMDs) are one of the class of genetic disorders affecting the musculoskeletal system. LGMD type 2E represents one of the most severe LGMDs, with an incidence of 1 in 200,000 to 1 in 350,000. [62] In this disease, mutations in the β-sarcoglycan (*SGCB*) gene lead to loss of functional protein with concurrent loss of other structural components of the sarcolemma-stabilizing dystrophin-associated protein complex (DAPC). [63, 64] The loss of one of the sarcoglycans leads to muscle fiber loss, similar to the pathophysiology of DMD. Importantly, the LGMD genes are relatively small and can be fully inserted as the transgene into the AAV vector, unlike DMD.

LGMD2E patients exhibit typical clinical Duchenne like features, with increasing difficulty in mobility in early childhood and subsequent loss of ambulation. [65, 66] Cardiac involvement is also common. [67, 68]

The LGMD2E disease phenotype is recapitulated in *sgcb*$^{-/-}$ mice, providing an ideal model to study therapeutic developments. In this model, studies have demonstrated the therapeutic efficacy using the tMCK promoter, with successful targeting of multiple muscles by vascular delivery to restore β-sarcoglycan expression along with the significant reduction of fibrosis,;

these results provided a foundation for translating AAV-mediated hSGCB transfer to LGMD2E patients. [69] This promoter allows for enhanced transgene expression in cardiac muscle, allowed the nearly complete transduction and restoration of hSGCB expression in limb skeletal muscles, diaphragm muscle and cardiac muscle after intravenous injection of scAAVrh.74.MHCK7.hSGCB in mice. [70]
The next step will now be to take this approach to clinic.

Giant Axonal neuropathy (GAN).

GAN is an autosomal recessive neurodegenerative disorder, characterized by abnormally large and dysfunctional axons with disordered microtubules and intermediate filaments. The disease is due to loss-of-function mutations in the *GAN* gene encoding the protein gigaxonin which plays a major role in the maintenance of orderly and functional intermediate filament (IF) architecture, which is critical for axonal function. In the peripheral nervous system the disease progressively affects predominantly sensory and motor nerves. Onset of symptoms, usually at 3-4 years of age, generally manifests with an ataxic gait. By the end of the 2nd decade of life, patients typically are wheelchair dependent with limited use of the arms and little to no use of their legs. During the 2nd decade a tracheostomy or other means of ventilation, as well as a feeding tube, are often necessary. Death normally occurs in the 2nd or 3rd decade of life. The diagnosis of GAN is suggested by clinical findings and the results of nerve conduction velocity (NCV) studies and brain MRI. [71] Intrathecal delivery of a gene transfer vector carrying a normal copy of the *GAN* gene to the spinal cord and brain offers a potentially effective treatment for GAN. A phase I clinical trial using Intrathecal administration of scAAV9/JeT-*GAN* is currently ongoing in the US. (NCT02362438)

AAV gene therapy for X-linked Myotubular myopathy.

X-linked myotubular myopathy (XLMTM) is a neuromuscular disorder caused by mutations in the myotubularin (*MTM1*) gene which encodes a protein called myotubularin. This protein plays an important role in the development, maintenance and function of skeletal muscle cells. XLMTM is a rare condition (~1:50,000 male births) characterized by profound muscle weakness, respiratory failure and early death. [72, 73]

Multiple studies in animal models of XLMTM have demonstrated that a single administration of an AAV8 vector containing a functional copy of the *MTM1* gene improves disease symptoms and survival rates, with no significant related adverse events or safety findings. [74]

A Phase I/II, multinational, open-label, ascending-dose, clinical study to evaluate the safety and preliminary efficacy of the *MTM1* AAV gene therapy in subjects with XLMTM aged less than 5 years old, has recently started in US (ASPIRO clinical study- NCT03199469). Subjects will receive a single dose of AT132 and will be followed for safety and efficacy for 5 years.
The study aims to evaluate the safety and preliminary efficacy of the AAV gene therapy in approximately 12 XLMTM patients. The study is expected to include nine treated subjects and three delayed-treatment concurrent control subjects. Primary endpoints include safety (adverse events and certain laboratory measures) and efficacy (assessments of neuromuscular and respiratory function). Secondary endpoints will include the burden of disease and health related quality-of-life, and muscle tissue histology and biomarkers.

Concluding remarks

In the last decade there have been dramatic developments on the genetic therapies for neuromuscular disorders and in particular for DMD and SMA. The deep knowledge of the genetic basis of these 2 conditions and advances in small molecules, AONs and very recently also of the use of AAV to deliver transgenes to muscle and the central nervous system have produced remarkable results especially in SMA, which certainly exceeded our expectations as investigators in these trials. Nevertheless these successful experiences also raise a number of issues that require urgent considerations. In the case of using AONs for SMA, for example, it is still unknown whether the intrathecal delivery and the lack of splicing correction in the peripheral tissues are relevant for the size and the longevity of the response. The comparative analysis between drugs that offer CNS and peripheral splicing correction vs nusinersen will provide answers to these questions in the years to come.
As far as DMD is concerned, the efficacy of the first generation AONs in targeting skeletal muscle can be improved, and with that the cardiac targeting, as cardiomyopathy remains a major unmet need of DMD patients. Additional DMD specific challenges are the abundance of the target tissue and the marked advanced pathology of even early symptomatic children.

This will also be a challenge for the upcoming AAV gene therapy trials that could only be overcome by considerably bringing down the age at recruitment in future clinical trials.

Nevertheless these early successes clearly suggest that these highly specialised and targeted therapies have the potential to produce substantial changes on the clinical course of these devastating disorders. For at least some of these therapies early indication suggests that if they could be administered early enough they can potentially lead to almost complete resolution of clinical features or prevention of most of the pathology (as in the SMA nurture AON study; and as the children receiving AAV in the very early symptomatic phase of the disease). These findings provide further impetus to consider newborn screening programmes for these devastating diseases. At the same time, the availability of these novel therapies has also been accompanied by very high price tags. The financial implication of these novel therapies at the time of financial constraint is currently an issue that is precluding the adoption of these novel therapies in several geographic regions. This paradox of extremely efficacious novel therapies that only with significant difficulties make their way to the very patients for whom they have been originally designed, is a pattern that is only going to continue until a broader discussion on the cost of novel therapies on one hand, but also the particular circumstances of therapy development for rare diseases is discussed more widely in the society. [75]

Key points

- Advances in the understanding the molecular genetic mechanisms in a number of neuromuscular conditions have provided substantial progress to therapeutic approaches
- Two ASO-mediated therapies have received approval from the US Food and Drug Administration for the treatment of Duchenne muscular dystrophy and spinal muscular atrophy.
- Further advancement of ASOs in the clinic will require optimization of ASO delivery, and tissues target maintaining optimal safety profile
- AAV gene therapy holds promise for the treatment of monogenic neuromuscular diseases and many such therapies have already made substantial strides toward clinical translation.
- Ongoing trials in the field are summarised in Table 1.

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Search strategy and selection criteria

Published and unpublished data, in English language only, for this review were identified by searches of PUBMED, MEDLINE, www.clinicaltrials.gov and references from relevant articles; FDA, EMA and drug companies' relevant Brief releases were also searched. Most common search terms used were: Duchenne and Becker muscular dystrophy; spinal muscular atrophy; antisense oligonucleotides; nonsense mutations; CRISPR; *SMN2* splicing modifiers**;** AAV gene therapy; Giant Axonal neuropathy; follistatin gene. Date of research ranges from 1990-2017.

Figure1. Duchenne Muscular Dystrophy: effect of out of frame mutations, (i.e. deletion of Exon 7) preventing the creation of a protein product**. Becker Muscular Dystrophy: effect of in-frame mutations (i.e. deletion of Exon 4; or deletion of multiple Exons i.e. 7 to 12)** leading to altered, but detectable dystrophin expression in muscle fibers.

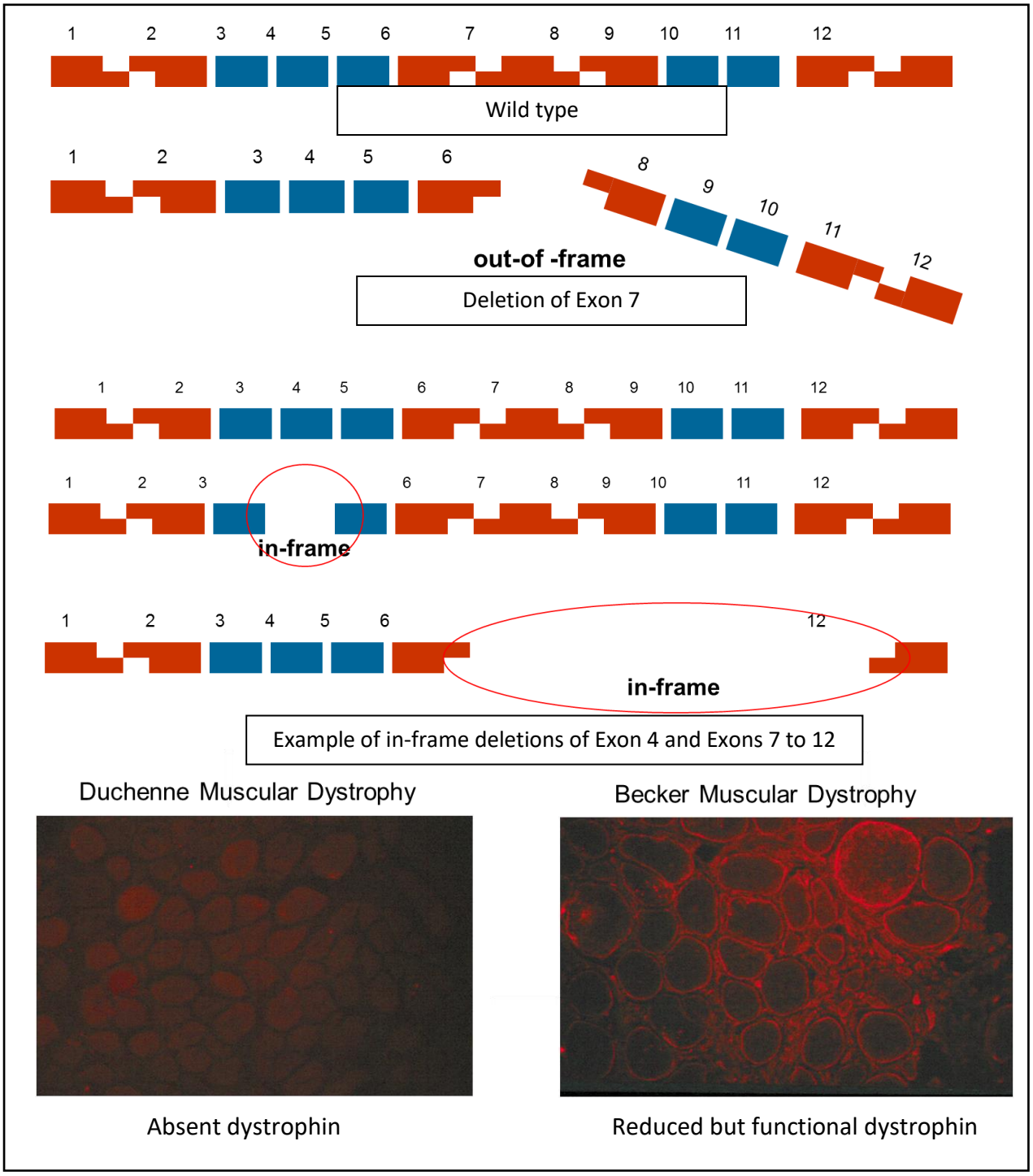

Figure 2. Exon skipping mechanism using AON in DMD to reframe transcripts

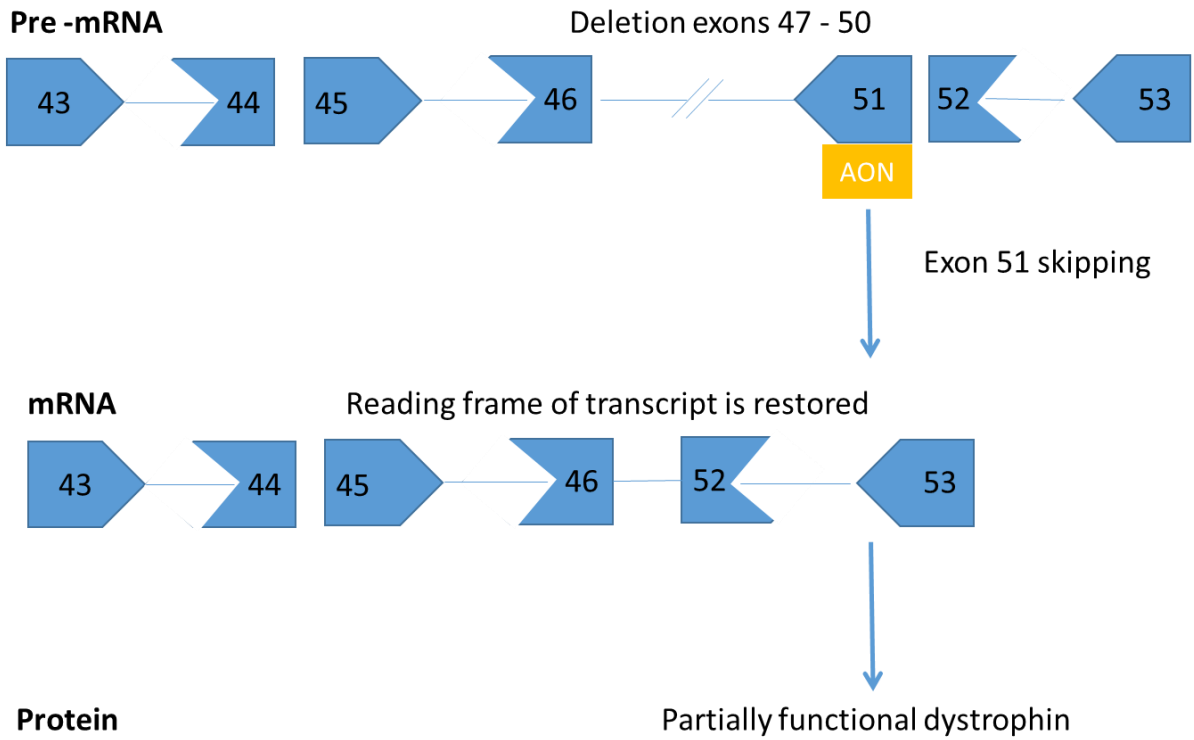

Figure 3. The mechanism of action of AON targeting Exon 7 inclusion in *SMN2* for the treatment of SMA