Briefing Document

Antisense oligonucleotide-mediated exon skipping therapy development for Duchenne muscular dystrophy (DMD)

A COST/SCOPE-DMD meeting hosted by the European Medicines Agency

April 2015

SCOPE-DMD
EXON SKIPPING IN DUCHENNE MUSCULAR DYSTROPHY

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Contents

1. Purpose of the meeting

This workshop is a follow-up to a seminal workshop hosted by the European Medicine Agency (EMA) in 2009 to discuss antisense oligonucleotide (AON) therapy development for Duchenne muscular dystrophy (DMD) [1]. In that meeting the focus was on the issue of the mutation specific nature of exon skipping for DMD and the implications of having to develop more than 30 different exon skipping compounds[2]. As a result of that discussion among stakeholders, various challenges were identified, such as the lack of natural history data for DMD, the lack of validated functional outcome measures and surrogate endpoints, lack of standardized protocols to detect dystrophin restoration and the fact that data on pharmacokinetics, pharmacodynamics and safety were limited to exon 51 skipping compounds, while preparations for trials for exon 44, 45 and 53 skipping were ongoing.

During the past 5 years, work has been ongoing to address these issues and substantial progress has been made. First, with exon skipping trials now also ongoing for exon 44, exon 45 and exon 53, pharmacokinetic, pharmacodynamics and safety data have been collected for multiple AONs. Secondly, dystrophin quantification methods have been optimized and compared among international groups[3]. Thirdly, natural history data has been collected for 5-16 year old DMD patients with clear correlations established between commonly used outcome measures and disease milestones. Collection of data in the very young and older patients is also ongoing [4, 5]. Finally the development of outcome measures in non-ambulant and very young DMD patients is filling important gaps [6, 7] and work on exploratory outcome measures like serum biomarkers and magnetic resonance imaging (MRI) shows promising results, especially because they monitor disease progression non-invasively [8, 9].

In light of this, we organized a follow-up workshop with the EMA in an open forum setting. The workshop, which will serve to exchange knowledge and perspectives among the different parties involved, has two main objectives:

1) to present the EMA with data that have been collected by academia, industry and patient advocacy groups over the past few years in DMD related pre-clinical and clinical research studies and to discuss the relevance and meaningfulness of the data for the evaluation of antisense-mediated exon skipping strategies in the treatment of DMD.
2) to accelerate the elaboration of regulatory guidelines on medicinal products, in particular antisense oligonucleotides for exon skipping, for the treatment of DMD. The increased interest in the development and application of disease modifying compounds in DMD highlights the urgent need for guidelines that address study strategies and designs compatible with small patient cohorts and advise on appropriate efficacy endpoints and the definition of reliable surrogate outcome measures.

A constructive dialogue on these objectives will help the neuromuscular community, especially patients and families, to plan for the future.

Two collaborative grants around the topic of exon skipping are jointly funding the workshop; COST Action BM1207: "Networking towards clinical application of antisense-mediated exon skipping"[10] chaired by Annemieke Aartsma-Rus (also current chair of the TREAT-NMD Alliance) and the FP7 project SCOPE-DMD: A Consortium for Products across Europe in Duchenne Muscular Dystrophy, EU FP7, 601573 coordinated by Volker Straub. The EMA is gratefully acknowledged for hosting the workshop free of charge, thus facilitating this stakeholder meeting.

2. Setting the scene

2.1 Patient perspective

2.1.1 Duchenne muscular dystrophy

Duchenne muscular dystrophy (DMD) is the most common fatal genetic disorder diagnosed in childhood, affecting approximately 1 in every 5,000 live male births worldwide [11, 12]. As the *DMD* gene is found on the X-chromosome, it primarily affects boys [13]. Although it is a genetic, inherited disease a lot of affected children are born in families without a history of DMD. In these cases the disease is caused by a new (*de novo*) mutation in the mother (or one of her maternal ancestors) that was passed on to the child.

DMD is characterized by progressive loss of muscle tissue and strength that causes loss of essential motor functions such as walking, use of the arms and the ability to sit independently or roll over in bed. The progressive muscle weakness leads to serious medical problems, particularly relating to heart and breathing functions [13].

Dystrophin is also present in the brain and its deficiency probably underlies the speech delay and learning difficulties frequently observed in children with DMD. A small subset of individuals affected by DMD has a significantly lower IQ. Psychosocial problems such as attention deficit hyperactivity disorder (ADHD), obsessive compulsory disorder (OCD) and autism are more frequent in the DMD population compared to healthy individuals[14, 15]. In contrast to the muscle pathology, cognitive problems are not progressive.

2.1.2 Diagnosis

Muscle weakness generally becomes apparent during the first few years of life. Parents of affected boys notice a delay in motor milestones (see section 2.2), but also other symptoms such as frequent falls and difficulty with climbing stairs. Delayed speech is another early manifestation that is observed in the majority of DMD children. Parents generally become concerned about the wellbeing of affected boys years before the definite diagnosis is made at the average age of 4.5 yrs. A diagnostic odyssey of 2-3 years is not unusual [16]. Some children are diagnosed earlier as result of a bloodtest showing a high creatine kinase (CK) level. However, newborn screening for DMD is not common practice and has only been performed in pilot studies in Europe, Canada, Australia and the US[11, 12, 17].

The diagnosis is without exception a shock to parents and often perceived as a death sentence as there is no cure for this fatal disease, leaving no hope for a positive outcome. Most parents have never heard about the disease before as they have no close relatives with the same disease or it is a first case in their family due to the high frequency of *de novo* mutations.

2.1.3 Course of the disease

When the children are young (~3-12 years) and still ambulant, they cannot keep up with their peers, have problems walking, hopping, running, climbing stairs and fall frequently. Falls can lead to injuries (including fractures). By the age of 12 years most boys are fully wheelchair dependent, although corticosteroids have improved this outcome (see below). Once ambulation or some other motor functional capacity is lost in an individual with DMD, it is gone forever. Death can occur without warning, at any moment, even (rarely) in younger boys (<12 years of age). Sudden death is usually caused by heart problems, but can also arise from cardiac complications, respiratory problems and rhabdomyolysis.
In non-ambulatory boys and young men, there is gradual loss of upper limb, trunk and neck functions, so that grooming, toileting, bathing, dressing, sitting unsupported and eating become impaired or impossible to perform by oneself — severely affecting the quality of life of patients, their caregivers and families.
Without nocturnal ventilation and cardiac support, survival into the third decade of life is unusual.

2.1.4 Natural history

There is currently no cure for DMD, although there are medical treatments that may help slow down the progression and consequently life expectancy has improved over the last two decades thanks to better standards of care (see section 2.2)[18, 19]. However, many of these interventions are associated with new complications, and quality of life often suffers. For instance, adverse events known to be associated with chronic glucocorticoid usage include excessive weight gain, growth inhibition, risk of diabetes, behavioural abnormalities, Cushingoid features, change in pubertal progression and cataracts. Of particular concern for the DMD community is the issue of weight gain, since DMD is a progressively debilitating disease and weight gain can compound the physical limitations of a patient with impaired muscle function.
Nowadays, due to assisted ventilation, adults with DMD typically live into their late twenties. By that time they have hardly any muscle function left with the exception of the facial muscles, which are relatively spared until a late stage of the disease. Muscles needed for chewing and swallowing are also affected, leading to problems with nutrition. In contrast to weight gain problems in younger DMD patients, malnutrition is often seen in older Duchenne MD patients.

2.1.5 Social aspects

Boys and young men with DMD are confronted with the fact that they lose motor functions from an early age and the gap in physical skills between them and their peers becomes bigger every day. They face many hurdles and challenges and have to use all their energy and strength to keep up with others. Despite all this most boys go to normal primary school, high school, sometimes to university. Quality of life studies in DMD individuals have clearly identified a gap between them and normally developing individuals. Nevertheless, individuals with DMD consistently rate their quality of life much higher than healthy people, including clinicians, would expect [20, 21].

Parents of DMD children report a high burden of care from an early age, not only compared to healthy children but also compared to children with other chronic disorders. Only parents of children with multiple complex handicaps score higher[22]. In addition having to help patients physically with dressing, feeding and lifting, some families have a hard time due to the behavioural issues often seen in DMD patients.

It is not unusual that parents of DMD boys and young men have to wake up 6-10 times per night to help to adjust their sons' position in bed, help with ventilation and/or coughing. Help from outside the family is often not available. DMD is also associated with a substantial economic burden[23].

2.1.6 Time spent on medical care

Standards of Care are available [18, 19] but the majority of individuals with DMD do not receive care accordingly, e.g. although there is a consensus that individuals with DMD should be seen by a multidisciplinary team, this often does not happen. Individuals with DMD often see a wide range of clinicians such as paediatricians, neurologists, neuromuscular specialists, cardiologists, pulmonologists, psychologists, rehabilitation doctors, physiotherapists, orthopaedic surgeons and nutrition specialists. Without a multidisciplinary team in place, DMD patients and parents spend a lot of time at and travelling to hospital visits, which keeps them away from school, work, social activities, sports and family life.
Almost all (medical) care for DMD individuals is not performed in hospitals but round-the-clock at home. Stretching exercises, medication, ventilation, endotracheal suction, assisted cough, care for bed sores, giving enemas is all done by parents at home.

2.2 Clinician perspective on natural history and standards of care

2.2.1 Clinical presentation

DMD is characterized by a predictable clinical progression with onset in infancy. Affected boys may present with delayed motor development at an early age followed by a progressive weakness of the skeletal muscles during childhood. Muscle weakness may be overlooked at an early age, as the motor delay may be considered as part of a more global developmental delay.
DMD specific learning and behavioural disabilities are well described, especially in patients with mutations in the distal part of the *DMD* gene, lacking expression of multiple brain specific dystrophin isoforms. A wide spectrum of general deficits in multiple areas of cognition and adaptive functioning, ranging from subtle learning and verbal memory disorders to severe mental retardation [24-26] have been reported. Overall mean IQ (82) is approximately one standard deviation (SD) below the normal population.

Young DMD boys may come to clinical attention because of the incidental finding of high CK levels, or by the finding of an associated rise in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) from muscular origin. CK levels are typically increased to more than 10 times the upper limit of normal, with a maximum value at around the age of 1 to 6 years old, followed by a progressive decline due to loss of muscle mass.
The first clinical manifestations of muscle weakness that trigger medical advice include gait abnormalities and difficulties in climbing stairs and rising from the floor, with the typical 'Gower's' sign (broad- based stance and "climbing" upright by the use of the support of the hands on the thighs) by the age of 3-4 years. Fatigability and the inability to participate in motor activities with peers are additional clinical features that may alarm the parents.

Further disease progression with loss of ambulation and subsequent progressive loss of upper limb, cardiac- and respiratory function is well characterized (see section 2.2.3). Without intervention cardiac involvement and respiratory complications will result in early mortality in the second or third decade of life.

2.2.2 Diagnosis

A diagnosis of DMD is usually made on the basis of family history, clinical features as described above, followed by measuring the serum CK activity, which is significantly elevated in DMD, usually >1000 U/L[27]. The diagnosis of DMD is confirmed by molecular analysis of the *DMD* gene, or by assessment of dystrophin protein expression on muscle biopsy, followed by identification of the mutation in the *DMD* gene (see section 2.3).

2.2.3 Natural History

The natural history of this predictable degenerative disorder is currently well characterized, based on data from patient registries, natural history studies and data drawn from the placebo arms of pharmaceutical industry trials. Current understanding of the natural history should take into consideration the effects of coordinated, multidisciplinary care interventions on disease evolution and longevity.

Different clinical stages of the disease can be identified over the course of a patient's lifetime.

- ***Neonates/Infancy:*** While DMD is rarely diagnosed in infancy, the disease is manifested from an early age. Most boys will show delayed motor and global development (assessed by developmental outcome measures such as Griffiths Mental Development Scales[28], and the Bayley Scales of Infant and Toddler Development, Third Edition (Bayley-III)[29, 30]).
- ***Young children, early ambulant*** *(aged 1 to approximately 3 ½ years):* Stage of disease characterized by a slower development of gross motor milestones compared to healthy peers. Some children may show signs of delayed language and cognitive impairment (assessed by developmental outcome measures such as the Bayley-III and Griffiths Developmental Scales[29, 30].)
- ***Young Ambulatory*** *(from 4 to approximately 7 years):* In this stage slower gains in ambulatory function are observed as compared to typically developing children (on 6MWT and 10 metre walk/run tests). There may be either gains or losses in milestones (on North Star Ambulatory Assessment), however, boys are increasingly falling behind normative performance levels of their normally functioning peer group.
- ***Late Ambulatory*** *(from approximately 7 to 13 years of age:* During this stage of disease, there is progressive weakness and the gradual loss of gross motor skills and ambulatory functions (including standing ability, stair climbing and ultimately, the ability to walk). Lower limb joint contractures develop almost invariably (ankle equinous contractures, shortening of iliotibial band, which impact further the gait pattern). The combined risk of osteopenia and fractures due to falls may precipitate the loss of ambulation. The progressive muscle weakness also affecting the breathing muscles is reflected by the decline in some pulmonary function parameters, particularly maximal expiratory pressure (MEP) and maximal inspiratory pressure (MIP) and forced vital capacity (FVC).

  While clinical symptoms of cardiomyopathy may emerge only in adolescence or young adulthood, early regional myocardial damage and myocardiac changes can yet be detected in young DMD patients by imaging techniques such as MRI, tissue Doppler measurements, and myocardial velocity gradients[31].
- ***Early Non-ambulatory****:* After loss of ambulation there is continued muscular deterioration with increasing loss of upper limb function and the development of skeletal deformities such as limb contractures and scoliosis. There is continued decline in pulmonary function with ultimate need for mechanical cough assistance and progressive risk of nocturnal hypoventilation requiring non-invasive ventilation. Progressive dilated cardiomyopathy will further develop insidiously
- ***Late non-ambulatory****:* Postural maintenance and sitting balance is progressively lost. Upper extremity function is severely limited to distal fine motor function and tabletop activities. At some point in the disease progression all affected individuals will require mechanical cough assistance and non-invasive ventilation. While DMD patients may now expect to live into adulthood thanks to improved care and management[18, 19, 32], new challenges have emerged as the disease progresses relentlessly. The management of swallowing and feeding difficulties, impaired phonation, smooth muscle involvement with bladder and intestinal dysfunctions, and issues of social integration and quality of life for adults with severe impairments require specific attention.
- With increasing age and associated disease progression, respiratory impairment and heart disease (heart failure and conduction abnormalities) will result in premature mortality.

2.2.4 The impact of optimal care and management on the course of DMD

Current medical management has changed the natural history in DMD affecting the timing of clinically meaningful milestones as well as longevity in individuals with access to high quality care. This has largely been due to the use of glucocorticoids, management of spine deformity, pulmonary management, and cardiac management. The occurrence of contractures may impact mobility and upper limb function, and efforts are made to prevent and manage contractures. Despite these interventions, the cardiomyopathy and pulmonary involvement in DMD still leads to substantially shortened lifespan.

With increased lifespan due to effective ventilation interventions, cardiomyopathy (which is almost universally seen in DMD patients) has become a more common cause of death among individuals with DMD [33]. Cardiac causes of death shifted from 8% to 44% over the last 30 years as indicated in recent retrospective studies, even though cardiac care has improved[33, 34].

2.2.4.1 Glucocorticoid therapy

The use of corticosteroids has been shown to improve muscle function and respiratory function tests [35] and to have a positive impact on all-cause survival as well [36]. Natural history studies have shown the effect of steroids on ambulatory milestones, prolonging ambulation by about two to three years over time and delaying losses in upper-limb functioning [32, 37]. Steroids have

also affected pulmonary function, delaying the need for mechanical cough assistance or non-invasive ventilation. The prevention and management of the known side effects such as Cushingoid features, weight gain and growth inhibition, impaired fat and glucose metabolism, fluid retention and hypertension, osteoporosis with increased risk of vertebral fractures, and cataract, should be integrated in the care of individuals with DMD. Different steroid regimens and compounds are in use with different effect and side effect profiles (see section 2.2.6. 2).

2.2.4.2 Contracture management

Progressive retractions of Achilles tendons, knee and hip flexors and iliotibial bands may negatively affect stability and the gait pattern in ambulant DMD and may contribute to the loss of stair climbing and ambulatory capacity [38]. Approaches to contracture prevention and management have been outlined in the care considerations [19]. Adequate joint positioning is further indicated in the non-ambulant stage to prevent severe joint deformities. Attention should be paid to adequate sitting posture and to the prevention of contractures in the upper limbs and hands, which could otherwise impair fine motor activities at a later stage.

2.2.4.3 Spine deformity management

The incidence of significant scoliosis requiring spinal arthrodesis has changed due to the use of glucocorticoids [39]. In addition, timely spine surgery for curves > 30-40 degrees has impacted survival [40].

2.2.4.4. Pulmonary management

The American Thoracic Society practice parameter [41] includes recommendations for management of DMD with airway clearance strategies or mechanical cough assistance and non-invasive ventilation. Implementation of these guidelines has considerably changed life expectancy [33]. Consequently, a larger number of young men with DMD are living into their twenties and thirties, but with profound motor and cardiorespiratory disability. In addition respiratory failure related to nocturnal hypoventilation represents a complication related to the respiratory muscle weakness.

2.2.4.5 Cardiac management

Unfortunately, death in the second decade of life is still commonly observed in individuals with DMD, mostly due to severe heart problems. Nevertheless, the cardiac management has evolved from treatment of symptomatic heart failure to prevention of progressive ventricular dysfunction with early afterload reduction e.g., angiotensin converting enzyme (ACE) inhibitors, Angiotensin II receptor blockers (ARBs), and beta blockers. ACE inhibitors have impacted positively on survival in young men with DMD-associated clinical cardiomyopathy [42]. Retrospective studies indicate a positive effect of chronic steroid treatment on the development of ventricular dysfunction as well.

2.2.5 Heterogeneity in DMD disease progression

While the muscle involvement and subsequent loss of function described above is well characterized, disease progression and rate of decline can differ from one patient to another. This clinical heterogeneity has to be taken into consideration in the design and interpretation of therapeutic trials. With more data emerging from natural history studies and the placebo arms from DMD treatment studies, many of the causes for variability in outcomes are becoming clearer. The age and stage of disease, steroid use, implementation of standards of care, and genetic factors such as type of mutation and genetic modifiers are factors that contribute to the heterogeneity in disease progression (see sections 2.3.3 and 2.3.4 for genetic causes of variation).

2.2.5.1 Age and stage of disease

The age at loss of clinically meaningful milestones (a proxy for disease severity) predicts the age at loss of future milestones. For example, the age at loss of ambulation predicts the age at which subsequent loss of upper limb function occurs and the age at which critical pulmonary milestones are reached [37]. It follows that changes in some clinical outcome measures in response to treatment over the short term, can predict subsequent disease progression years later, as has been demonstrated in children using corticosteroid treatment followed for many years [43]. These insights on effect of age- and baseline functions on disease evolution should be taken into account for the selection and randomization of study participants in controlled clinical trials to reduce variability in subsequent rate of progression.

2.2.5.2 Corticosteroid therapy

There are data to suggest that differences in patterns of steroid use — including whether the patient is on daily versus intermittent regimens, dosage, time on treatment, and possible drug choice (deflazacort or prednisone) — may have variable effects on clinical progression and function [44, 45]. Both prednisone/prednisolone and deflazacort, have been shown effective in increasing muscle strength [35]. Uncontrolled studies suggest slightly different effects and side effect profiles, such as less weight gain but an increased incidence of cataract for deflazacort [46]. A wide range of regimens are currently used across the world among which daily, alternate day or intermittent schedules (10 days on 10 days off or week-end dosing) are the most commonly used [19]. Daily steroids have been proven more effective compared to alternate day in improving strength and function, however alternate day or intermittent schedules have been associated with a milder side effect profile [47]. High dose weekend prednisone was shown to be as effective as daily prednisone with a better side effect profile [48,] however the relative short term of the study precludes firm recommendations. Age and disease stage at initiation of corticosteroids may also impact the variability in disease progression as suggested by the data collected through the North Star Network [45].

2.2.5.3 Night splinting, physical therapy, and other standard interventions

Night splinting, physical therapy and other standard of care interventions as described by the DMD Care Considerations are recommended because they are expected to have significant effects on functional performance[19].

Implementation of recommendations for cardiac and respiratory management has shown a clear impact on longevity. Significant variability in the course of progression could be introduced depending upon whether or not a person with DMD receives standard of care and management, or is adherent to recommended prevention and management strategies.

2.3 Dystrophin, genetics and genetic modifiers

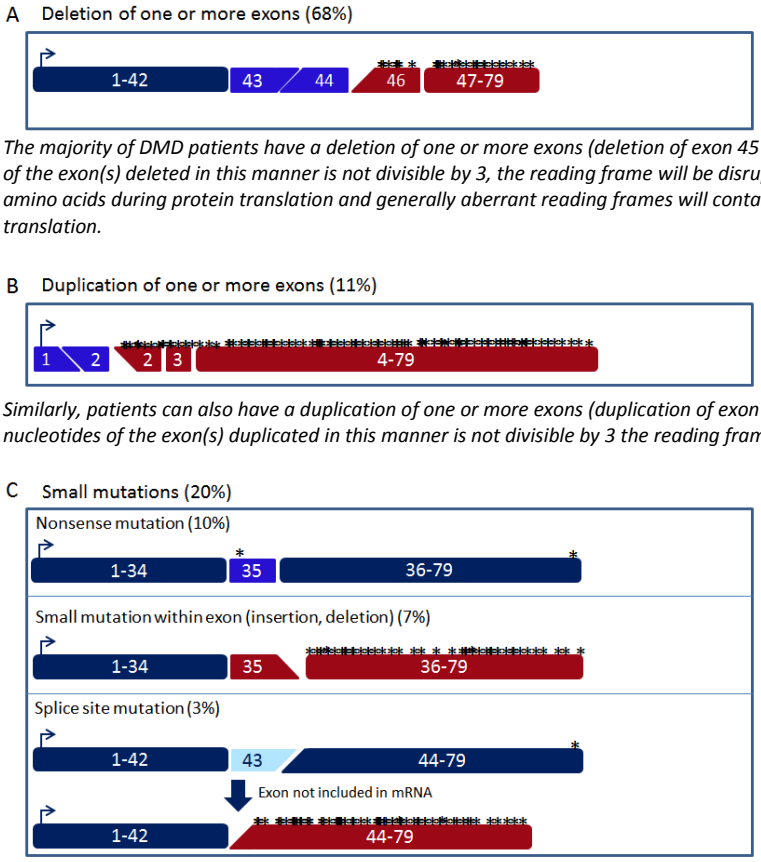
2.3.1 DMD gene and dystrophin protein

The *DMD* gene is one of the largest human genes, with 79 exons spread out over 2.2 Mb. The 427 kDa muscle dystrophin protein is the major protein product [49, 50]. Mutations in the *DMD* gene that abolish the production of a functional dystrophin protein are the underlying genetic cause of DMD. In muscle, dystrophin acts as a shock absorber during muscle contraction by connecting cytoskeletal actin to the extracellular matrix, while the middle of the protein acts like a spring that can unfold during muscle fibre lengthening and refold when force is no longer applied [51]. DMD is caused by mutations that cause premature truncation of dystrophin protein translation. This can happen through mutations that disrupt the reading frame or through point mutations that generate a premature stop codon (Figure 1)[52]. With the lack of dystrophin, the link between actin and the extracellular matrix is lost and muscles become sensitive to damage, especially during lengthening contractions. DMD patients suffer from chronic, continuous muscle damage, which is accompanied by chronic inflammation, eventually leading to replacement of muscle tissue by fibrotic and fat tissue and irreversible loss of muscle tissue and function [53].

Different DMD causing mutations have been identified (Figure 1A-D). The majority of patients have a deletion (~68%) or duplication (~11%) of one or more exons, while ~21% have a small mutation, either involving a point mutation that induces a premature stop codon (nonsense mutations, 10% of all mutations) or disrupting a splice site (3%) or involving a small deletion or insertion that disrupts the reading frame (7%)[52].

Mutations in the *DMD* gene have also been found in Becker muscular dystrophy (BMD) that is characterized by a less severe clinical phenotype compared to DMD [50]. However in BMD, mutations generally affect the central part of dystrophin and the open reading frame is maintained. Therefore protein translation is not stopped, resulting in a dystrophin protein that possesses actin- and extracellular matrix binding domains and as such is partially functional (*Figure 1 D*).

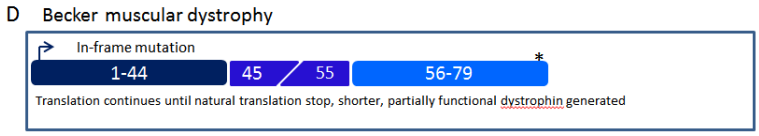
Figure 1: Mutations in the dystrophin gene



The majority of DMD patients have a deletion of one or more exons (deletion of exon 45 in the above example). When the number of nucleotides of the exon(s) deleted in this manner is not divisible by 3, the reading frame will be disrupted. This will lead to the incorporation of aberrant amino acids during protein translation and generally aberrant reading frames will contain many stop codons, leading to premature truncation of translation.

Similarly, patients can also have a duplication of one or more exons (duplication of exon 2 in the above example). When the number of nucleotides of the exon(s) duplicated in this manner is not divisible by 3 the reading frame will be disrupted like for deletion mutations.

Small mutations (i.e. involving part of an exon) can affect transcript translation in different ways. Point mutations that create a premature stop codon (nonsense mutations, top panel of the above Figure). Note that the reading frame is not disrupted for these patients. Protein translation stops when it encounters the premature stop codon (in exon 35 in this example). Small mutations can disrupt the reading frame (middle panel), e.g. through the deletion or insertion of a number of nucleotides that is not divisible by 3. In this example the insertion of one nucleotide in exon 35 results in a disruption of the reading frame. Finally, small mutations can affect a splice site (for exon 43 in the above example). This generally leads to the exon not being recognized by the splicing machinery and excluded from the mRNA transcript. Consequently, this leads to a deletion on transcript level that disrupts the reading frame.



Mutations that maintain the open reading frame and allow the production of a partially functional dystrophin are associated with BMD. When the number of nucleotides deleted or duplicated is divisible by 3, the reading frame will be maintained; e.g. in the example exon 46-54 are deleted, which involves 1413 nucleotides. Since this is divisible by 3 (1413/3=471), protein translation can continue until the end of the transcript. The resulting protein will lack the 471 amino acids encoded by exons 46-54, but will contain the actin binding domain and the extracellular binding domain and as such be partially functional.

2.3.2 Genetic testing

Having confirmation of the exact mutation in the *DMD* gene is a crucial part of diagnosis, since it will allow carrier analysis for females at risk, and will reveal whether patients could be eligible for mutation-specific therapies such as exon skipping (see below). Since the majority of DMD patients have a deletion or duplication of one or more exons, DNA diagnosis generally starts by multiplex ligation dependent probe amplification (MLPA)[54], a method that will assess the presence and abundance of each of the 79 *DMD* exons. This method that is recommended by the care guidelines[19], can exactly pinpoint the size of the deletion or duplication in patients, but also in women who carry the genetic defect. When suspecting DMD or BMD in patients for whom

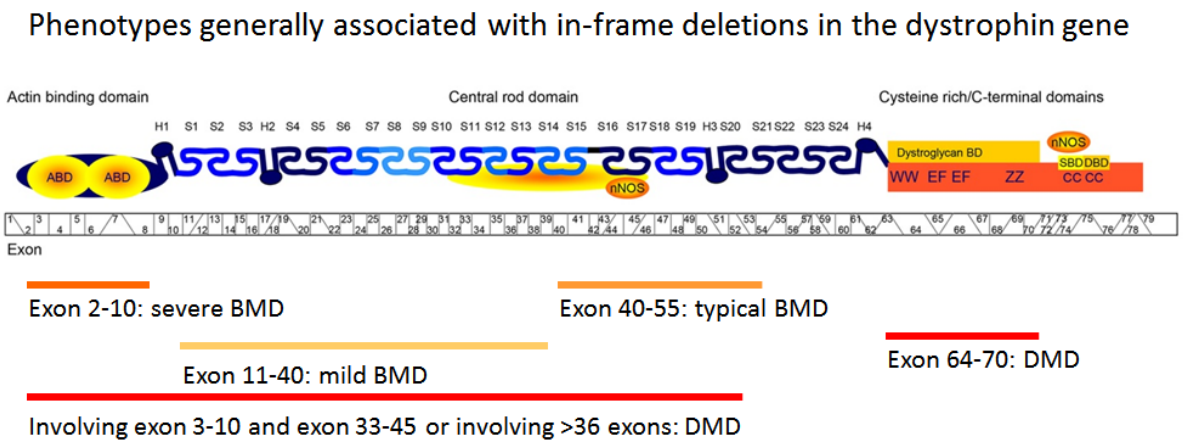
no deletion or duplication mutation has been detected, the care guidelines prescribe sequencing each and every exon and their splice sites to identify potential small mutations. Genetic testing is the gold standard for diagnosis and should be the prerequisite for all participants in trials, especially for compounds that are mutation specific [19].

2.3.3 Genetic modifiers: dystrophin mutations and phenotype-genotype correlations

The distinction between DMD and BMD is currently based on whether dystrophin mutations maintain the reading frame or not. In the past, before the identification of the dystrophin gene, the distinction was based on the age when patients became wheelchair dependent (<12 years DMD, >16 years BMD, between 12 and 16 years intermediate phenotype)[13]. However, with the improved care for DMD patients, the distinction between DMD and BMD is less clear (e.g. some individuals with DMD are still ambulant beyond 16 years), and the 'dystrophinopathies' are more like a continuum (mild BMD, typical BMD, severe BMD, mild DMD, DMD) than a black and white distinction between the two diseases.

BMD severity is more variable, with patients often presenting with first symptoms in childhood but also in adulthood or even for some cases in old age [13, 50]. The main reason for this variation lies in functionality of the dystrophin protein produced, which differs depending on the location and size of the in-frame mutation [55] (Figure 2). Because different Becker-type dystrophins have different functionalities, it is challenging to assess if and how dystrophin levels influence disease severity. It is clear that a threshold amount of dystrophin is required, and there is essentially no overlap in the level of dystrophin between DMD and BMD (see below), but this minimum level may well vary for different dystrophins (depending on their functionality).

Figure 2 (based on [55]): Overview of the phenotypes generally associated with in-frame deletions in the dystrophin gene

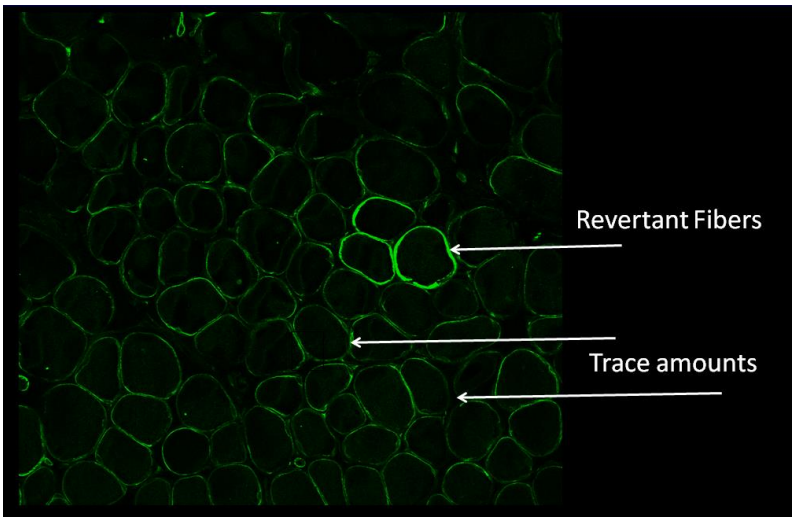


In-frame mutations between exon 40-55 are usually associated with typical BMD. In-frame mutations removing the first 2 actin-binding domains (affecting exon 2-10) usually lead to severe BMD, while in-frame mutations removing all actin binding domains are associated with a DMD phenotype. Similarly, in-frame mutations affecting the dystroglycan binding domain (exon 64-70), or involving 36 exons or more are associated with DMD. In-frame mutations affecting exon 11-40 are usually associated with a very mild phenotype (mild BMD, cramps, myalgia).

Among DMD individuals there is less variation in disease severity compared to BMD, which is not unexpected since the out-of-frame mutations will abolish dystrophin function regardless of the size of the mutation and its location. Although DMD shows less variation in disease severity, specific genetic mutations may influence disease progression, e.g. the group of patients with deletions flanking exon 44 and for which exon 44 skipping would restore the reading frame, appear to have a slower than average disease progression (less decline in the distance walked in 6 minutes or North Star ambulatory assessment score over 12 months, older age at loss of ambulation)[45, 56, 57].

Even though theoretically DMD individuals should not produce any dystrophin, it is now apparent that most do produce very low levels of dystrophin [58]. Here it is necessary to discriminate between revertant fibers (individual fibers expressing high levels of dystrophin) making up <3% of all fibers on a cross section, and trace amounts of dystrophin that appear to be produced by all fibers (Figure 3). The level of trace amounts varies among patients, but in two independent studies it has been shown that they are higher for patients who need exon 44 skipping to restore the reading frame [58, 59]. The implication is that when dystrophin analysis is being studied in trials aiming at dystrophin restoration both pre- and post-treatment biopsies should be obtained so the patient can act as his own control for trace dystrophin levels.

Figure 3: Immunofluorescence staining of a muscle biopsy from an individual with DMD.



It is clear that all muscle fibers show some dystrophin staining (trace amounts), likely due to low levels of spontaneous exon skipping. A few fibers (revertant fibers) are very brightly stained, likely due to a secondary, reading frame restoring mutation on DNA level in the muscle progenitor cells. Picture kindly provided by Afrodite Lourbakos (Biomarin, Leiden, the Netherlands).

The overall correlation between genotype and phenotype in dystrophinopathies is relatively well understood and tools to examine this are widely available. Exceptions to the overall pattern of genotype-phenotype correlation (<10% of all cases) have been elucidated and are well documented [55].

2.3.4 Other genetic modifiers for DMD

Polymorphisms in genes involved in the biochemical pathways leading to muscle damage or influencing a response to steroid treatment could influence disease progression as well. Two potential genetic modifiers have been reported:

- Secreted phosphoprotein 1 (SPP1 or osteopontin) polymorphisms: In this case, the genetic modifier appears to be influencing the patient's response to corticosteroid therapy rather than affecting the disease itself directly [60].
- LTBP4 polymorphisms: a minor allele present in about 30% of the population appears to have a protective effect on ambulation, with an effect roughly equivalent to steroid treatment, prolonging ambulation as much as two years [61, 62].

There may be other genetic modifiers yet to be identified for DMD. However, while the two known genetic modifiers do appear to be responsible for some of the heterogeneity in the rate of disease progression in subjects receiving standard of care medical management, how they exactly influence disease progression and outcome measures have yet to be clearly determined.

Apart from the impact of the type of dystrophin mutation, clear information on other genetic modifiers of the phenotype is still lacking.

3. Exon skipping

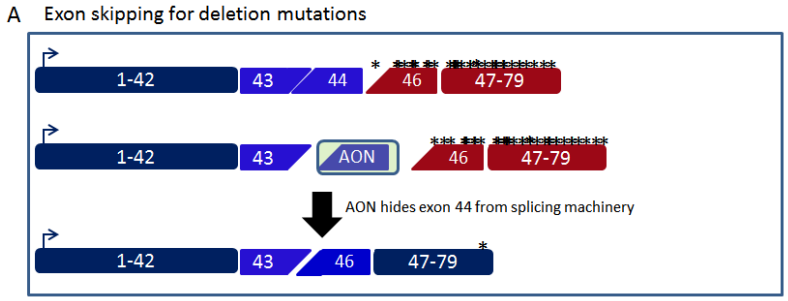
3.1 Rationale

The rationale of the exon skipping approach is based on the fact that mutations in the *DMD* gene can result in mild BMD or more severe DMD phenotypes, depending on whether the reading frame of the dystrophin transcript is maintained or disrupted (Figure 1AD). The aim of the exon skipping approach is to restore the reading frame of the DMD dystrophin transcript to allow the production of an internally deleted, partially functional dystrophin as found in BMD patients, rather than a prematurely truncated, non-functional dystrophin found in DMD patients [63]. For deletion mutations, exon skipping can often restore the reading frame by skipping one of the exons flanking the deletion (Figure 4A). For small mutations in in-frame exons, exon skipping of the mutated exon would bypass the mutation without affecting the reading frame (Figure 4B)[64].

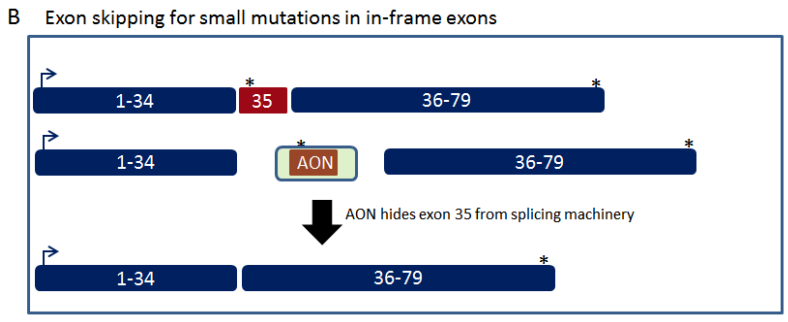
Exon skipping can be achieved by manipulating the pre-mRNA splicing process. For most genes the protein coding information is dispersed over exons, while interspersing introns do not contain any coding information. When a protein is required by the cell, gene transcription is initiated, and a pre-mRNA copy of the gene is made, containing both exons and introns. Before the transcript can be translated into protein, the introns have to be removed and the exons joint together in a process called splicing. This will result in a messenger (m)RNA transcript that is then translated into protein by the ribosomes.

During the pre-mRNA splicing process the spliceosome and splicing factors recognize individual exons. When two adjacent exons are recognized, this will result in the removal of the intermittent intron and the joining of the exons. AONs, small pieces of chemically modified RNA or DNA, can hybridize to exons in a sequence specific manner. This will hide the exon from the splicing machinery, which will search for the next available downstream exon. As such the targeted exon will be removed with its flanking introns [63].

Figure 4: The exon skipping approach



For deletions skipping one of the exons flanking the deletion can often restore the reading frame. In the above example (deletion of exon 45), exon 44 skipping (or exon 46 skipping) would restore the reading frame (indicated by the fact that exon 43 and 46 'fit together' as do exon 44 and exon 47). Exon skipping can be induced using an antisense oligonucleotide (AON) that specifically binds to an exon and hide it from the splicing machinery (exon 44 is targeted in this example). The splicing machinery will look for the next available exon (exon 46 in this example) and join that to exon 43, thus restoring the reading frame.



When small mutations reside in in-frame exons, exon skipping can bypass these mutations. In the example of a nonsense mutation in exon 35, skipping this exon will bypass the nonsense mutation without affecting the reading frame. Likewise, when a small mutation in exon 35 would disrupt the reading frame (e.g. insertion or deletion of 1 base pair), exon 35 skipping would restore the reading frame.

Exon skipping is a mutation specific approach. Depending on where the mutation is located and which exons are involved, the skipping of different exons will be required to restore the reading frame or bypass a mutation (e.g. Figure 4A vs. 4B). However, most DMD individuals have a deletion of one or more exons (68%), and 66% of these deletions cluster in a hotspot region between exon 45 and 55 [52, 64]. As such, skipping of certain exons applies to larger groups of patients (Table 1). Nevertheless, because DMD is a rare disease, these subgroups are not large. AONs targeting exon 51, 45, 53 and 44 have currently been tested in clinical trials. Skipping these exons would in theory be applicable to 39% of patients in total [52].

Two types of chemically modified AONs have advanced into clinical trials: 2'-*O*-methyl RNA with a phosphorothioate backbone (2OMePS) and phosphorodiamidate morpholino oligomers.

Table 1: Applicability of single exon skipping

Exon	Applicability (all mutations)	Applicability (deletions)
51	14.0%	20.5%
45	9.0%	13.1%
53	8.1%	11.8%
44	7.6%	11.1%
50	3.8%	5.6%
43	3.1%	4.5%
8	2.0%	2.9%
55	1.7%	2.5%
52	0.9%	1.3%
11	0.9%	1.3%

For certain deletions the reading frame can be restored by skipping either of the flanking exons (e.g. an exon 52 deletion can be reframed by skipping exon 51 OR exon 53). This Table has been adjusted to make sure this type of deletions are not counted twice (e.g. exon 51 skipping applies to the largest group of mutations, including exon 52 deletions; exon 52 deletions are therefore not taken into account when calculating the applicability for exon 53 skipping). Source: [52]

3.2 Current state of affairs for the 2'-O-methyl phosphorothioate AONs

Biomarin has six 2OMePS antisense oligonucleotide (AON) compounds in development for Duchenne muscular dystrophy (DMD) with four (drisapersen, BMN 044, BMN 045 and BMN 053) in clinical trials. A multi-skip programme is in early development. The lead compound, drisapersen, is currently in the process of regulatory submission in the USA and Europe. Drisapersen is a chemically modified AON with a sequence optimised to skip exon 51 in the human dystrophin pre-mRNA and is intended for the treatment of patients with DMD bearing certain mutations that can be corrected by skipping exon 51.

Specific exon 51 skipping occurs following administration of drisapersen in myogenic cell cultures and human tissue from DMD patients with relevant mutations, and exon 51 skipping results in an internally truncated dystrophin protein [65]. Extensive mdx mouse model studies have demonstrated that 2'*O*-methyl phosphorothioate oligonucleotides can be delivered by subcutaneous injection, have a favourable pharmacokinetic profile, induce specific exon skipping, restore dystrophin expression and improve muscle pathology (reducing creatine kinase [CK]) with overall positive effects on muscle performance [66-68]; factors that have subsequently been confirmed with drisapersen in placebo controlled studies ([69] and unpublished).

A total of 326 subjects have been included in the drisapersen clinical program which comprises two Phase II (DMD114117 and DMD114876), one Phase III placebo-controlled (total N=290), and two open-label extension (OLE; N=245) studies. The Phase II studies enrolled similar populations with mean baseline 6-minute walking distance (6MWD) ~400m; DMD114117 required a loading dose of drisapersen to be given. At Week 25, study DMD114117 (N=53) showed a statistically significant treatment difference of 35m (p=0.014) in 6MWD for drisapersen 6mg/kg/week versus placebo (maintained at Week 49 [36m; p=0.051]). After 24 weeks of drisapersen 6mg/kg/week in study DMD114876 (N=51), a treatment difference was seen in 6MWD (27m; p=0.069).

The Phase III study, DMD114044 (N=186), enrolled a more severe population (mean baseline 6MWD <350m). After 48 weeks, there was a non-statistically significant 10m difference in favour of drisapersen 6mg/kg/week versus placebo. However, for subjects from DMD114044 enrolled into the open label extension study (OLE) DMD114349 completing at least 48 weeks, the 6MWD difference (n=98) at 96 weeks was 30.3m for subjects receiving 96 weeks of drisapersen versus those receiving delayed treatment (placebo in feeder studies/48 weeks of drisapersen) and 66.8m after 120 weeks treatment (n=62). For those originally enrolled in DMD114117 a treatment difference of about 50 m was maintained at these time points (n=31,24

respectively) with the continuous drisapersen group remaining close to the original baseline (-5m). In OLE DMD114673 (n=10; average age: 12.9y), mean change in 6MWD at 3.4 years was –24.5m (median 8m) from OLE baseline in those able to complete the 6MWD at baseline.

Drisapersen was measured in muscle biopsies of drisapersen treated subjects along with exon 51 skipping and an increase in dystrophin was clearly demonstrated in the biopsies with sufficient muscle content and integrity (quality) such as in DMD114117. Drisapersen treatment induced a decline in serum CK in all placebo studies and a reduction in muscle fat infiltration in a MRI sub study in DMD114876.

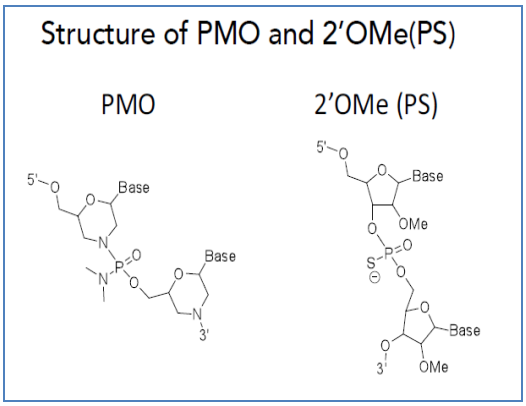
The most commonly reported drisapersen adverse events were injection-site reactions and sub-clinical proteinuria. Infrequent moderate to severe thrombocytopenia was also seen. In some subjects more severe ISRs were seen reported especially in those subjects for whom injection site rotation had not been initiated from the outset of treatment.

Overall, the clinical data suggest that early benefit is seen in DMD boys with good function, taking longer to appear in those with more progressed disease. Future plans include further investigation into alternative routes of administration and studies in non-ambulatory, early disease and under 5 years of age in the context of optimized standard of care and inclusion of appropriate endpoints.

3.3 Current state of affairs for phosphorodiamidate morpholino oligomers

Sarepta is developing the PMO chemistry. The main characteristic of the PMO chemistry (Figure 5) is that they are charge-neutral, with a highly modified backbone that makes the compounds extremely resistant to nucleolytic enzymes in serum and intracellularly. Unlike other AON chemistries in clinical development, PMOs do not bind to serum proteins [70].

Figure 5: Chemical structures of morpholino phosphorodiamidate oligomers (PMO) and 2'-O-methyl phosphorothioate (2'OMe(PS)).



Eteplirsen is a PMO AON targeting exon 51 (Table 1). The PMO SRP-4053(exon 53 skipping PMO) completed investigational new drug (IND)-enabling studies and is being tested in a Phase I clinical study. In addition, planning is currently under way for a clinical trial with SRP-4045 (exon 45 skipping PMO) in 2015.
In addition to these clinical candidates, Sarepta has a pipeline of PMO based DMD product candidates that target the majority of DMD patients who have mutations amenable to exon skipping [52, 64, 71].

The above PMO product candidates and a few others have been evaluated in a series of *in vitro* and *in vivo* safety studies. PMOs have been shown to skip exons of murine dystrophin pre-mRNA (e.g., exon 23 with AVI-4225)[72]. Despite having different lengths, ranging from 22 to 30 nucleobases, and unique sequences, the safety profiles of PMOs are consistent and no sequence-dependent toxicities have been observed to date. No adverse effects on major organ systems have been observed, including immune, cardiovascular, neurobehavioral, cardiovascular, and male reproductive systems. Repeat-dose toxicity studies of 12 to 39 weeks duration in mice and cynomolgus monkeys have shown that the maximum feasible doses (MFD) were 960 mg/kg in mice and 320 mg/kg in monkeys. This maximum was limited by solution solubility for IV injection of PMOs. The only target organ of toxicity identified was kidney, consistent with renal elimination as the predominant excretion pathway for PMOs. These effects seen at many times the exposure at therapeutic dose were usually limited to mild to moderate microscopic

findings of tubular basophilia and vacuolation, with occasional incidences of tubular degeneration. No effects were detected on renal function (i.e. serum creatinine, urea nitrogen and bilirubin). Finally, PMOs tested to date have shown no evidence of serum protein binding, no increased aPTT, no pro-inflammatory effects, no complement activation, thrombocytopenia, or no Kuppfer cell basophilia and/or hyperplasia which are known to affect targeted organs by other classes of antisense therapies in animal studies [73].

In addition to tolerability studies, efficacy of eteplirsen was tested in DMD patients amenable to exon 51 skipping first by IM injection [74] followed by Phase 2, intravenous treatment at dose range 0.5 mg to 20 mg per kilogram of body weight [75] and Phase 2B at doses 30 and 50 mg/kg per week.

Restoration of dystrophin in dystrophic animal models has demonstrated improvement in muscle function. For example, Sharp et al. [76] compared muscle function between PMO-treated and untreated muscle tissues. In dystrophic mdx mice, tibialis anterior (TA) muscles treated with a mouse specific PMO maintained ~75% of their maximum force capacity after stress inducing lengthening contractions, compared to untreated contralateral TA muscles that maintained only ~25% of their maximum force capacity ($p < 0.05$). Further analysis of these data demonstrated that restoring dystrophin expression even in a small amount of muscle fibers had a direct linear ($r^2=0.87$) impact on preservation of muscle function.

In a similar way, using dystrophic CXMD dogs receiving IV PMO up to 22 weeks, muscle function (tested using a 15 min running test) was maintained or improved [77]. Murine and canine models have demonstrated that dystrophin restoration directly leads to improved muscle function.

Importantly, in DMD patients it was shown that restoration of functional dystrophin expression through PMO-mediated exon 51-skipping resulted in proper localization of proteins normally interacting with dystrophin at the muscle fiber membrane [78, 79]. As restoration of dystrophin plays an essential role in maintaining muscle function, well-established dystrophin measurements are critical.

In Phase 2b studies of eteplirsen (original study 4658-US-201 and an extension study 4658-US-202), the primary endpoint, absolute percentage of dystrophin-positive fibers compared to baseline, was met [78]. Results of the 6-minute walk test (6MWT) at 144 weeks of uninterrupted treatment showed a decline in walking ability of 10 ambulant participants at a rate slower than would be expected based on available DMD natural history data. In addition, results at 168 weeks of uninterrupted treatment, showed continued ambulation across all patients evaluable on the 6MWT. However patients showed a reduction in distance walked since the 144 week time point. Nevertheless, the decline compared to baseline was less than would be expected from natural history (2 year decline of 121.8m for DMD with >7 y.o., or 125 m for DMD with mean 9.5 y.o.[4, 4, 80, 81]). Through week 168, a continued stability of respiratory muscle function was observed, as assessed by pulmonary function test. No clinically significant treatment related adverse events were observed.

Additionally eteplirsen is also currently being studied in a larger population phase III (160 patients), and a phase II non-ambulatory (20 patients). In view of the encouraging data obtained so far, regulatory advice for accelerated approval of eteplirsen has been requested from the FDA

3.4 New AON chemistries

As outlined above, the 2'-*O*-methyl phosphorothioate (2OMePS) and phosphorodiamidate morpholino oligomer (PMO) chemistries are currently in clinical trials have been tested in DMD boys, after initial tests in patient-derived cell cultures and animal models. Meanwhile, new modifications and chemistries are being have been explored in pre-clinical tests for dystrophin exon skipping as well [70, 82, 83]. Peptide groups can be relatively easily added to PMOs. Addition of a peptide group containing positively charged amino acids flanking a hydrophobic domain (pipPMO) leads to efficient exon skipping in skeletal muscle and heart in the mdx mouse model after injections with relatively low doses (12.5 mg/kg pipPMO vs 50 mg/kg generally used for unmodified PMOs in mouse)[82]. Likewise the tricyclo DNA chemistry induces efficient exon skipping in muscle and heart in mdx mice, albeit with higher doses (200 mg/kg). Preclinical trials to assess safety in toxicity studies in additional animal models are currently ongoing [83].

4. Outcome measures and clinical benefit

4.1 Functional outcome measures

4.1.1 Towards more defined functional outcome measures

In the last few years there has been an enormous impetus to identify the most suitable outcome measures for clinical trials in DMD.

Over the past 5 years in particular, the focus has gradually shifted from the simple identification of suitable outcome measures and analysis of existing ones to the identification of a roadmap which includes different outcome measures that can be chosen according to requirements of individual studies. One of the main advantages of this collaborative effort is that for the first time there has been an attempt to bring together different perspectives, taking into account not only clinical and statistical issues, but also input from Regulatory Agencies and patient advocacy groups. Meetings with Regulatory Agencies have confirmed the need for outcome measures that are statistically robust, validated and suitable for multicentric studies, and that are able to reflect changes which are clinically meaningful for patients and their caregivers. Over the last few years, national and international networks have produced an impressive amount of work, developing and validating old and new measures and trying to solve some of the bottlenecks identified at the time the first clinical trials were designed.
After a careful review process of the literature, a number of measures covering the whole range of functions as well as more disease specific ones have been identified. These can be selected according to the design of the study.

From the many outcome measures reported, they were prioritized based on the following criteria:

- The outcome measure had already been validated in DMD
- Reliability data were available
- The outcome measure had been used in combination with other measures
- Longitudinal natural history data were available
- The outcome measure had already been successfully used in previous trials

Other criteria were the ease and time taken for their execution and their suitability for multicentric studies.
The selection of measures has also been driven by the Regulatory Authorities' recommendation that they should be 'clinically meaningful' for patients and their families. This has driven the identification of a number of clinical outcome measures that have been brought forward for use in natural history data collection and for validation studies which comply with the standards outlined by the Regulatory Agencies.
These have been considered in a previous meeting organized by COST Action BM1207 and TREAT-NMD to discuss the draft EMA guidelines on the clinical investigation of medicinal product for the treatment of Duchenne and Becker muscular dystrophy. They have also been used when composing the guidance for industry submitted by the DMD community to the FDA [84].

4.1.2 Natural history for outcome measures

Since the meeting in 2013 further effort has been made to make new natural history data available using existing outcome measures, such as the 6MWT. This is currently chosen as the primary outcome measure in most ongoing clinical trials in ambulant DMD boys, along with the North Star Ambulatory Assessment [5]. These studies have identified the rate of change over time and helped to power studies, establishing how a treatment would cause a difference from the expected course of the disease. Longitudinal data is now available showing changes over 12, 24 and 36 months [4, 57, 80].
These studies have established how such measures can predict important clinical endpoints such as loss of ambulation, and have identified cut off points that have been used in trial design for inclusion and stratification criteria. As some variability has been found within these measures, there has been an effort to focus on disease stage and a description of the impact that variables (such as age, values at baseline or type and site of mutation) can have in affecting longitudinal performance. For example, recent natural history studies in DMD have shown that young boys show some improvement in their 6MWT and NSAA scores up to the age of 7 whilst after the age of 7 there is a slope of deterioration. Similar results have been observed using cut off values of baseline on the 6MWT (above/below 350 meters). The combination of these two variables has allowed the identification of distinct trajectories of progression in different subgroups subdivided by age and baseline values which has proved very useful at the time of interpretation of results of clinical trials conducted e.g. for ataluren, drisapersen and eteplirsen [84, 85].

4.1.3 Development of clinical outcome measures for older DMD patients

While reviewing the existing scales it has become increasingly obvious that there were a number of activities that were not currently captured. Most notably, the assessment of upper limb function appeared to be scarcely represented and the need for dedicated assessments of this has been highlighted in non-ambulant DMD boys.

A number of international meetings have addressed the need to have reliable measures for DMD individuals who have lost ambulation. A critical review of the measures that have been used in other neuromuscular disorders revealed that none of these measures have been systematically used in this cohort or been validated and that the activities included in the existing measures are not always suitable in DMD[86].

In the last two years, a collaborative international group including DMD boys and their families developed the Performance of Upper Limb (PUL) assessment, a tool specifically designed for assessing upper limb function in non-ambulant DMD boys and that can already be used in ambulant DMD boys [6, 7, 87]. The PUL provides a tool that can be used across spectrum of functional abilities. A recent study has shown that that the scale has excellent inter-observer and intra-observer reliability, is suitable for a multicentric setting and can easily be performed in DMD boys and adults from the age of 5 years with no floor effect even in the oldest (>25years) individual . The scale has been validated against other functional measures such as the 6MWT [88]. Longitudinal data are also becoming available showing that the scale can follow progression of the upper limb involvement in both ambulant and non-ambulant patients, and also to assess upper limb function in non-ambulant patients with and without steroid therapy (Pane et al, submitted). The importance of taking into account the use of steroids in clinical trials in non-ambulant DMD patients has also been highlighted recently by another group using different measures [89].

Similarly, there has been an international effort to validate the MyoSet - composed of MyoGrip and MyoPinch devices (strength) and the MoviPlate device (motor ability) - a battery of tests aimed at assessing distal upper limb strength and function [90]. New tools aimed at assessing upper limb movements (Actimyo) have also been developed and their validation in an international setting is in progress.

4.1.4 Development of new methods for assessment of young infants with DMD

It has become increasingly accepted that should novel therapeutic interventions be effective, it would be important to administer them as early as possible, possibly from the neonatal period. However, we are lacking normative data or outcome measures that could be used for very young DMD children. In the last few years international networks have reported the results of two large studies using neurodevelopmental scales in young DMD boys [29, 30, 91]. Both studies show that a proportion of DMD boys have delayed motor milestones that are more marked in the gross locomotor and language subscales and are partly dependent on the type and site of *DMD* mutations.

Another study has explored the suitability of the NSAA functional scale in infants, finding that it can be reliably used after the age of 3 years, 6 months [92].

4.1.5 Measuring muscle strength

DMD is characterized by a decline in muscle strength and indeed measuring muscle strength is used to assess disease evolution. However, the use of strength as outcome measure is hampered by the lack of a linear correlation between muscle strength and function in ambulant DMD individuals. Indeed, the relationship between quantitative knee extension strength and walking function (walking velocity or 6MWT) is not linear but logarithmic [84]. Large decrements in strength are associated with little change in function in young boys (4 to 7 years) [93] whereas small changes in strength are associated with large loss of function thereafter. While strength could be an appropriate outcome for compounds that lead to short term benefit in force production and/or lean muscle tissue mass, functional measures should be used only as additional endpoints to confirm the clinical meaningfulness of a given change in strength.

In non-ambulant patients a measurable decline over a 12 month interval in distal arm and hand function, grip and pinch force has been measured with the MyoTools (MoviPlate, MyoGrip, MyoPinch), which correlated to functional scales such as MFM or the Cochin scale, and to lung vital capacity [90, 94].

Data are available on the predictive values of outcome measures currently used in clinical trials for ambulant DMD boys such as 6MWT, timed tests and NSAA [4, 5, 80]. The minimal clinical important difference (MCID) for the 6MWT has been determined on distribution based methods and was shown to be approximately 30 meters [93], a distance in the range that has been considered for the registration of drugs for other neuro-metabolic diseases. Data from a large multicentric Italian natural history study confirmed that a 30 m decrement from baseline predicted the likelihood of experiencing 10% deterioration in ambulatory function over the next 12 months [80].

A strong correlation was shown between the 6MWT and the global Pediatric Outcome Data Collection Instrument (PODCI) a health-related quality of life measure of functional ability. Moreover, it was shown that at lower level of function, even smaller increases in 6MWD result in meaningful change in quality of life instrument scores [37]. The rate of decline on the NSAA and its predictive value on subsequent loss of ambulation has been established from a large database (UK NorthStar network) [5]. A minimal important difference (MID) for the NSAA has been calculated between DMD boys on daily vs intermittent steroids, as

well with a distribution based method and has been demonstrated to be comprised between 7 and 9 points on the transformed NSAA scale [45].

4.2 Patient Reported Outcomes (PROs) in DMD

The importance of the patients' perspective in establishing clinically meaningful outcomes is widely recognized. Tools assessing this in the context of clinical trials should be disease specific and should capture aspects of functioning and quality of life that are important specifically to DMD patients. Moreover, these tools should be constructed and validated according to the modern standards of clinimetrics in order to be applicable in clinical trials.

While quality of life is of uttermost clinical relevance, measuring quality of life in the context of clinical trials in DMD carries specific issues. Quality of life is a multidimensional concept that is not linked only to the presence or absence of disease or level of functionality. Further research is needed to understand the factors that impact on the relationship between functionality and measurable changes in quality of life.

A number of PROs with existing longitudinal data have been used or are being evaluated in DMD and efforts are ongoing to create DMD specific and robust PROs.

- **The Pediatric Outcome Data Collection Instrument** (**PODCI**), is a patient self-reported or parent-proxy reported health related quality of life measure, which has several domains that measure functional ability. The PODCI has shown strong correlation with the 6MWT in boys with DMD. Additional data are collected to assess responsiveness over time [37]
- **Pediatric Quality of Life inventory (PedsQL)** has a generic core scale assessing physical, emotional, social aspects, school functioning, and specific modules for children with neuromuscular disorders and DMD, focusing mainly on aspects related to physical functioning. A weak responsiveness over time has been observed in DMD, which may hamper its use for registration studies.
- The **NeuroQol** contains an extensive set of measures that are validated for neurological disorders. http://www.neuroqol.org/Pages/default.aspx. The **pediatric Neuro-QOL** was designed to include both generic and targeted item banks, including multidimensional aspects of quality of life, such as anxiety, depression, general concerns, as well as more targeted aspects of upper and lower limb function and activity of daily living. Studies to evaluate its clinical validity and suitability for disease specific clinical trials are underway.
- **DMD-UL PROM**: This DMD specific PROM for upper limb assessment has been created to evaluate the functional ability to manage daily activities requiring use of the upper limbs in DMD. The clinical relevance of items has been guaranteed by the ongoing input from patients and their families. This tool is in further development according to the current insight in clinimetrics (Rash analysis and item response theory) in order to provide a robust ordinal scale. This UL-PROM has been developed in parallel with the DMD specific observer-based outcome measure PUL, which evaluates upper limb and manual performance and has been validated for DMD.

4.3 Biomarkers

There has been important progress in the last few years in defining biomarkers for DMD [95]. Generally, in addition to diagnosing a disease (i.e. elevated CK levels for muscle damage), biomarkers may be used for monitoring of disease progression, regression, or therapeutic responses. While outcomes focused on clinical parameters, such as measuring muscle function, remain essential in the evaluation of experimental therapies for DMD, they have some limitations including slow response time.

By contrast, molecular biomarkers may show earlier response to treatment and reflect the different pathophysiological aspects of the disease. A further advantage is that biomarkers are likely not to be biased by placebo-like effect or individual expectations from patients, family and clinicians, providing a reliable index of patient state.
Biomarkers can also serve for patient stratification and selection of appropriate subjects for clinical trials. The European Neuromuscular Centre recently hosted a workshop focused on biomarker development for DMD [9].
Regarding therapeutic biomarkers, these are divided into pharmacokinetic, pharmacodynamics and prognostic surrogate endpoints for therapy monitoring - both for safety and efficacy. Considering the scope of this workshop, consideration will be given to two broad categories of therapeutic biomarkers; those found in body fluids (serum/ plasma/urine); and those found in skeletal muscle.

Regarding the first category, in the last few years there have been several examples of serum or plasma biomarkers including protein and miRNAs which it has been suggested to correlate with clinical severity and, at least in animal models, response to therapeutic intervention. Plasma CK, whilst useful for diagnosis has less value as therapeutic biomarker, its levels being dynamically influenced by muscle activity and muscle mass with great inter-individual variability and intra-individual fluctuations. There is generally a trend for lower plasma CK levels in older patients due to loss of muscle mass. Specific examples are represented by serum or urine protein and protein fragments including matrix metallopeptidase 9 (MMP9)[96], fibronectin [97], carbonic anhydrase III [98] and muscle protein fragments in serum and urine, such as titin [99]. Regarding miRNAs, these can be detected in serum and several studies have identified their significant dysregulation in DMD boys. Some correlation between these and disease severity has been demonstrated, although the data are not completely concordant [100, 101]. These biomarkers are of interest as they are derived from pathophysiological processes which characterise the dystrophic muscle (for example: muscle necrosis; muscle regeneration; fibrosis; inflammatory response), although at this point in time they should still be considered as exploratory because there is little no demonstration of their behaviour following successful therapeutic intervention in DMD boys.

In terms of therapeutic biomarkers found in muscle, dystrophin protein quantification and its mRNA expression represent a well-known example in DMD. As described above, the lack of dystrophin leads to the progressive muscle degeneration that characterises DMD; while the presence of residual dystrophin expression, even at very low levels, is associated with a slower disease progression (e.g. in BMD but also “milder” DMD).

Measuring dystrophin protein production is therefore an obvious therapeutic biomarker, in trials focused on its re-expression. At the same time, its determination is not straightforward for a variety of reasons. Firstly its large molecular size and the severity of the fibrotic disease process (which differs from the mouse muscle) makes the assessment and isolation challenging; secondly there is variability of muscle pathology even within the same muscle, hence the determination of its pattern of expression in a given muscle portion of a given muscle in a given patient may not necessarily be representative of every single muscle in the same patient. This process can be studied systematically in animal models which do indeed well-replicate the human findings. In addition, different therapeutic strategies will aim at producing “quasi dystrophins” (i.e. not entirely normal dystrophins), whose efficacy can differ from the wild type dystrophin. Quasi-dystrophin will also be typically reintroduced postnatally, in a partially compromised muscle. Finally, while it is known that low levels of dystrophin since birth are associated with a slower disease progression, in DMD individuals dystrophin expression will only be restored at the time of intervention, when a certain amount of muscle damage will already have accumulated. It is anticipated that the muscle quality at time of intervention influences the therapeutic effect. Currently, insufficient data is available to assess a correlation between dystrophin levels and muscle function for various stages of disease.

Furthermore, the freezing, cutting, transporting and processing of muscle is a laborious process that, while mastered by many regional pathology centres worldwide, has not been necessarily replicated with success by several multicentric therapeutic trials in the DMD field, in which large number of muscle biopsies have been obtained but not stored or processed adequately, leading to loss of precious material and frustration by the patient and investigator community.

All the issues above need to be kept clearly in mind when attempting to utilise dystrophin as a therapeutic biomarker. Additional challenges are the lack of a protein standard for the absolute quantification of dystrophin. Notably, multiple methods need to be utilized in parallel to capture different and relevant aspects of dystrophin production

- Western blot or mass spectrometry for quantification, but at the expense of relatively limited sensitivity and lack of information on its localization
- Quantitative immunocytochemistry to acquire information about localization and relative quantification. However, this method is not necessarily linear across the entire spectrum of protein expression from DMD to normal muscle.

Recent efforts of an international working group have been able to demonstrate that, by utilising a carefully devised standard operating procedure, and sharing (in a blinded fashion) the same material, it is possible to stratify patients with different levels of dystrophin protein production very accurately (including the extremely low levels of trace production present in DMD boys), with a good intra- and inter-laboratory reliability and a good correlation between western blot and immunocytochemistry [3]. These techniques as well as further improvements to increase the size of the sample that is analysed in an unbiased way, appear therefore fit for purpose as a pharmacodynamic biomarker of therapeutic intervention. The ongoing clinical trials (assuming they show clinical benefit) will eventually inform us on whether these dystrophin detection techniques will also be fit for providing a surrogate endpoint for clinical trials. This would facilitate the development of AONs for rare exons.

4.4 Using MRI and MRS in DMD clinical trials

Diagnosis and therapy development for DMD and other neuromuscular conditions has rapidly expanded in recent years and there has been an urgent need to develop objective, non-invasive outcome measures to monitor disease progression and treatment effect. The limited number of sensitive OMs and the need to avoid invasive techniques in a population with compromised muscle function makes Magnetic Resonance Imaging (MRI) and Spectroscopy (MRS) techniques particularly attractive as a tool for patient assessment. MRI and MRS techniques applied to muscle could potentially quantify aspects of disease progression and pathology and thereby contribute to the time- and cost-effective evaluation of therapeutic interventions. The benefit of this concept is reflected in increased interest in quantitative MR from the pharmaceutical industry and recommendations from regulatory bodies to include these techniques in trial protocols. One important aspect of why MRI could potentially replace physical assessments as a primary or secondary outcome measure, is its sensitivity to pathological changes compared to many standard motor function measures. This may enable trial results to be achieved more quickly. The DMD field is now at a stage where international consensus has to enable recommendations on the use of MRI to be provided to regulatory bodies, industry and academia regarding protocol optimisation for robust multi-centric trial results.
Despite their promise, several factors had prevented MR techniques from reaching their full potential in DMD previously. There was a lack of understanding of the added value of muscle imaging in DMD and other neuromuscular conditions because most centres were lacking the required expertise, and MR techniques were seen as complex and expensive. Together with the variations in platforms and protocols used in different centres that made cross-compatibility of results difficult to achieve, these shortcomings meant that on previous occasions MRI data were not discussed [1].

While the concept that quantitative MR protocols have much to offer as outcome measures in DMD clinical trials is now well accepted, the development of robust protocols validated on the numerous different MR-platforms (e.g. for Dixon acquisition, for quantitative evaluation of fat infiltration and for T2 acquisition for quantitative evaluation of muscle edema/inflammation) has only been achieved over the past few years.
The most relevant imaging methods that are currently used are based on:

- Quantitative mapping of muscle replacement by fat
- Muscle T2 mapping
- P31 NMR spectroscopy

Overcoming the main hurdles to the rollout of MR techniques (validating protocols across platforms and sites, sharing expertise so that staff can run protocols reliably) has now led to the use of MRI and MRS as a quantitative, and in most cases exploratory, outcome measure in a number of ongoing natural history studies and interventional trials. Specialised protocols that have enabled the use of these techniques to quantitatively assess treatment effect earlier and with greater sensitivity than other measures have been developed and tested independently across neuromuscular centers around the world and the first promising results have now been published [102-106].

In particular the ImagingDMD consortium in the US, led by Krista Vandenborne and Lee Sweeny and supported by various patient organisations and the NIH, has been able to collect longitudinal data in a large cohort of DMD patients and was able to show that:

- MR measures of T2 and lipid fraction show excellent sensitivity to DMD disease pathologies and potential therapeutic interventions in DMD, even in younger boys
- MRI measurement of muscle T2 in boys with DMD is sensitive to disease progression and shows promise as a clinical outcome measure
- MRI/MRS is able to detect therapeutic effects of corticosteroids in reducing inflammatory processes in skeletal muscles of boys with DMD

Based on collected imaging data it is now important to determine which parameters are the most sensitive indicators of disease progression and of a positive response to treatment, how they correlate with clinical changes over a certain time period and with outcomes from physical assessments like the 6MWD and blood, serum and urine biomarkers that are currently under investigation. Linking positive findings of clinical trials to novel biomarkers such as those derived from omics technologies and MRI/MRS should both reduce the future need for invasive monitoring, and also allow investigators to assess response in smaller cohorts of DMD patients, which will be essential when experimental approaches such as exon skipping move to rarer exons for which large studies will never be feasible.

Imaging data that have been obtained in natural history studies and clinical trials are now being correlated with clinical outcome measures and other biomarkers that have been suggested for DMD or are currently under investigation[107].

4.5 Trial design and extrapolation

The challenges of trial design and the extrapolation of data from a defined DMD patient group to the entire spectrum of the disease have been discussed at a recent TREAT-NMD workshop in London[84]. It was noted that despite the fact that DMD is one of the most common genetic diseases, patient numbers are limited and mutation- or stage-specific inclusion criteria for clinical trials further narrow down the number of eligible patients. The accomplishment of multi-centric international trials is also hindered by the lack of clinical trial expertise in many sites that look after DMD patients and by the fact that there is variety in how the care standards are implemented across various sites. In addition, given the relatively slow, progressive nature of DMD, pivotal studies may need to be extended over more years to confirm therapeutic value and reduce adverse events. However, extending trials up to 3 or 5 years would imply delaying access to potential therapies for a disease with a clear unmet medical need. Because of these limitations, randomised controlled phase III trials will always be challenging in DMD. There is therefore a need for flexible trial design, which takes into account the rapidly evolving field and emerging data. In principle, regulators agree that long-term monitoring would potentially provide the information that is required before market authorisation could be granted and time for monitoring, e.g. through surveillance registries, may therefore need to be accounted for in clinical trial protocols and even after marketing authorization.

With a decreasing patient population available for new studies due to competing ongoing trials, it is necessary to look at alternative study designs. These would include smaller studies and adaptive seamless phase II/III studies with short and long-term follow-up as well as extrapolation of data from other studies. A challenge with trial design in small patient populations is the use of small placebo controlled groups and data from contemporaneous controls, who have received similar standards of care as those subjects in the trial. However, due to the heterogeneity in the DMD population there is variability across the various studies to date. There are currently a number of natural history studies ongoing. It is hoped that data from these studies could be collated and used as reference information for future studies, particularly those where the study population would be too small for a properly powered placebo-controlled trial.

The development of new therapeutic candidates also requires that trials consider other stages of the disease, such as the non-ambulant and very young DMD patients (<5 years). These populations bring additional challenges to designing pivotal trials based on the reduced amount of muscle tissue in the non-ambulant patients and regulatory and ethical concerns in recruiting very young patients into trials without the availability of sufficient safety data in this population. Natural history studies are ongoing in both young and non-ambulant DMD patients, but more data are needed, and new outcome measures along with validated biomarkers, are required to address the needs of these populations. Because of the change in muscle mass and muscle quality over the course of the disease, extrapolation of data from a group of 6-12 year old ambulant DMD patients to younger or older boys and stages of the disease might not be adequate.

Extrapolating to young patients is an important consideration as the ultimate goal of many therapeutic approaches is dystrophin restoration, which relies on the muscle quality at treatment initiation. As such, restoring dystrophin in the very young, where muscle quality is still relatively good, would be expected to give the best opportunity to prevent muscle damage and slow down disease progression. However, there are some feasibility issues with conducting trials in young children due to compliance with outcome measures and the age at diagnosis. In addition, there can be confounding issues, such as the age at which corticosteroids are started and the potential impact of variable delayed psychomotor development on an outcome measure.

Extrapolating to non-ambulant patients opens up another set of issues. Maintenance of upper limb function, cardiac and respiratory function is important for all patients, but a primary concern for non-ambulant patients. For this patient population outcome measures are in development using DMD specific assessment tools for upper limb function and patient-reported outcome measures. The use of multiple assessment tools will be essential in this population as the individual response to treatment will vary due to the heterogeneity of affected muscles and it is anticipated that an observable treatment effect on muscle function will take longer than in younger patients. Notably, there is evidence that suggests that certain mutations lead to a slightly different disease progression, implying that when a potential treatment is tested in patients with a certain mutation, one should probably not use natural history data from patients with another DMD mutation as a reference. Therefore, personal natural history data may help to show an effect once the patient is recruited into a trial.

4.6 Patient perspectives on drug development for DMD

Having drugs in the pipelines for DMD that could possibly slow down the progression of the disease brings high hopes to the DMD community. For caregivers and patients the greatest concern is not the total lifespan, but losing functions. Halting or slowing down the progression of the disease is highest on their wish-list.

There are critical unmet medical needs across the entire spectrum of the disease — at all ages, stages and in each sub-population of DMD — that must be addressed with greater creativity and coordination on the part of regulators, sponsors, and researchers with more meaningful engagement of patients and caregivers.

4.6.1 Clinical trials

The DMD community wants trials that are inclusive of people with DMD of all ages across the spectrum of disease — to whatever degree possible. It is up to the sponsor to determine how best to do this, whilst bringing its product to market efficiently. This could help assure a broad label for products if they receive approval.

There is widespread support in the community to move away from placebo-controls or to use trial designs that minimize exposure to placebo. It is hard for patients and families to accept being placed on placebo, knowing that every day, a child with DMD loses muscle cells and functions.

4.6.2 Clinical benefit

In a degenerative disease with progressive loss of functions, eventually leading to death, any slowing down or stopping the progression of the disease is considered meaningful to patients as this would preserve their abilities and delay the next loss of function. Major efforts have been made to improve our insights in the outcome measures used in clinical trials for DMD and to link these measurements to a clinical meaningful outcome.

In non-ambulant DMD patients, preservation of upper limb function is key to participation and quality of life. The Performance of Upper-Limb (PUL) assessment has been developed with input from patients and their families throughout the whole process to establish the clinical meaningfulness and relevance of the PUL items to activities of daily living [6]. In addition, a patient reported outcome measure (PROM) is in development to link measured performance (PUL) to meaningful activities. Longitudinal data are emerging, establishing the rate of decline in upper limb function as measured with these tools.

5. Patient and regulatory perspectives

5.1 Patient perspective to benefit risk

The community wants safe and effective treatments. In a progressive disease like DMD doing nothing can be considered a fatal risk and the disease itself comes with a high burden. Ultimately 'clinically meaningful to the patient' is one of the most important criteria in regulatory discussions. So it is of utmost importance to know what patients and their caregivers consider as benefit and how they balance this against potential risks. Consideration of their opinion in the whole process of drug development and approval is essential. Changes and improvements considered small in the eyes of healthy individuals can be considered as a huge benefit in the eyes of the patients. Each day without treatment brings a person with DMD a step closer to losing essential functions and to death. Because of this, the community has expressed a willingness to accept a certain degree of uncertainty regarding both benefit and risk [106]. Some in the community may be willing to take an even greater risk — on account of accelerated rates of progression, or their proximity to loss of a vital function or death.

5.2. The regulatory perspective

The assessment of the benefits and risks in the context of a new drug application is a central element of the scientific evaluation for a marketing authorisation application. The assessment must reach, as objectively as possible, a sufficient level of confidence that a set level of quality, efficacy and safety of the new medicinal product has been demonstrated. This requires evaluation of all relevant data as well as the use of judgement and arguments. Balancing benefits with risks is complex, as it involves levels of uncertainty (in some cases the limited and sometimes conflicting data make it difficult to estimate probability of desirable and undesirable effects, effect size, etc), multiple objectives (maximising benefits and minimising risks), differences in perspectives (patient, societal and regulatory), ill-defined preferences and utilities of outcomes and the difficulty in trading off effects of differential importance. The lack of agreement on what evaluation criteria to use, and the heterogeneity of effects across patient populations, further complicate benefit-risk assessment.

The assessment of the benefit-risk balance should be based on the available data obtained in tests and trials, which are designed to determine the efficacy and safety of the product under normal conditions of use, and are generally performed under ideal conditions.

The EU legislation states that the marketing authorisation shall be refused “if the benefit-risk balance is not considered to be favourable or if therapeutic efficacy is insufficiently substantiated”. Under the present law, in the interest of public health, authorisation decisions under the centralised procedure should be taken only on the basis of the objective scientific criteria of quality, safety and efficacy of the medicinal product. In practice, this means that economic and other considerations such as “cost-effectiveness” are not included when these decisions are made.

It is important to be explicit about the perspectives of different stakeholders that are taken into account in the assessment of the benefit-risk balance, in particular the perspectives of patients and treating physicians.

6. Summary

DMD is a chronic, complex multisystem disease, with unmet therapeutic needs and a global patient community. Over the past few years, clinical research on outcome measures, national and multinational natural history studies, collaborations on patient registries, and interventional studies coordinated by academia and industry all contributed to an increase in our knowledge on DMD and the collection of large datasets relevant for the planning of future clinical trials. The clinical research efforts improved our understanding of the clinical variability in DMD, the heterogeneity of muscle biopsy findings, the relevance of serum biomarkers and the views and needs of patients and families. Therapeutic strategies have recently started to change from symptomatic treatment approaches to disease modifying interventions developed and supported by pharmaceutical companies. Despite the fact that so far no drug has received full marketing approval for DMD, the results from the clinical research efforts over the past few years form a very good foundation to inform new trials and drug development programmes. The neuromuscular stakeholder community and especially patients and families encourage the regulators to publish guidelines on the clinical investigation of medicinal products for the treatment of DMD based on our improved knowledge. This should help to provide a helpful reference for the planning and the design of future studies and will hopefully accelerate the elaboration of more effective therapies for patients with DMD.

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