KU Leuven
Biomedical Sciences Group
Faculty of Medicine
Department of Development, Reproduction and Regeneration

THERAPY DEVELOPMENT AND CLINICAL OUTCOME MEASURES FOR DUCHENNE MUSCULAR DYSTROPHY

Nathalie GOEMANS

Promotor:

Professor. G. Buyse, MD, PhD

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LIST OF ABBREVIATIONS

2-OMePS	2-O*methyl –phosphorothioate
ATP	adenosine triphosphate
AE	adverse events
ALT	alanine aminotransferase
ACE-I	angiotensin-converting-enzyme inhibitors
AFO	ankle-foot orthose
AON	antisense oligonucleotide
AUC	area under the curve
AST	aspartate aminotransferase
BMD	Becker muscular dystrophy
CK	creatine kinase
DMD	Duchenne muscular dystrophy
ECG	elecrocardiogram
FVC	forced vital capacity
GSK	GlaxoSmithKlein
ICF	international classification for Functioning, Disability and Health
MRI	magnetic resonance imaging
MCID	minimal clinical important differences
nNOS	neuronal nitric oxide synthase
PODCI	Pediatric Outcomes Data Collection Instrument
PMO	phosphorodiamidate morpholino
QoL	quality of life
RNA	ribonucleic acid
SAE	serious adverse events
SC	subcutaneous
MWD	six minute walk distance
MWT	six minute walk test
SD	standard deviation
XLMC	X-linked dilated cardiomyopathy
2-OMePS	2-O*methyl –phosphorothioate
6MWD	six minute walk distance
6MWT	six minute walk test

ABSTRACT

Duchenne muscular dystrophy (DMD) is a lethal inherited neuromuscular disorder caused by mutations in the dystrophin gene and subsequent absence of the dystrophin protein, for which so far no causative treatment is available. We have investigated the therapeutic potential of a oligonucleotide-based splicing modulation approach, aiming to restore the reading frame by excluding dystrophin exon 51 from the splicing machinery in a suitable genetic subset of DMD subjects. This approach is hypothesized to restore the production of a truncated but partially functional dystrophin protein. The systemic administration of PRO051/drisapersen, a 2-O*methyl-phosphorothioate oligoribonucleotide, in a small uncontrolled phase I/IIa study with DMD patients carrying amenable mutations showed molecular efficacy in a dose dependent manner and was generally well tolerated, although potential renal effects, thrombocytopenia and local injection site reactions warrant further monitoring. The systemic administration of drisapersen indicated functional improvements after 12 weeks of treatment , which were sustained at 141 weeks, as observed in the subsequent long-term open label extension study.

We further generated new insights in the 6 minutes walking test (6MWT), a global measure of function, which is currently used as the primary endpoint in clinical trials in ambulant DMD. We demonstrated test-retest reliability in typically developing young boys, generated normative data for this gender in the age range 5-12 years, established correlations with anthropometric and myometric parameters and developed a optimized prediction equation. We further contributed to the understanding of the modified natural history of DMD in the context of corticosteroid treatment and provided insights in the 6MWT in DMD. These data contribute to optimize the use of the 6MWT as a disease measure and endpoint in clinical trials for ambulant DMD.

CHAPTER I

INTRODUCTION AND BACKGROUND

A. DUCHENNE MUSCULAR DYSTROPHY

Dystrophinopathies form a group of X linked recessively inherited degenerative muscle diseases caused by mutations in the dystrophin (DMD) gene (locus Xp 21.1) which encodes for the dystrophin protein [1]. This subsarcolemmal protein links the cytoskeleton to the extracellular matrix and plays a critical role in muscle membrane integrity and prevention of contraction-induced cell membrane damage. Its absence induces a pathophysiological cascade of events resulting in irreversible necrosis and fibrosis of the muscle fibers. The most common and devastating type is Duchenne muscular dystrophy (DMD;OMIM320 200), with an incidence worldwide of 1 in 3500 to 1 in 6.000 male live birth [2,3].

1. Clinical aspects and natural course of the disease

DMD is a progressive and lethal myopathy with onset in early childhood, characterized by a predictable clinical course, affecting primordially striated and cardiac muscles (*Figure 1)*. Affected boys may present with delayed motor development at an early age followed by a progressive weakness of the skeletal muscles during childhood. This muscle weakness may be overlooked at an early age, as the motor delay may be considered as part of a more global developmental delay, which can be the presenting symptom and reason for referral. Indeed, the presence of a cognitive impairment is well described in a proportion of patients with DMD, with 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 [4-6]. Overall mean IQ (82) is approximately one standard deviation (SD) below the normal population [7]. The central nervous system involvement is not progressive, exhibiting a specific pattern with an early generalized developmental deficit that may evolve to a more specific language impairment in older DMD boys and adolescents.

Young DMD may come to clinical attention by the incidental finding of high creatine kinase (CK) leaking in the blood circulation due to the damaged striated muscle membrane, or by the finding of an associated rise in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) from muscular origin. Raised transaminases are often mistaken for an underlying hepatic disease by uninformed clinicians and may lead to unnecessary ancillary hepatic investigations. CK are typically increased to more than 10 times the upper limit of normal, with a maximum value around the age of 1 to 6 years old, followed by a progressive decline due to loss of muscle mass[8].

The first clinical manifestations of muscle weakness include gait abnormalities and difficulties in climbing stairs and rising from the floor, with the typical ‘Gowers’ 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. Affected boys will develop a

typical waddling gait pattern with a tendency to tip toeing due to progressive pelvic girdle weakness and muscle contractures. Muscle pseudohypertrophy may be striking, especially at the level of the calves, however spectacular pseudohypertophic muscle mass may also develop at the level of the shoulder girdle and paraspinal musculature, resulting in a "body builder" appearance. If untreated, loss of ambulation will occur at a mean age of 9 years, with subsequent progressive loss of upper limb function [7]. Full-time wheelchair dependency and axial weakness lead to the development of scoliosis and joint contractures in upper and lower limbs. Progressive weakness of respiratory muscles results in a reduced cough with difficulties in airway clearance and a restrictive pulmonary syndrome that evolves into respiratory insufficiency during the late second or third decade [9,10]. With increasing age DMD patients typically develop a progressive dilated cardiomyopathy, eventually leading to symptomatic congestive heart failure and an increased risk of cardiac arrhythmia and sudden death [11]. Without intervention cardiac involvement and respiratory complications will result in early mortality in second or third decade of life.

Figure 1. The clinical evolution of Duchenne's Muscular Dystrophy

2. Molecular genetic and pathophysiological aspects

DMD is caused by mutations in the dystrophin gene, a 2.4 Mb gene located on the short arm of chromosome X. It is the largest gene described in human beings, the coding sequence however is limited to 0.6 % of the gene (11kb), dispersed over 79 exons, separated by large introns [12]. Gene transcription is driven by different promotors, resulting in dystrophin isoforms named after their predominant site of expression. Three isoforms, Brain (Dp427c), Muscular (Dp427m) and Purkinje (Dp457p) are full length dystrophin, while four internal promotors generate shorter tissue specific isoforms (Retinal Dp260,Brain specific Dp140,Schwann cell Dp116 and General Dp71 *(Figure 2)* [13].

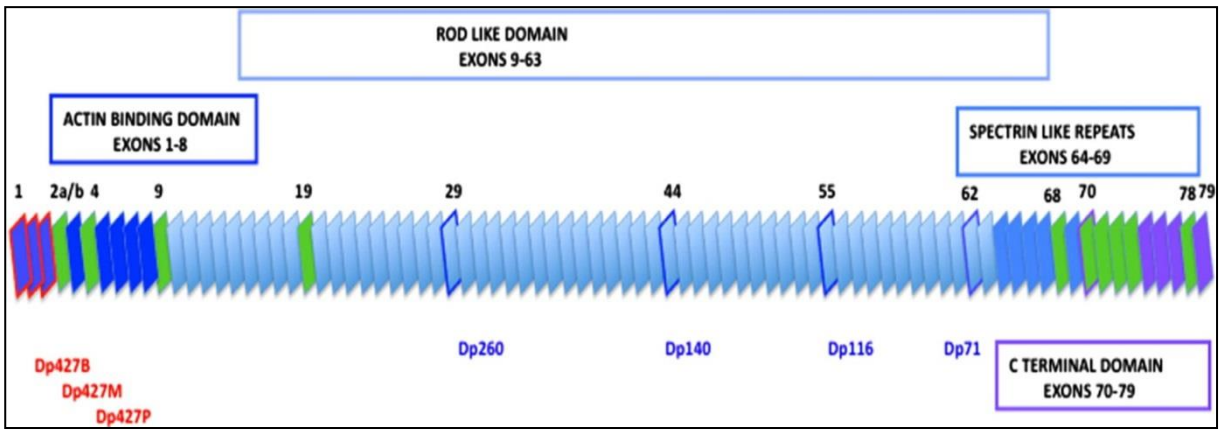

Figure 2. The Dystrophin gene structure and protein domains.
Schematic representation of 79 exons of the dystrophin gene with isoforms and protein domains.
Red lines represent full length promoters and their first exon (Dp427B-M-P). Blue lines represent the promoters and first exons of isoforms Dp260 (retinal), Dp116 (Schwann cell), Dp71 (general)
(with permission of A; Ferlini, Neuromuscular Disorders 2013;23(1):4-14)

The muscle isoform is the best–known protein product of this locus. Muscle dystrophin protein is a subsarcolemmal protein anchoring the cytoskeleton to the plasma membrane and basal lamina. It is a component of the large transmembraneous dystrophin associated protein complex, which forms a structural bridge between the extracellular matrix and the cytoskeleton [1]. Full length dystrophin is a large (427 kDa) rod-shaped protein comprising four domains: (1) an actin binding domain, (2) a central rod domain with spectrin-like triple helical repeats binding amongst other with neuronal nitric oxide synthase (nNOS), (3) a cystein rich domain, which interacts with α dystroglycan, and (4) a C terminal domain that interacts with syntrophin and dystrobrevin [14-16].

The pathophysiologic processes induced by loss of dystrophin protein are complex and not fully elucidated, however dystrophin is considered to contribute to membrane stability as part of the dystrophin-associated

protein complex *(Figure 3)*. The rod-shape structure of the dystrophin protein hints at a protecting role of dystrophin against the repeated contraction-induced mechanical damage to muscle fibres by stabilizing the sarcolemma. Multiple linking domain anchoring with structural and signalling proteins suggest that the role of the dystrophin protein is not limited to a mechanical linking and protecting function but that this protein plays a key role in cell signalling as well [17,18]. *(Figure 3)*

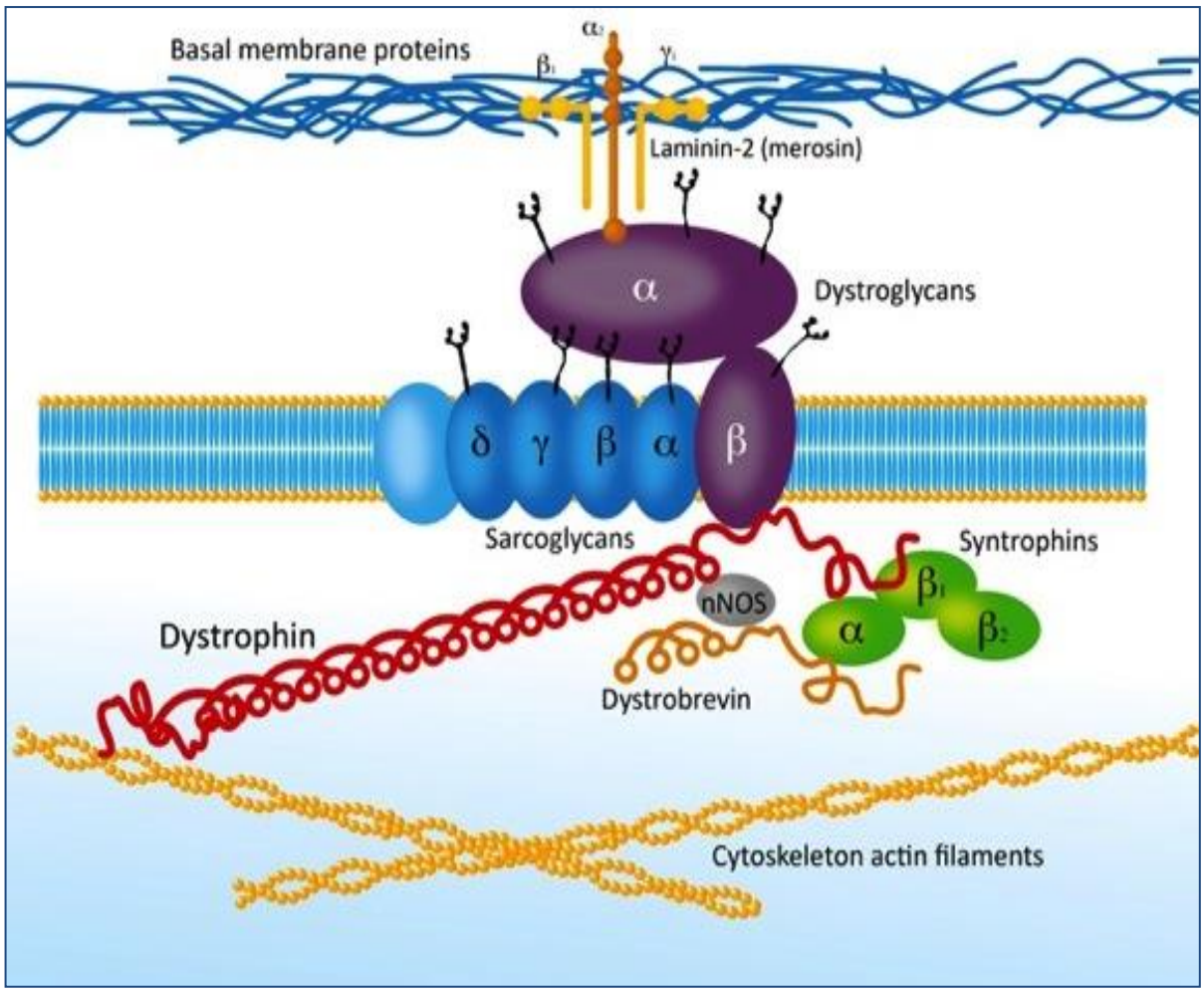

Figure 3. Dystrophin protein: interactions and binding sites with sarcolemmal and cytoskeletal protein. Dystrophin is part of a mechanical link between intracellular contractile structure and extra cellular matrix through the β-dystroglycan- α– dystroglycan,-α 2 laminin axis

Absence of the dystrophin protein will lead to membrane leakage and increased permeability inducing a pathofysiological cascade of events resulting in muscle necrosis and fibrosis. An increased influx of Calcium through the dystrophin–deficient membrane will result in disrupted Calcium homeostasis and activation of calcium dependent proteases, ultimately inducing cell death. Impaired haemodynamic responses to exercise, due to mislocalisation of nNOS, will cause additional cell damage. Ultimately, chronic inflammatory responses and extra cellular matrix gene upregulation will result in an end-stage necrosis and fibrosis of the muscle [19].

Intragenic deletions and duplications of one or more exons account for respectively 72 % and 7 % of the reported mutations, the remaining approximately 20 % being microdeletions, point mutations and translocations [20]. Most mutations cluster around the "hot spot" regions exons 45-55 and exons 2-19.

The phenotype resulting from a mutation is not related to the size of the deletion but will depend on the ensuing effect of the mutation on the translation according to the reading frame hypothesis [21]. Mutations in the DMD gene disrupting the transcriptional open reading frame will lead to prematurely aborted dystrophin translation, resulting in the absence of the dystrophin protein at the sarcolemmal membrane. However, mutations that maintain the translational reading frame usually lead to internally deleted but partially functional dystrophin proteins, with preserved N-terminal, cystein rich and C-terminal domains and are associated with the typically much milder Becker muscular dystrophy (BMD) phenotype.[21] (*Figure* 4)

The majority of patients with BMD show slowly progressive symptoms of proximal weakness in adolescence or early adulthood but do preserve ambulation and do not evolve towards fulltime wheelchair dependency. Respiratory function is preserved as well, however cardiac involvement is common, with dilated cardiomyopathy as most common cause of death. Mean age of death is in the mid-60s but life expectancy is likely to improve with improved cardiac care and management (unpublished data from Dutch database, J.J.Verschuuren, LUMC, The Netherlands)

Large in-frame deletions involving almost 50 % of the gene have been described in milder BMD phenotype, indicating that some parts of the central rod domain may be truncated with minimal impact on protein function[13,22]. This reading-frame rule has shown a 88 to 91% predictive accuracy in different cohorts [20 23],

Other clinical phenotypes of dystrophinopathies include intermediate muscular dystrophy (IMD) and pure cardiac X-linked dilated cardiomyopathy (XLCM). Being an X linked recessive inherited disorder, female carriers are generally asymptomatic, however mild to more severe presentations are well described, due to non-random X inactivation of the normal X chromosome [24], with a spectrum of symptoms ranging from mild myalgia and cramps to overt muscle weakness, which can present in a limb girdle pattern and follow a full blown pattern of DMD as well as in a asymmetrical, focal involvement of a limb. An increased incidence of dilated cardiomyopathy (5%) has been reported and regular cardiac surveillance is advised in female carriers [25].

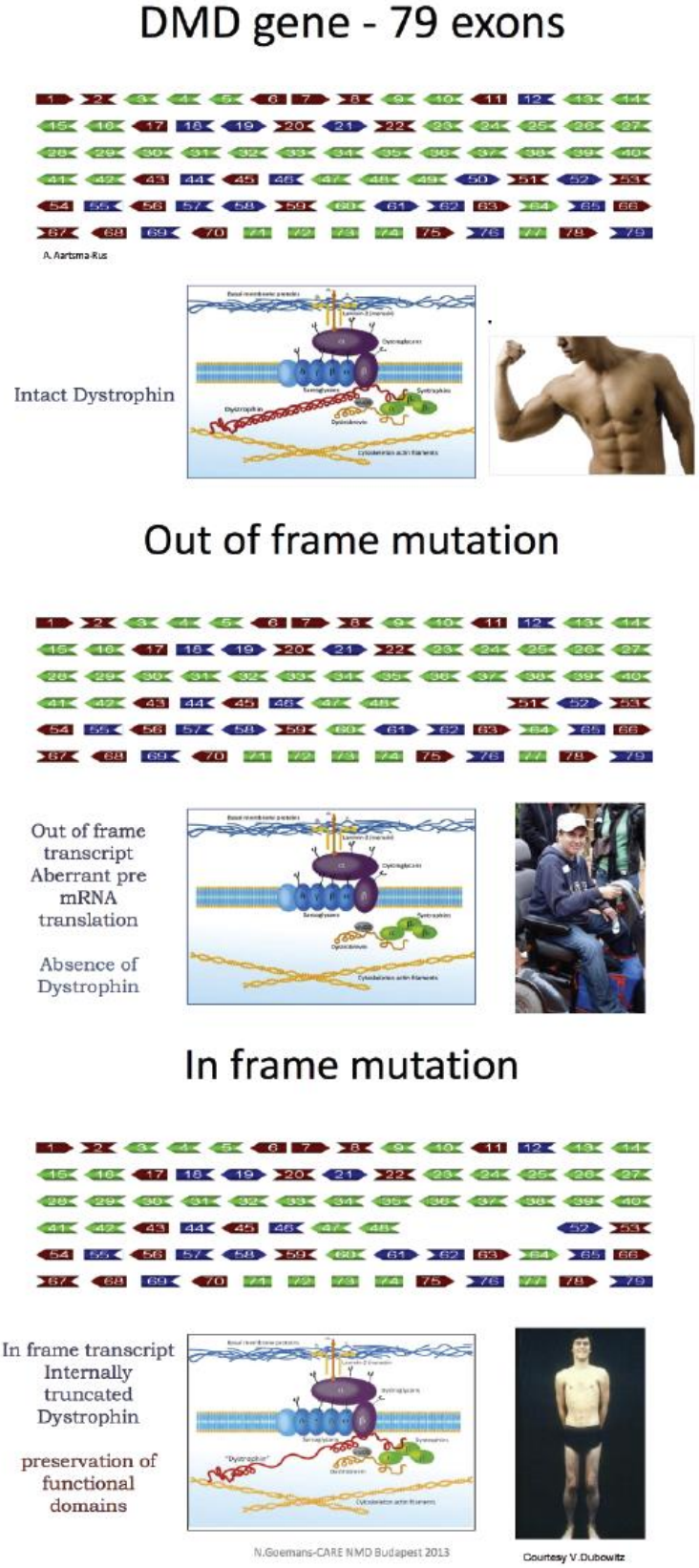

Figure 4. the Reading Frame Hypothesis of DMD and BMD
(DMD gene reading frame illustration adapted from A. Aartsma-Rus)

3. Diagnosing Duchenne muscular dystrophy

A diagnosis of DMD should be suspected in any young boy with a motor or more general developmental delay and/or with clinical signs and symptoms of a proximal muscle weakness. Delay in walking, difficulties with running, hopping, jumping or climbing stairs, a positive Gower's sign or gait abnormalities should alert the clinician and trigger an assessment of creatine kinase levels in serum. Raised CK > 10 x the upper limit of normal will confirm this suspicion and trigger further investigations. The incidental finding of raised CK and/or transaminases in an apparently asymptomatic young boy should raise suspicion as well. The suspicion of DMD should be further investigated by genetic analysis. Quantitative assays of all exons using multiplex ligation –dependent probe amplification (MLPA) will detect whole exon deletions and duplications (approximately 75 % of mutations). This technique characterizes the borders of most rearrangements and can be used for carrier testing of females. If this assay is negative and in the presence of a strong clinical suspicion, further sequencing of the DMD gene is indicated to detect point mutations or small rearrangements[26].

A muscle biopsy with immunohistochemical staining of the dystrophin protein (Dys1,Dys2, Dys3) and of the associated proteins of the transmembraneous complex is advised to confirm the absence of subsarcolemmal dystrophin, or to identify less frequent types of muscular dystrophies, as congenital and limb-girdle muscular dystrophies should be considered in the differential diagnosis in clinical atypical cases. While an additional muscle biopsy is not strictly required in cases where clinical suspicion has been confirmed by genetic diagnosis, it may provide useful additional information by giving an indication of the amount of dystrophin and dystrophin-positive revertant fibres especially in less common mutations where the genotype-phenotype- correlations are not always clear or when the open reading frame rule does not seem to fit the clinical picture. This may be important in view of the different upcoming clinical trials in DMD, targeting different genetic and pathofysiological mechanisms, where information on pre-study diagnostic biopsies is included in the study population criteria. A minimally invasive techniques using a conchotome [27,28] through a small incision in easily accessible muscles such as the musculus tibialis anterior, together with the use of nitrous gas (Kalinox) sedation have proven to be well tolerated in this pediatric DMD population (unpublished personal data).

4. Current management

Despite major advances in insights of pathogenetic mechanisms and the emergence of promising novel therapeutic strategies targeting the causative molecular defect, there is still no cure for DMD. However a multidisciplinary medical, surgical and rehabilitative approach of symptoms, long term corticosteroid treatment and the use of ventilatory support, have altered the natural course of the disease and life expectancy. While DMD has always been considered a pediatric disease, patients with DMD may now expect to live into adulthood [29-31]. Guidelines on coordinated multidisciplinary care have been established, based on the recommendations of the DMD care considerations Working Group [32] and they are disseminated throughout international collaborative networks aiming at improving the care for and the well-being of DMD patients worldwide. Harmonization and implementation of standards of care are a key issue in clinical research in DMD as differences in treatment and management may act as confounding factors in the interpretation of results from multicentric international clinical trials in DMD.

This section summarizes current practice and recommendations in the main areas of medical management of DMD. All management aspects should be embedded in a coordinated multidisciplinary approach taking into consideration the multisystem manifestations and the secondary complications of this chronic disease as well as the side effects of pharmacological interventions such as chronic treatment with glucocorticosteroids.

4a. Treatment with corticosteroids

Chronic corticosteroid treatment, initiated in the ambulant stage of the disease has been established as standard of care [32]. Treatment should include the monitoring, prevention and treatment of side effects associated with the long term use of these compounds 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. The exact mechanism of action is unknown, but probably related to a combination of anti-inflammatory and immunosuppressive properties and interaction with expression of genes involved in protein synthesis and calcium metabolism [33]. Its use has been proven to increase muscle strength and function [34,35], to delay the loss of ambulation and the progression of cardiac and respiratory function [35-40] as well as to prevent the development of severe scoliosis[29,41,42].

Both prednisone/prednisolone and deflazacort, an oxazoline derivative of prednisolone, have been shown effective in increasing muscle strength and delaying disease progression [36,37,43,44]. The superiority of one above the other has not been proven, however uncontrolled studies suggest slightly different side effect profiles for those compounds, such as less weight gain but an increased incidence of cataract for deflazacort [36,45]. A wide range of regimens are currently used across the world among which daily, alternated day or intermittent schedules (10d on 10d off or week-end dosing) are the most commonly used

[46,47]. Daily steroids have been proven more effective to improve strength and function compared to alternate day administration, however alternate day or intermittent schedule have been associated with a milder side effect profile [48-50]. High dose weekend prednisone was shown to be as effective as daily prednisone with a milder side effect profile [51], however the relative short term of the study precludes firm recommendations.

Data on optimal timing to initiate and stop corticosteroids in DMD are lacking underscoring the need to further assess very long term effects and risk versus benefit of chronic use of corticosteroids in the adult DMD population

4b. Respiratory care

Multidisciplinary care has significantly improved life expectancy throughout the past decennia [30]. Respiratory causes of death were the most prevalent in the natural course of the disease due to progressive weakness of respiratory muscles and subsequent respiratory insufficiency and /or inadequate clearance of secretions. Life expectancy has changed considerably since the 1980's with the provision of ventilatory support together with coughing and clearing assisting techniques and devices. A recent retrospective study conducted over the last 30 years in a French center providing invasive ventilation through tracheostomy indicated an increase in mean life expectancy from 25 to 40 years for DMD patients born respectively before and after 1970 [52]. As a consequence, cardiac causes of death shifted from 8% to 44% in this time frame. A similar trend was reported in an Italian retrospective study indicating a modification in the pattern of survival by improved management of cardiorespiratory problems [53].

Guidelines for respiratory care in DMD have been published [54] and include the use of assisted coughing and clearing techniques and the timely instauration of ventilatory support in the presence of hypoventilation, which may start insidiously and first be limited to deep sleep stages, reflecting dystrophic involvement of the diaphragm [55,56]. The use of non-invasive ventilation through a nose or a mouth piece is advocated, however invasive ventilation through tracheostomy may be indicated in individuals where non-invasive techniques fail.

Despite severe physical impairments adult DMD patients on chronic ventilator support indicate a high quality of life. This life satisfaction should be taken into consideration when making therapeutic decisions [57-59].

4c. Cardiac management

Lack of dystrophin will also induce membrane damage and progressive necrotic and fibrotic histologic changes in the cardiac muscle. Cardiac involvement in DMD is characterized by the development of a progressive dilated cardiomyopathy, which will ultimately lead to congestive heart failure, conduction system disease with arrhythmia and early death [52,53]. The current management of cardiac manifestations

includes early detection and follow-up of cardiac abnormalities and the standard symptomatic treatment of cardiac insufficiency and arrhythmias, among which the use of angiotensin-converting-enzyme inhibitors (ACE-I), beta –blockers and diuretics [25,60,61]. While clinical symptoms of cardiomyopathy may emerge only in adolescence or young adulthood, partly due to the physical inactivity associated with the severe skeletal muscle weakness, early regional myocardial damage and myocardiac changes can be detected in young DMD by imaging techniques such as MRI, tissue Doppler measurements, and myocardial velocity gradients [62]. This raises the question whether pharmacological interventions initiated in a pre-symptomatic stage of the disease could be beneficial in preventing the onset of cardiac symptom or in changing the slope of the decline of cardiac function in DMD. Preclinical evidence has been obtained of the cardioprotective effect of ACE-I in Syrian hamsters cardiomyopathy, an experimental model of delta-sarcoglycanopathy phenotypically similar to DMD [63], and of the prophylactic effect of angiotensin II antagonists (losartan) in the *mdx* mice [64]. Encouraging results from limited uncontrolled clinical studies of the use of ACE-I in the prevention of cardiomyopathies in DMD have been reported [65-67].

Current recommendations advocate the initiation of ACE-I at the first signs of decreased cardiac function [68]. However, the lack of long-term prospective randomized controlled studies hampers definite recommendations about the prophylactic use of those compounds. Corticosteroid treatment has been shown beneficial in delaying cardiac symptomatology in DMD, refuting earlier concerns that improving skeletal muscle strength and exercise capacity with corticosteroids could, at the long term, be detrimental to the cardiac muscle by increasing the load to the dystrophic cardiac muscle

4d. Orthopedic management and rehabilitation

The natural course of DMD is characterized by the development of limb muscle contractures and scoliosis. An orthopedic surgeon with experience of patients with DMD should be part of the multidisciplinary team responsible for a regular integrated monitoring, care and management of these patients, as the indication for orthopedic interventions and surgical procedures has to be assessed on an individual base, aiming at optimizing or preserving yet weakened muscle function or at preventing severe deformities such as collapsing scoliosis.

1. Muscle contractures

Progressive retractions of Achilles tendons, knee and hip flexors and iliotibial bands may negatively affect stability and the gait pattern in the ambulant DMD. Preventive measure such as mobilization and stretching are advocated, as well as the use of resting ankle-foot orthoses (AFO's) at night and standing programs to optimize joint positioning. As ambulant DMD patients do need compensatory positions to negotiate ambulation with increasing pelvic weakness, fixed positions during the day and/or surgical intervention for contractures should be prescribed with great cautiousness as they could jeopardize ambulation in weak DMD.

Prolongation of ambulation in knee ankle foot orthoses after release of contractures is an option in DMD patients in the transitional stage, offering the possibility to prolong standing and limited house hold ambulation, facilitating transfers and other activities of daily living. However this should only be prescribed after careful consideration of the individual's abilities and motivation and the facilities to organize intensive rehabilitation postoperatively. This approach requires an optimal coordination between orthopedic surgeon, physiotherapist and orthotics.

In the non-ambulant stage, adequate joint positioning is further indicated to prevent severe joint deformities. Attention should be paid to adequate sitting posture and to the prevention of contractures in upper limb, and hand which could otherwise impair fine motor activities in a later stage.

2. Scoliosis

Treatment with corticosteroids has been effective in preventing the development of spinal deformities in DMD, reducing considerably the occurrence of severe scoliosis requiring surgery [69,70] which reached up to 90 % in the natural course of the disease [71]. Regular clinical and radiological monitoring of the spine is indicated with any incipient scoliosis and in subjects with progressive spinal curve posterior spinal fusion is recommended to prevent further evolution. An anticipatory coordinated approach with surveillance of (pediatric) neurologist, anesthesiologist, cardiologist, pneumologist and physiotherapist with experience in neuromuscular disorders is needed to limit operative risks and to optimize the preoperative care and postoperative recovery and rehabilitation. Data are lacking on the development of scoliosis in the emerging adult generation on very long term corticosteroids. In this population, the issue of bone demineralization and collapsing vertebral fractures due to the chronic use of corticosteroids forms an additional risk to be monitored in the spinal management.

4e. Endocrinological aspects

The beneficial effects of corticosteroids on muscle strength and function are associated with treatment related side effects on multiple organs, raising new issues in the care and management of patients with DMD. Endocrinological aspects in DMD such as bone health, growth and puberty, glucose and fat metabolism are gaining attention. Several reports from international collaborative workshops supported by patients' advocacy groups have highlighted the urgent need for harmonized follow-up and treatment recommendations. So far, guidelines for the management of endocrine aspects are based on expert opinions, clinical experience with corticosteroids in other diseases and limited non-controlled studies, underscoring the need for additional research in these areas [72,73].

1. Bone health

Reduced bone mineralization with hypocalciuria, low 25-OH vitamin D and increased bone turnover markers, is a well-known feature of DMD. This impaired bone metabolism is further negatively affected by

corticosteroid therapy, resulting in osteoporosis and an increased incidence of long bone and vertebral fractures causing significant morbidity [74-76]. Leg fractures can precipitate definite loss of ambulation in weak ambulant DMD and require prompt and adequate treatment aiming at limiting immobilization to a minimum. Guidelines on treatment and management follow the general approach for osteoporosis in children on chronic corticosteroid treatment, including adequate calcium and vitamin D intake, and fracture prevention with safe weight bearing exercises within the limits of the underlying disease. Bifosfonates have been proven useful for the treatment of painful vertebral fractures, however prophylactic use of bifosfonates in the absence of symptoms remain controversial and deserve further research in controlled prospective studies in this population.

2. Growth and puberty

Short stature is a well described feature in the natural history of DMD, with a characteristic growth pattern, starting with a normal height and weight at birth followed by a slowing in growth velocity in the first two years of life and a downward diversion from the percentile curves during childhood and adolescence [77]. Most of the patients will fall within the 5th percentile for height when reaching adulthood, irrespective of any corticosteroids treatment [78].

The long term chronic use of corticosteroids has an additional detrimental effect on growth, resulting in extremely short stature below the 3th percentile in adolescent DMD on steroids. Therapeutic options to improve growth in DMD include growth hormone, IGF-1 and testosterone treatment. None of these therapies have been studied extensively in this population. Growth hormone treatment resulted in improved growth in a small, short term retrospective study in steroid treated DMD, without negative effects on muscle strength and cardiopulmonary function [79]. A randomized controlled study assessing the efficacy of IGF1 in steroid treated DMD is currently ongoing (NCT01207908).

Delayed puberty is an additional endocrinological issue associated with the long term use of corticosteroids due to their suppression of pituitary function. Testosterone is commonly used to induce puberty. In hypogonadal boys this treatment was associated with increased body mass and bone mineral density, as well as reduction of body fat mass, additional effects that could be beneficial to steroid treated DMD. Although the induction of puberty with testosterone in DMD is implemented in clinical practice, based on individualized decision, data on the outcome of treating delayed puberty are lacking in this population.

3. Weight gain, glucose and fat metabolism

Long term treatment with corticosteroids is associated with a predictable increase in appetite, and the induction of excessive weight gain and insulin resistance. This a major concern as even without steroids boys with DMD are at risk to develop overweight from the age of 7 years onwards. Excessive weight gain increases the burden on already weakened muscles and has a negative impact on motor function. Overweight can obscure the benefit gained by corticosteroid treatment and increases the risk of co-

morbidity such as the development of arterial hypertension, glucose intolerance and hyperlipidemia. Inappropriate weight gain and fat accumulation can be influenced by adequate dietary advice. Energy requirements depends on the level of activity, energy expenditure, metabolism and genetic factors. Documented energy recommendations for DMD are not available. Dietary advices on caloric intake should take into consideration the lower physical activity and lower muscle mass in DMD. Particular emphasis should be made on appetite control at the time of corticosteroids initiation

Metformin, an insulin-sensitizing agent, has been shown to reduce weight gain, insulin resistance and visceral adiposity in a short term randomized placebo controlled study in patients with neuromuscular disorders [80]. Further research is needed to assess whether these beneficial effects persist on the long term.

4f. Gastro-intestinal management and nutrition

Dystrophin expression has been identified in the visceral smooth muscles [81] and there is evidence of functional smooth-muscle impairment in Duchenne dystrophy [82] . With increasing age DMD patients typically develop gastro-intestinal symptoms such as delayed gastric emptying and intestinal paresis, requiring preventive measures and pharmacological interventions targeting gastro-esophageal reflux and constipation. As the condition progresses oro-pharangeal weakness with swallowing difficulties and dysphagia may jeopardize safe and adequate intake. The placement of a percutaneous endoscopic gastrostomy tube has to be considered to prevent aspiration and optimize nutritional status. The prescription of long term corticosteroids should be associated with gastric protection with proton pump inhibitors.

4g. Renal and urinary tract management

Despite clear evidence of signs and symptoms of bladder dysfunction in clinical practice, this area has been poorly investigated and deserves further attention to better understand and provide guidelines on monitoring and optimal management of bladder involvement in DMD.

Little is known about renal function in patients with DMD and reports on renal function in DMD most often relate to the occurrence of myoglobinuria. However several dystrophin isoforms have been found in kidney tissue (Dp 71, Dp 40, Dp140), raising the hypothesis of renal dysfunction being an integral part of the multisystemic nature of DMD. The most common dystrophin isoform that is found in the kidney tissue, Dp 71, seems to be localized in the mesangial and endothelial cells, interstitial capillaries and the macula densa. The exact function of the renal dystrophin associated complex is not clarified yet, although its molecular structure suggests multiple functional roles related to ion transport mechanisms and to the mechanical protection of renal epithelial cells for the high osmotic pressure of the hypertonic interstitial fluid [83,84]. Two Japanese studies reported renal dysfunction as a frequent complication in patients with advanced stage DMD [85,86]. Currently, serum creatinine is used as standard marker of renal function and

integrated in equations to assess glomerular filtration rate. In DMD however, the use of this marker is hampered by the low serum and urinary level of creatinine as a consequence of the low muscle mass.
Recently, interest in renal function and renal markers in DMD has emerged from clinical studies with new pharmacological compounds, highlighting the lack of insights in renal function in DMD and the need for robust tools to monitor renal safety of therapeutic interventions.
Further studies are required to investigate the pathogenesis and the long-term prognosis of possible renal disease in DMD, as well as the confounding issue of chronic steroid treatment on renal function.

4h. Ophthalmological management

Regular ophthalmological monitoring is part of the standard care of DMD as patients treated with chronic corticosteroids are at risk of developing cataract, particularly with the use of deflazacort [36]. The role of the retinal dystrophin isoforms Dp260 and Dp71 is not well understood, however, impairments in photopic and scotopic ERG responses have been reported in DMD as well as mild red-green color vision impairment [87-89]. Ophthalmologic manifestations have not been primordial within the spectrum of clinical symptoms of DMD, however this area deserves further attention as the development of DMD specific clinical symptoms of retinal abnormalities could be anticipated with increased longevity.

4i. Dental and maxillofacial issues

The typical distribution of facial and masticatory muscle weakness and hypertrophy together with the occurrence of tong hypertrophy results in a typical dental conformation and maxillofacial appearance, with a risk of dental malocclusion deserving specialized orthodontic management by dental and maxillofacial specialists with experience in neuromuscular disorders.

4j. Exercise and physical activity

Exercising has always been an area of controversy in the management of neuromuscular disorders, fueled by the fear for overwork damage to the weakened muscle. However, in patients suffering a neuromuscular disorder, lack of exercise is well known to induce a 'vicious circle' with deconditioning further impairing the practice of physical exercise and subsequent lean muscle loss. A recent review reports the beneficial effect of a strength training physical program limited to concentric contractions in *mdx mice,* while high resistive eccentric contraction were proven detrimental, by exacerbating the pathophysiological cascade of events in muscle fibres lacking dystrophin. Studies in human are limited and focus on respiratory muscle training reporting that specific training improves respiratory endurance in DMD [90]. One study reports beneficial effects of low-intensity endurance training without detrimental effect on cardiac function or skeletal muscle in subjects with BMD [91]. Further research is needed to define optimal training in neuromuscular disorders and in DMD [92,93].

B. DMD: NEW THERAPEUTIC APPROACHES IN CLINICAL STAGE OF DEVELOPMENT

Molecular genetic advances and new insights in the underlying disease mechanisms have generated new concepts for therapeutic approaches for DMD, some of which are currently being investigated in clinical trials or close to moving into clinical studies. Promising disease-modifying approaches targeting the causative genetic defect include exon skipping and suppression of stop codon mutation. These therapies are mutation specific, which limits their use to subsets of DMD patients with suitable mutations. Other pharmacological approaches, including Utrophin upregulation, muscle building strategies and strategies targeting the pathophysiological mechanisms downstream from dystrophin deficiency are potentially applicable to all DMD patients independent of their individual genotype and/or could have an additional beneficial effect to gene based therapies.

1. Dystrophin restoration therapies

1a. Anti-sense mediated exon skipping strategy

Antisense-mediated exon skipping strategy for DMD is based on the open reading frame rule and uses antisense oligonucleotides (AON) to interfere with transcription and translation. AON's oligomers with sequence complementarity to regions of the pre mRNA transcript, target splicing motifs of the pre-mRNA and hide selected exons from the splicing machinery in order to restore an open reading-frame (*Figure 5*). This would theoretically allow the production of truncated but partially functional dystrophin proteins, associated with typically much milder phenotypes as seen in Becker muscular dystrophy [94,95]. Spontaneous frame-restoring exon skipping is a phenomenon observed in patients with DMD, explaining the occurrence of rare dystrophin positive fibres (revertant fibres) in the biopsies of up to 50% of DMD patients [96-98]. As exon skipping is a naturally occurring event in DMD, the potential immunogenicity of the new protein product and the risk of immune-mediated rejection of this new protein after therapeutic targeted exon skipping could, theoretically, be limited.

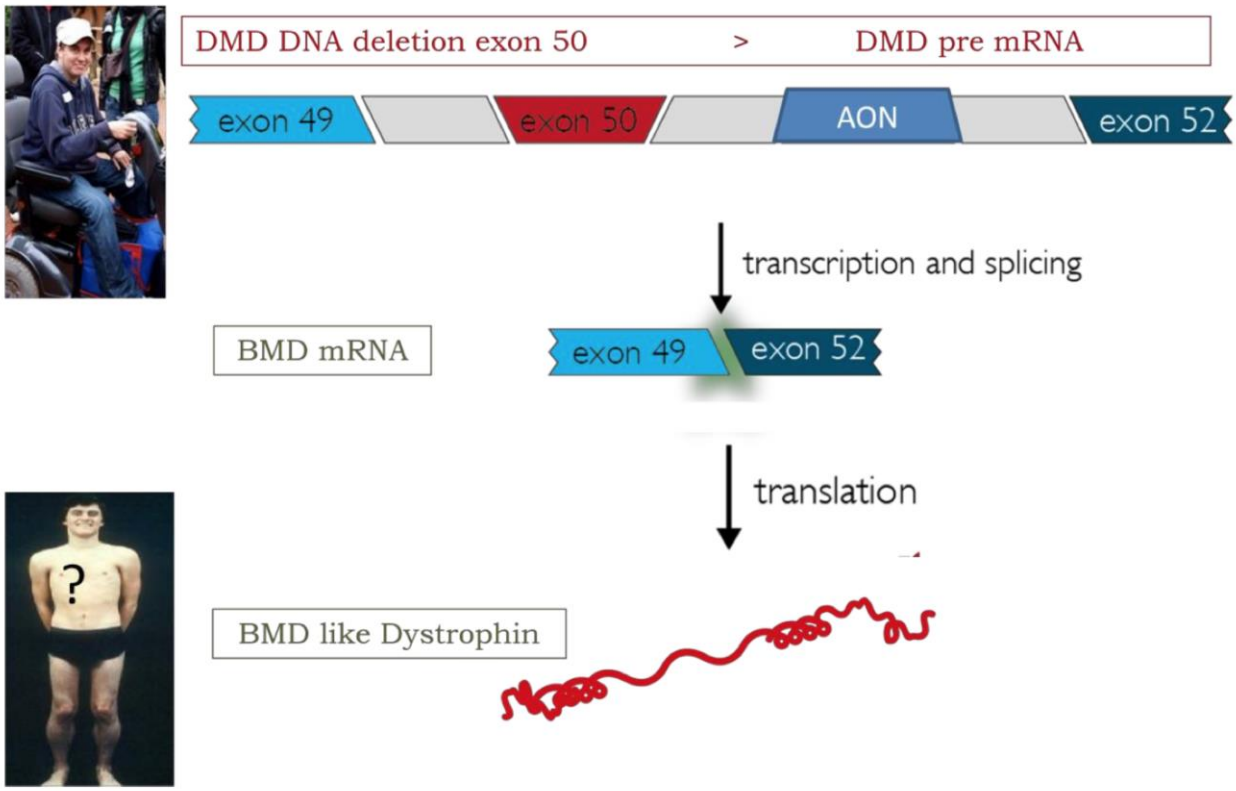

Figure 5. Antisense mediated splicing modulation approach to restore the open reading frame.

AON are sequence-specific single stranded oligonucleotides, chemically modified to resist nucleases, binding to partially open pre-mRNA structures in a target exon and interfering with splicing regulatory factors. Currently two types of antisense oligonucleotides are in clinical development for DMD: 2'O-methyl-ribo-oligonucleoside-phosphorothioate (2'OMe) and phosphorodiamidate morpholino oligomers (PMO), with distinct backbone chemistry and different physicochemical properties. 2'OMe phosphorothiates are negatively charged compounds and bind to plasma proteins which leads to high and prolonged plasma exposure in contrast to the uncharged backbone of the PMO with high renal clearance and low plasma half-life. 2'OMe AON drugs have been extensively studied in the past decade for different disease indications and their toxicology is well characterized in laboratory animals. More than 3000 subjects have been dosed with AON phosphorothioate and class effects are well described, with toxicology profiles that are similar from sequence to sequence as the interaction on the AON with plasma proteins depends on the physicochemical characteristics of the backbone [99].

Figure 6. Structure of 2'O-methyl-ribo-oligonucleoside-phosphorothioate (2'OMePS) and phosphorodiamidate morpholino oligomers (PMO)

Pre-clinical proof of concept with specific exon skipping and restoration of dystrophin expression has been demonstrated with both types of molecules in cell cultures from DMD patients [100] as well as in multiple DMD animal models such as *mdx* mice, *mdx4* mice, *mdx52 mice, hDMD mice* and the cCXMD and GRMD dogs [97,101-107].

Exon skipping is a mutation specific therapeutic approach, however AON targeting a particular exon would theoretically reframe a series of different deletions (table 1) [20,100]. For example, skipping exon 51 would theoretically by applicable to the largest genetic subset of all DMD patients, correcting approximately 1/5th of all DMD deletions (13% of all mutations), explaining why the (clinical) research on AON mediated exon skipping in human DMD has first targeted this exon.

Preceding this doctoral work, we did contribute to the first proof of concept of this approach in a first ever human study, indicating exon 51 skipping and local expression of novel sarcolemmal dystrophin protein in myofibres after a IM injection of PRO 051, a 2 OMe AON targeting exon 51 (Prosensa B.V, later in-licensed by GSK and renamed drisapersen). Four patients with suitable mutations received a single dose of 0.8 mg of PRO O51 injected into the tibialis anterior muscle. This was well tolerated and not associated with clinically significant adverse events. Muscle biopsies performed at the site of injection 28 days post dosing showed specific skipping of exon 51 and the presence of sarcolemmal dystrophin in 64 to 97% of the myofibres [109].

Such pilot data require confirmation in larger studies and in addition therapeutic applicability of this approach, would require a systemic delivery to all muscles. Based on these promising data further development towards systemic therapeutic application of PRO 051/drisapersen was explored.

The doctoral research work further described in this thesis has been a major part of the development of exon 51 skipping from the pre-clinical stage to the current late stage with phase III studies.

Since the start of this doctoral work a PMO AON inducing exon 51 skipping (Eteplirsen/AVI-4658,Sarepta Therapeutics, Cambridge, MA, USA) has demonstrated successful dystrophin restoration after local

administration and has moved into phase II studies as well. [110,111]. We are currently contributing to the clinical development of AON targeting other exons of the DMD gene, hence expanding the DMD population that might benefit from this approach. Discussion on the current status of exon skipping strategy for DMD is included in the general discussion of this thesis (*Chapter5)*.

EXON	Applicable to deletion of exon(s)	% of deletions	% of mutations
51	13-50,29-50,43-50,45-50,47-50,48-50,49-50,50,52	19.1%	13%
45	12-44,18-44,46-44,46-47,46-48,46-49,46-51,46-53,46-55,46-59,46-60	11.8%	8.1%
53	10-52,42-52,43-52,45-52,47-52,48-52,4-52,50-52,52	11.4%	7.7%
44	3-43,5-43,6-43,10-43,14-43,17-43,22-43,28-43,30-43,33-43,34-43,35-43,36-43,37-43,38-43,40-43,41-43,42-43,45,45-54,45-68	8.8%	6.2%
46	19-45,21-45,43-45,45,47-54,47-56	6.2%	4.3%
52	8-51,51,53,53-54,53-55,53-57,53-59,53-60	5.7%	4.1%
50	51,51-57,51-53,51-55	5.6%	4.0%
43	44,44-46,44-47,44-48,44-49,44-51,44-53	5.3%	3.8%
8	3-7,4-7,5-7,6-7,9-21	2.3%	2.3%
2	3-7	1.3%	1.9%

Table 1: applicability of exon skipping to deletions of dystrophin gene exons according to the Leiden Data Base (adapted from A.AArstma-Rus,2009;Human Mutation) [108]

1b. Suppression of stop codon mutations

Other RNA modulating approaches aiming at restoring dystrophin by interfering with mRNA translation include the use of read through molecules such as PTC124/Ataluren (PTC Therapeutics, South Plainfield, NJ). This small molecule is known to specifically induce ribosomal read-through of premature stop codons caused by non-sense mutations. This approach has been investigated in genetic subsets of patients with other monogenetic diseases such as cystic fibrosis [112] and could theoretically be applicable in approximately 13% of all DMD patients. Following encouraging pre-clinical results in the *mdx* mouse this compound has moved into clinical development. Its safety and efficacy has been investigated in a large international multicentric phase 2 randomized double blind, placebo-controlled study, followed by an open label extensions study (NCT00592553; NCT01557400). This study established the 6MWT, a standardized assessment of ambulation, as new clinical endpoint for DMD. A multicentric international phase III study has been launched recently to assess safety and efficacy of Ataluren in patients with nonsense mutation DMD (NCT01826487).

2. Targeting muscle growth

Non-dystrophin specific therapeutic approaches interfering with mechanisms involved in growth and regeneration of skeletal muscle mass are currently under investigation in neuromuscular disorders, The rational to interfere with the myostatin pathway, a major negative regulator of skeletal muscle mass or with its activin II receptors, is based on the observation of a remarkable increase in muscle mass in naturally occurring myostatin null animals.[113,114]. The safety and efficacy of ACE-031, a recombinant activin receptor inhibitor, was investigated in DMD in a phase IIb trial (NCT01099761), however this study has been interrupted, based on preliminary safety data.

3. Utrophin upregulation

Utophin is a subsarcolemmal protein expressed during fetal development and muscle cell regeneration. The strong homology of utrophin to dystrophin has fueled the research of utrophin upregulation as a potential disease modifying approach for DMD. Utrophin is persistently expressed at the neuromuscular junction in normal mature muscle. The utrophin muscle specific promotor can be manipulated to increase utrophin RNA and expression of full length utrophin has been shown effective to prevent muscular dystrophy in *mdx* mice [115]. The utrophin modulator SMT C1100 (Summit Corporation PLC, Oxford) has successfully increased utrophin expression in human cells and in the *mdx* mice and is now close to moving into clinical development [116].

4. Targeting the pathofysiological cascade

Numerous indirect approaches targeting the pathophysiological cascade of events downstream of the dystrophin deficiency and aiming at reducing the dystrophic process are under investigation in pre-clinical and clinical phases. These include strategies aiming at reducing oxidative stress or at preventing necrosis and fibrosis. This paragraph is limited to the compounds that have moved into phase III clinical studies for DMD.

4a. Reduction of oxidative stress and improvement of respiratory chain function

Idebenone [2-(10-hydroxy-decyl)-5,6-dimethoxy-3-methyl-[1.4]benzoquinone] is a potent antioxidant of the quinone family. It interacts also as an electron carrier in the mitochondrial chain transport and supports adenosine triphosphate production (ATP). Idebenone may hence interfere with the pathophysiological

cascade of events in DMD by improving mitochondrial function and stimulating cell energy production. Presymptomatic initiated and long term treatment with idebenone has shown a cardioprotective effect and improved exercise performance in the *mdx* mouse [117]. In DMD patients, trends in cardiac effects and a significant respiratory treatment effect were observed in a phase IIa double-blind randomized placebo-controlled clinical trial [118,119]. These encouraging results are currently further under investigation in a large international phase III study (DELOS, NCT01027884).

4b. Restoration of NO-mediated hemodynamic responses to exercise

The absence of dystrophin at the sarcolemmal muscle membrane results in a mislocalisation of neuronal and nitric oxide synthase mu (nNOSμ). This enzyme is involved in the post exercise vasodilatation in muscles and its dysfunction results in loss of normal hemodynamic responses with post exertional ischemia of the muscle and subsequent irreversible pathological changes [120].

Tadalafil is a selective, reversible inhibitor of cGMP-specific phosphor diesterase 5. Its application in DMD aims at restoring NO mediated cGMP signaling and skeletal muscle hemodynamic responses to exercise. A Phase 3 double blind randomized controlled study is currently recruiting patients to assess safety and efficacy of Tadalafil in DMD, based on clinical observations of the beneficial effect of Tadalafil on functional muscle ischemia with the restoration of normal blood flow regulation in forearm muscle in BMD [121].

C. CLINICAL OUTCOME MEASURES

The emergence of new therapeutic strategies for DMD moving into clinical development have exposed an urgent need for suitable outcome measures to assess the efficacy of new treatment interventions within the framework of clinical trials.

The international classification for Functioning, Disability and Health (ICF) provides a framework to describe the multifactorial dimensions of human disease. Outcome measures can be identified in all the four categories: Pathology, Functioning, (dis)Ability and Health/Quality of Life (*Figure 6)*. A review of all existing measures is beyond the scope of this manuscript. A registry of all available outcome measures for DMD trials, with their references is available through the TREAT-NMD website (www.researchrom.com)

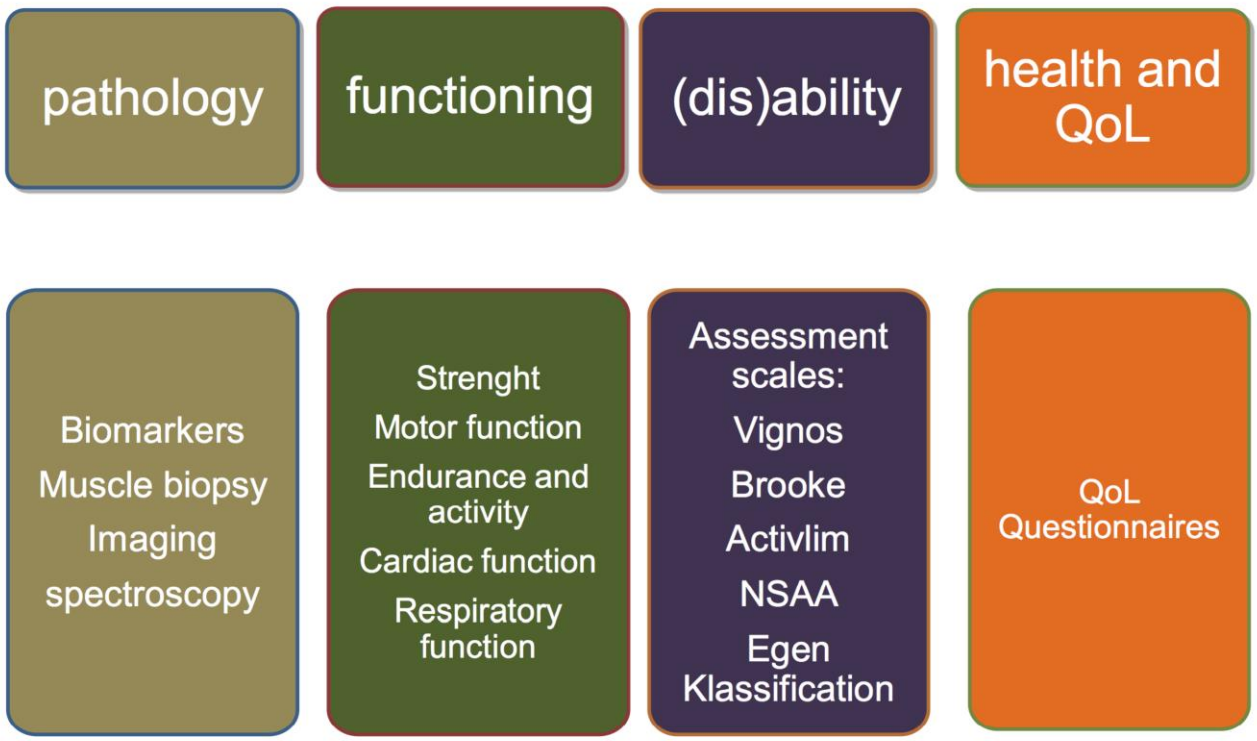

Figure 6. Outcome Measures in DMD according to ICF classification

Clinical trials for progressive and complex childhood orphan diseases such as DMD face specific challenges. Indeed, because of the limited duration and other inherent limitations of human trials, sensitive, reliable and clinical meaningful age and stage specific assessment tools are required to allow efficacy assessments of therapeutic intervention in the natural history of the disease.

Measures of strength and timed motor performances (10 meter walk, ascending 4 stairs and raising from the floor from a supine position (Gower's maneuver)) have been validated and used as surrogate outcome measures to assess disease progression in DMD and as endpoint in previous interventional trials in ambulant DMD [122-124]. However the clinical relevance of short-term assessments measuring peak activities has been questioned and it has been showed that the correlation between muscle strength and function is not linear and differs depending on the stage of the disease [125]. Moreover, outcome measures able to detect clinically meaningful changes in patients' daily life are needed to meet regulator's requirement for approval of new therapeutic compounds for specific diseases. So far, most therapeutic trials in DMD target the ambulatory stage of the disease, aiming at restoring or maintaining muscle function before disease progression results in an irreversible stage of fibrosis of the muscles. This highlights the need for validated tools to assess the ambulatory capacity of DMD subjects.

The 6-minute walk test (6MWT), a measure of function and endurance originating from the cardiorespiratory field, assesses the distance a subject is able to walk in 6 minutes at a normal pace (6-minute walking distance, 6MWD). This measure reflects the physical capability and walking function at a submaximal level and has been accepted as a clinically meaningful outcome measure by the regulatory authorities in registration-directed clinical trials in neuromuscular and neurometabolic disorders[126]. More recently, a modified 6MWT has been described and validated in DMD[127]. Modifications from the original American Thoracic Society guidelines include the use of an orientation video to instruct the children, standardized constant verbal encouragements and a "safety chase" assisting the child with DMD in the event of a fall [127]. This modified 6MWT has been used as primary endpoint in a large international multicentric clinical trial assessing safety and efficacy of PTC 124 in DMD/BMD caused by non-sense mutations (NCT00592553; NCT01557400) and its use is currently advocated as primary endpoint for clinical trials in ambulant DMD. However, at the start of this doctoral work, data on reliability and developmental evolution in young children as well as normative data in the age range of particular interest for clinical trials in ambulant DMD were lacking.

An additional challenge in the design of clinical trials in DMD, especially in trials with a specific gene-based approach, is the limited number of eligible patients available, challenging the use of control arms and fueling the idea of using contemporaneous natural history data as a surrogate control arm. This underscores the need for a better understanding of the contemporary natural disease history and the disease/outcome measures currently used in clinical trials in DMD. Treatment with glucocorticosteroids is currently standard of care and their use should be implemented in clinical trials for DMD. However, the data on the modified course of the disease as measured by the tools currently used in DMD trials are

lacking, and ‘steroid-era’ contemporary dataset are eagerly needed as background against which the efficacy of new compounds can be assessed [128]. At the start of this doctoral project data on the 6MWT in DMD were lacking, especially in larger cohorts treated with a homogenous regimen of corticosteroids according to current standard of care.

Figure 7. The 6 minute walk test:
Method: fixed course, with a length of 25 meters marked on the floor. Participants are instructed to walk back and forth around the cones at each end of the course at their own pace under standardized encouragements

CHAPTER II

RESEARCH AIMS AND STRATEGY

DMD is a devastating neuromuscular disorder for which no causative treatment is available. Insights in the underlying molecular and pathophysiological aspects of the disease have generated new therapeutic approaches some of which have or are entering clinical development. This PhD project has focused on the development of new therapies and on methodological improvements to monitor the efficacy of new therapies for DMD, with the following aims:

(1) To investigate the potential of dystrophin exon 51 skipping as a novel therapeutic approach for DMD

The first part of this project aimed at contributing to the development of the exon skipping strategy as a potential novel therapy for a genetic subgroup of DMD patients, by investigating the first ever systemic application of a AON in DMD. PRO 051 (Prosensa Therapeutics B.V) is a 2-O*methyl –phosphorothioate oligoribonucleotide with a specific sequence targeting exon 51 of the DMD gene. We have investigated in a phase I/IIa study the safety, tolerability, pharmacokinetics and molecular and clinical effects of the systemic administration of AON PRO 051 in a genetic subset of patients with DMD (Chapter III A.1). The long-term safety and efficacy of systemic administration of PRO051 (GSK2402968/ Drisapersen) was further investigated in a still ongoing long-term open label extension study (Chapter III A.2.).

(2) To investigate the 6 minute walk test as a clinically meaningful, relevant and suitable outcome measure for disease progression monitoring and for therapeutic trials in DMD

The second part of this project aimed at investigating the 6MWT both in healthy typically developing young Caucasian boys and in ambulant DMD subjects, treated with glucocorticosteroids. Such data will contribute to methodological improvements of therapeutic trial protocol and design in ambulant DMD subjects.

A first study (Chapter III,B.1.) investigated the test-retest (*intrater*) reliability and developmental evolution in typically developing healthy boys aged 5-12 years and this in narrow age subcategories, and assessed the correlation of the 6MWD with anthropometric characteristics in this age group of particular interest in relation to natural history studies and clinical trials in the ambulatory phase of DMD.

A second study (Chapter III,B.2.) aimed at generating normative 6MWT data by measuring 6MWD in a large cohort of typically developing Caucasian boys aged 5-12 years. This study improves our understanding on the developmental evolution of this functional capacity measure, and provides data, including a predictive equation, to be used for the interpretation of 6MWD measurements in diseased (DMD) children.

A third study (Chapter III,B.3.) aimed at providing more insights on the 6MWT in DMD treated according to the current standards of care, by investigating its evolution in a large homogenous cohort of glucocorticosteroid treated DMD boys. This work has generated data on the contemporary modified disease progression and its variability. Such data improve our understanding on the disease course and optimize the use of the 6MWT as a disease measure and endpoint in clinical trials.

CHAPTER III

RESULTS AND DISCUSSIONS

Chapter III — Part A

Splicing Modulation with Antisense oligonucleotides as a novel therapeutic approach for DMD

III.A.1

SYSTEMIC ADMINISTRATION OF PRO 051 IN DUCHENNE'S MUSCULAR DYSTROPHY

Published as:

Systemic Administration of PRO051 in Duchenne's Muscular Dystrophy

Nathalie M. Goemans, M.D., Mar Tulinius, M.D., Ph.D., Johanna T. van den Akker, Ph.D., Brigitte E. Burm, Ph.D., Peter F. Ekhart, M.Sc., Niki Heuvelmans, Tjadine Holling, Ph.D., Anneke A. Janson, Gerard J. Platenburg, M.Sc., Jessica A. Sipkens, M.Sc., J.M. Ad Sitsen, M.D., Ph.D., Annemieke Aartsma-Rus, Ph.D., Gert-Jan B. van Ommen, Ph.D., Gunnar Buyse, M.D., Ph.D., Niklas Darin, M.D., Ph.D., Jan J. Verschuuren, M.D., Ph.D., Giles V. Campion, M.D., Stef J. de Kimpe, Ph.D., and Judith C. van Deutekom, Ph.D.

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III.A.2

EXON SKIPPING IN DUCHENNE MUSCULAR DYSTROPHY: 144-WEEK SAFETY AND EFFICACY RESULTS FROM AN OPEN-LABEL PHASE I/IIA STUDY OF ANTISENSE OLIGONUCLEOTIDE DRISAPERSEN

Adapted from:

Exon Skipping in Duchenne Muscular Dystrophy: 144-Week Safety and Efficacy Results from an Open-Label Phase I/IIa Study of Antisense Oligonucleotide Drisapersen

Nathalie M. Goemans[1]*, Már Tulinius[2], Marleen van den Hauwe[1], Anna-Karin Kroksmark[2], Gunnar Buyse[1], Rosamund J. Wilson[3], Judith C. van Deutekom[4], Sjef J. de Kimpe[4], Allison Morgan[4], J.M. Ad Sitsen[4], John E. Kraus[5], Giles V. Campion[4,]

Manuscript in preparation

ABSTRACT

Background: Drisapersen induces exon 51 skipping during dystrophin pre-mRNA splicing. In a dose-escalation study, multiple doses of drisapersen produced novel dystrophin expression in boys with Duchenne muscular dystrophy.

Methods: This open-label extension to the dose-escalation study assessed the long-term safety, efficacy, and pharmacokinetics of drisapersen (PRO051/GSK2402968), 6 mg/kg subcutaneously, in 12 subjects with Duchenne muscular dystrophy. Planned treatment was once weekly for 72 weeks. All subjects had a scheduled treatment interruption from weeks 73 to 80 inclusive, and an intermittent dosing regimen thereafter.

Findings: Subjects received a median (mean; standard deviation) total dose of 19.2 (20.1; 5.35) g drisapersen. Most adverse events were mild, most commonly local injection-site reactions, raised urinary α1-microglobulin, and proteinuria. Seven subjects had one or two unplanned treatment interruptions: reduced thrombocyte count (n = 2), skin ulcer at prior injection site (n = 2), inflammatory reactions (n = 1), changes in renal/hepatic markers (n = 1), both thrombocytopenia and inflammatory reaction (n = 1). In general, parameters moved towards normal during drug-free periods. After 141 weeks, median (mean; standard deviation) 6-minute walk distance changed from extension baseline by +20 (–8.2; 161) meters (subjects able to complete the test at extension baseline, n = 10); 6/7 subjects considered in plateau phase at extension baseline walked further at all postbaseline visits, 2/3 subjects considered in decline lost ambulation, while one walked further at some visits.

Interpretation: Drisapersen, 6 mg/kg, was generally well tolerated over 144 weeks. Possible renal effects, thrombocytopenia, and injection-site reactions warrant continued monitoring. Improvements in the 6-minute walk distance at 12 weeks were sustained at 141 weeks; 6/10 subjects showed improvement or no deterioration from extension baseline. For a small, uncontrolled study, the outcomes are encouraging, as natural history studies show a progressive decline in 6-minute walk distance over shorter observation periods in this age group.

Keywords: Duchenne muscular dystrophy, antisense oligonucleotide, exon skipping, dystrophin, the 6-minute walk distance

INTRODUCTION

Duchenne muscular dystrophy (DMD) is an X-linked recessive muscle disease with a global incidence of 1/3500 newborn boys [1]. DMD has a predictable clinical trajectory. Initial development of motor skills is followed by a plateau phase then progressive functional decline between 6 and 10 years of age (decline to be expected from 7 years of age), leading to loss of ambulation and early death[2-8]. Interventions such as glucocorticosteroid treatment[9] and ventilator support[10] can delay key milestones of progression by approximately 2 years, but do not fundamentally alter the course of the disease. and no curative treatment is currently available.

DMD is caused by mutations (mostly deletions of one or more exons) in the *DMD* gene encoding the dystrophin protein [11], which has key structural and signaling functions in skeletal and cardiac muscle. Disruption of the transcriptional open reading frame leads to prematurely aborted dystrophin translation, resulting in a DMD phenotype. Mutations that maintain the translational reading frame usually lead to truncated, but partially functional, dystrophin proteins, and are associated with typically much milder Becker muscular dystrophy phenotypes [12].

Antisense oligonucleotides are designed to induce specific exon skipping during pre-mRNA splicing [13,14]. This strategy aims to correct the reading frame and produce a Becker-like transcript in patients with a DMD mutation [14,15]. About 13% of the DMD population carry mutations that are corre ctable by skipping exon 51[13] . Drisapersen is a 2'-*O*-methyl phosphorothioate RNA antisense oligonucleotide that induces exon 51 skipping during pre-mRNA splicing [16] .

After a clinical study established proof of concept for local administration of drisapersen in DMD[17], systemic subcutaneous (sc) drisapersen was administered in an open-label, dose-escalation study, with dose-related novel dystrophin expression[18]. Subjects subsequently entered an extension phase, receiving drisapersen, 6 mg/kg/week. Over the first 12 weeks of the extension, treatment was well tolerated without serious adverse events (AEs), and clinical effects were promising[18]. The open-label extension phase is ongoing, and here we report results after 144 weeks of follow-up.

MATERIALS AND METHODS

Ethics Statement

The study was sponsored by Prosensa Therapeutics BV (Leiden, the Netherlands) and performed in accordance with Good Clinical Practice guidelines, the Declaration of Helsinki (2008), the European Directive 2001/20/EC, and local regulations. Study sponsorship was transferred to GlaxoSmithKline (Middlesex, UK) in July 2011. The study was approved by the local independent ethics committees (Medical Ethics Committee of University Hospitals Leuven and Regional Ethics Committee of Gothenburg) and authorized by the competent authorities of Belgium and Sweden. Written informed consent from parents/guardians and assent (subjects over 12 years of age) was obtained for all subjects prior to any study procedure.

Study Design and Conduct, Study Drug, and Dosing

The initial Phase I/IIa, open-label study (5-week dose escalation then 13 weeks off-treatment; EudraCT 2007-004819-54) aimed to assess the safety, tolerability, pharmacokinetics, and clinical and molecular effects of multiple sc doses of drisapersen (PRO051/GSK2402968) in 12 subjects with DMD[18]. The objectives of the 144-week extension period (performed between July 2009 and May 2012) were to assess the long-term safety, efficacy, and pharmacokinetics of drisapersen, 6 mg/kg sc, in subjects with DMD.
The study was performed at two centers: University Hospitals Leuven, Belgium, and The Queen Silvia Children's Hospital, Sweden. No formal statistical testing was performed owing to the small number of subjects and the absence of a control group.

Abdominal sc injections of drisapersen (SynCo Bio Partners BV, Amsterdam, the Netherlands)[17], 6 mg/kg (maximum 366 mg, capped at 300 mg from week 54), were given once weekly initially; after approximately 9 months of treatment, rotation among other injection sites was recommended. All subjects had a scheduled interruption of treatment from weeks 73 to 80 inclusive. The interruption was planned and documented in a protocol amendment. After this period, a change from continuous to intermittent dosing was made after evaluating safety and pharmacokinetic data. An intermittent dosing regimen, 6 mg/kg once weekly for 8 weeks followed by 4 weeks off-treatment, was introduced at week 81. At 144 weeks, all subjects had completed the first five 12-week intermittent-treatment cycles and were 4 weeks into the treatment phase of the next 12-week cycle.

Subjects

Boys aged 5–16 years with DMD resulting from a mutation correctable by treatment with drisapersen were eligible for inclusion in the original dose-escalation study. Briefly, subjects were eligible if they had no evidence of dystrophin in more than 5% of fibres on previous diagnostic muscle biopsy, an estimated life expectancy of 6 months or more, no serious pre-existing medical conditions, and no dependency on assisted ventilation, and had not participated in any other study with an investigational product in the past 6 months. Concurrent glucocorticosteroid treatment was permitted if stable for at least 2 months prior to enrolment, and was to be kept constant during the study if possible.

Inclusion and exclusion criteria, have been described previously [18]. Briefly, subjects were eligible if they had no evidence of dystrophin in more than 5% of fibres on previous diagnostic muscle biopsy, an estimated life expectancy of 6 months or more, no serious pre-existing medical conditions, and no dependency on assisted ventilation, and had not participated in any other study with an investigational product in the past 6 months. Concurrent glucocorticosteroid treatment was permitted if stable for at least 2 months prior to enrolment, and was to be kept constant during the study if possible.

After completion of the dose-escalation study, all subjects were included in the extension study on the basis of the drisapersen safety profile and pharmacokinetic data, and effects seen at muscle biopsy. The start of the extension study was defined as “extension baseline”. The time interval between the two baseline visits ranged from 6 to 15 months.

Endpoints

Safety and tolerability endpoints included AEs, serious AEs (SAEs), local tolerability, laboratory parameters, vital signs, electrocardiograms (ECGs), echocardiography, physical examination, and serum antidystrophin antibodies.

Pharmacokinetic evaluations included the maximum plasma concentration (C_{max}), time to achieve C_{max} (T_{max}), and the area under the plasma concentration–time curve from 0 to 24 hours ($AUC_{0–24h}$). Efficacy endpoints were muscle function, muscle strength, respiratory function, and exon-51-skipped dystrophin mRNA and protein production.

Assessments

The antidystrophin antibody assay was based on a method described previously[19], the presence of antibodies was assessed at weeks 12, 24, 48, 72, 96, 120, and 144.

Blood samples for pharmacokinetic assessments were collected predose and 3 hours postdose at monthly intervals up to week 24, and predose only from weeks 76 to 144. At week 20, samples were collected predose and at 0.5, 2, 3, 4, 6, 9, 12, and 24 hours postdose.

Timed functional tests included a 10-meter walk/run, rising from the floor, and a four-stair climb, and were performed at baseline, week 8, monthly until week 28, then at weeks 36, 48, 60, 72, 80, 93, 105, 117, 129, and 141. The 6MWD was recorded at baseline and then every 12 weeks until week 72, then at weeks 80, 93, 105, 117, 129, and 141 [7].

Muscle strength was evaluated by handheld myometry using a microFET dynamometer (Biometrics BV, Almere, the Netherlands). Respiratory function was assessed using a hand-held Koko spirometer (PDS Instrumentation, Louisville, USA) and a magnehelic manometer (Dwyer Instrument, Michigan City, USA); the following parameters were assessed: forced vital capacity (FVC), forced expiratory volume in 1 second, maximal inspiratory pressure, maximal expiratory pressure, peak flow, and peak cough flow. Assessments were performed at baseline, every month between weeks 8 and 28, and then at weeks 36, 48, 60, 72, 80, 93, 105, 117, 129, and 141.

Biopsies were taken from the tibialis anterior muscle at week 24 and used for detection of specific exon 51 skipping at the RNA level [17,20,21] (data to be reported separately) and dystrophin expression at the protein level [17,18] .

RESULTS

Subjects

All 12 subjects who completed the dose-escalation study entered the extension study (Supporting Figure S2. Demographic data and study medication dose at extension baseline and week 141 are shown (Table 1). The age of subjects at extension baseline ranged between 5.9 and 14.3 years (Mean (standard deviation [SD]): 10.1 [2.27]). As reported previously, study sample was clinically heterogeneous at entry of the initial dose finding study, including a non-ambulant boy and ambulant subjects in different stages of disease. In the time interval between the two baselines, an additional subject lost the ability to complete the 6MWT. Subjects were prospectively classified by their ambulatory disease status at extension baseline (in plateau phase or in decline), based on the judgment of the investigator, the subjects' parents, and physiotherapists according to pre-clinical study notes and assessment. All subjects classified in decline walked less than 300 m at baseline.

All subjects were on stable daily corticosteroid treatment for at least 1 year before entry into the initial dose-escalation study. A minor adjustment to the steroid regimen was made for one subject in accordance with growth. Another subject had a period of intermittent steroids implemented by his general practitioner because of delayed growth. All other subjects remained on a stable continuous dose

One subject was on stable angio-converting enzyme inhibitor (ACE-I) at baseline visit. In two subjects ACE-I were initiated after week 48.

Subjects received a median (mean [SD]) total dose of 19.2 (20.1 [5.35]) g drisapersen over the course of the extension study. Overall compliance was calculated to be 92.9%, and the mean (range) dose was 5.94 (5.21–6.02) mg/kg.

Safety and Tolerability

All subjects reported one or more AEs. Local injection-site reactions in the abdominal sc tissue were the most prominent AE of clinical note and were reported for all subjects. The majority of injection-site reactions were considered mild to moderate and included inflammatory changes, pigmentation, induration and sclerosis which occurred after chronic administration and persisted in all subjects. The most common treatment-related AEs are shown in Table 2. Increases in urinary α1-microglobulin levels and proteinuria (in spot-urine samples; defined as ≥0.2g/L) were each reported for all subjects and were considered to be mild in severity and treatment-related. Overall, all treatment-related AEs were classified as mild, except for the following moderate AEs: injection-site reactions (6 subjects), gastroenteritis (2 subjects), upper abdominal

pain, influenza-like illness, pain in extremity, pyrexia, rash, bacterial infection, localized infection, and thrombocytopenia (1 subject each; minimum thrombocyte count: 91×10^9/L). None of six SAEs (tympanic membrane perforation, tibia fracture, febrile convulsion, scrotal pain, tendon operation, and postoperative care for dental extraction) was considered treatment-related.

Eleven subjects had at least one 24-hour urine collection as a result of a predefined criterion of spot protein levels measured at ≥0.2 g/L in two consecutive samples. For seven subjects, 24-hour urine protein levels were ≥0.15 g/day, however, all were <0.3g/day with the exception of a single measurement for two individuals.

No subjects withdrew permanently from the study. All subjects missed at least one planned dose up to 144 weeks. Eleven subjects missed visit(s) and seven had one or two treatment interruptions (excluding planned washout and off-drug periods) owing to emerging safety data; interruptions were based on investigator opinion in consultation with the medical monitor. Reasons were: reduction in thrombocyte count (<150 x 10^9/L; n = 2), skin ulceration at prior injection site (n = 2), inflammatory reactions (n = 1), or changes in renal and hepatic markers (n = 1), both thrombocytopenia and systemic inflammatory reaction, i.e. chills (n = 1). However, no subjects had a thrombocyte count of <75 x 10^9/L and none of these subjects reached any predefined stopping criteria prior to the treatment interruption. In general, these parameters moved towards baseline levels off-treatment, and inflammatory reactions were manageable with nonsteroidal anti-inflammatory drugs (NSAIDs) and/or antihistamines. None resulted in hospitalizations.

For subject 3 with the highest weight (61 kg) and body mass index (31 kg/m^2) at extension baseline and a high proportion of body fat on dual-energy X-ray absorptiometry (43%), the dose was capped at 300 mg from week 54 following a treatment interruption owing to changes in renal and hepatic markers. There was a trend towards a return to baseline values for the majority of laboratory parameters during treatment interruptions, and evidence that values stabilized during the intermittent dosing regimen.

There were no apparent significant effects of treatment on coagulation, vital signs, ECG, cardiac tissue, or inflammatory parameters (C-reactive protein, fibrinogen, haptoglobin, and monocyte chemotactic protein-1). Cardiac function measured using echocardiography was variable but showed stability over 144 weeks. (Table 1). No antidystrophin antibodies were detected.

Pharmacokinetic Profile

After subcutaneous injection of 6 mg/kg drisapersen, the drug was quickly absorbed, with peak levels (T_{max}) generally reached at 2–3 hours. Thereafter, drisapersen was rapidly distributed with a decline in plasma levels to approximately 18% of the C_{max} at 24 hours, and 0.6% at the end of the dose interval (7-day trough levels). Peak plasma levels and AUC_{0-24h} showed no clear increase after repeated dosing, but mean trough plasma levels increased with increasing numbers of injections up to week 20 (week 24 in some subjects),

indicating continued tissue exposure at once-weekly dosing intervals. Plasma levels of drisapersen decreased in all subjects during the washout and off-treatment periods indicating clearance of the drug (Figure 1). Trough concentrations increased during the dosing periods, suggesting a likely decrease in tissue exposure during off-periods of the intermittent dosing regimen. One subject (subject 4) had notably higher trough plasma levels; the reason for this apparent outlier is not known. This subject had a leg ulcer and an extended treatment interruption (week 101 to week 128), during which trough plasma concentrations declined and were within the range of the other subjects when treatment was restarted at week 129.

Efficacy

Of the 12 subjects, 10 (mean [standard deviation; SD] age 9.5 [1.88] years) were able to complete the 6MWD test at extension baseline (). At original study baseline, the median (mean [SD]) 6MWD was 394 (402 [73]) meters in the 10 subjects able to complete the 6MWD at extension baseline (Table 3). Six of the 10 subjects who completed the 6MWD at extension baseline improved or did not show deterioration in the 6MWD at week 141 compared with extension baseline. 6MWD increased by over 100 meters for four subjects, including over 120 meters and 200 meters for two subjects at week 141 (Figure 2). Two subjects aged 9.6 and 11.4 years at baseline did lose ambulation Two subjects age 9.9 and 12 years at extension baseline preserved ambulation but did show a decline over the 144 week duration of the study Although data are limited, the introduction of an intermittent dosing regimen following week 72 did not appear to adversely affect efficacy parameters.

Despite the variability in the 6MWD, a general improvement was maintained at least up to week 129, when the median (mean [SD]) change from extension baseline was 59 (–0.6 [165]) meters (Table 4). At week 141, the median (mean [SD]) change from extension baseline in the 6MWD was +20 (–8.2 [161]) meters.

These summary statistics include those who were unable to complete the test at later visits and scored ‘zero’, (Table 4). The mean change for the subjects (n=8) still able to walk at week 141 was 72 (53 [109]) meters. Stratification of subjects by baseline status demonstrated that the two groups had notably different outcomes over the extension phase Subjects whose disease status was classified as in plateau phase at extension baseline (n=7, Mean (SD) age 8.83 (1.77)) all walked further than 300 m at base line and generally improved over 141 weeks, whereas those classified as in decline (n=5, Mean (SD) age 11.0) generally deteriorated further (Table 4; Figure 2).

An age- and height-based equation fitted to normative data by Geiger et al [22]. was applied to the 6MWD data from the boys in the current study, and a percentage predicted value of normal for each subject was calculated. Seven of the subjects in plateau phase had percentage predicted values of greater than 70% of normal by week 93 in contrast to two at extension baseline, despite six of these subjects being older than 7 years.

There was no clear evidence of clinically important deterioration in the time to complete the four-stair climb, the 10-meter walk/run, or the rising-from-floor test in subjects classified as in plateau phase at extension baseline; however, a reduction in function was noted in those classified as 'in decline' at extension baseline (Supporting Table S1). For patients able to complete the timed tests (n=8), no clear deterioration was observed overall and test times were stable..

Myometry data were highly variable, but there was no evidence of any clinically relevant deterioration during 141 weeks. There were no clinically relevant changes in spirometry parameters over 141 weeks (Supporting Figure S1). A decrease in FVC (% predicted)) (median; mean [SD] –13.0; –13.4 [16.31] %) was noted, however the interpretation of this age and height-related equation is hampered by difficulties in acquiring adequate height measurements in this population, especially in the non-ambulant and transitional stage of the disease. Increases in absolute values of FVC (0.1; 0.05 [0.212 L), peak cough (29.0; 33.5 [34.17] L/min) and peak cough flow (50; 50.0 [59.33] L/min) were observed.

Dystrophin Expression

Exon 51 skipping (confirmed by mRNA analysis) and dystrophin production (confirmed by immunofluorescence and Western blot) were demonstrated previously in this population in a 5-week dose-escalation study [18]. RNA assessment of the week-24 biopsies is currently under evaluation and will be the subject of a separate paper, however, after 24 weeks of treatment, immunofluorescence of muscle biopsy cross-sections revealed clear dystrophin expression at the fiber membrane for 11/12 subjects, and up to 100% dystrophin-positive fibers were observed for most subjects (Figure 3).

DISCUSSION

Exon skipping is currently considered the most promising strategy for the treatment of DMD[20]. The molecular and clinical effects of sc drisapersen, 6 mg/kg/week, over 12 weeks of open-label treatment in subjects with DMD, were reported previously [18]. As a continuation of this study, we show that long-term treatment with drisapersen appears to be well tolerated in subjects over 144 weeks. To our knowledge, this is the longest reported follow-up of any exon-skipping therapy currently being studied in DMD and provides the longest-duration published dataset on any oligonucleotide administered sc.

No subjects withdrew during the long-term extension study. Safety monitoring was directed primarily towards target organs of accumulation (liver and kidney), thrombocytes, markers of inflammation, and local skin tolerability. There was no evidence of overt hepatic injury, although two subjects had elevations in alanine aminotransferase, γ-glutamyl transferase, and glutamate dehydrogenase, which were temporally related to drisapersen administration. Similarly, although there was some evidence of mild proteinuria and raised urinary α1-microglobulin levels, renal effects did not appear to be progressive, with values moving towards normal during drug-free periods, which may indicate mild reversible proximal tubule dysfunction. Cardiac function, determined using echocardiography, was variable but generally appeared stable with some tendency for improvement. However, three patients were on preventative angiotensin-converting enzyme inhibitors during the extension study and this should be considered in the interpretation of the results. The majority of AEs were considered to be mild, and there were no treatment-related SAEs.

Seven subjects had treatment interruptions due to increases in at least one marker of renal or hepatic function, skin lesions at prior injection site, inflammatory reactions, or thrombocytopenia; however, none met the predefined stopping criteria, all moved towards baseline levels off-treatment and systemic inflammatory reactions appear to be manageable with NSAIDS and/or antihistamines.

The intermittent dosing regimen would be expected to give lower overall drug exposure than a continuous regimen, and pharmacokinetic data from this study appear to support this. Plasma concentrations of drisapersen decreased in all subjects during the washout and off-treatment periods, indicating tissue clearance of the drug. Trough concentrations increased during the treatment periods. There is no evidence that efficacy was affected by the lower overall drug exposure, but there is evidence that some safety parameters stabilized from week 72, either for the group as a whole or for individuals where parameters were trending up or down, eg, hematology cell counts, γ-glutamyl transferase, glutamate dehydrogenase, serum cystatin C, α1-microglobulin, and proteinuria. In particular, the increases in α1-microglobulin and proteinuria reported in all subjects as mild AEs appeared to decrease during the drug-free periods, suggesting that any renal changes may be reversible, with no secondary structural damage. Such renal effects, however, as well as thrombocyte levels and local injection-site reactions, warrant continued monitoring, and further evaluation of the intermittent phases is needed. Drisapersen administration caused

local injection-site reactions including induration, hematoma, erythema, pain, discolouration and sclerosis; none led to treatment withdrawal, and most were described as mild, although persistent. Although individual injection-site reactions did not impact the subject's daily activities or cause significant discomfort, the development of sclerosis/fibrosis at multiple sites may eventually lead to the exhaustion of suitable injection sites in some or all individuals and the potential inability to administer study medication is of clinical concern. Rotation of injection sites may help to prevent the development of chronic injection-site reactions. No subject had any detectable antibodies to dystrophin during this extension study, mitigating concerns about the novel production of dystrophin protein triggering an immune response.

Clinical and laboratory safety results from a 12-week dose-escalation trial of another exon-51-skipping oligonucleotide (eteplirsen, AVI-4658) based on an alternative chemistry have shown to restore dystrophin expression in the skeletal muscle of some individuals following systemic administration of the drug [23]. Eteplirsen was well tolerated with no serious drug-related AEs; however, the short duration of the study limit safety conclusions [23]. Data from a single-center, open-label, long-term extension study of twelve ambulant boys with DMD (mean age at baseline 8.8 years), showed a decrease in maximum 6MWD of 15.6 meters in boys receiving 50 or 30 mg/kg/week eteplirsen for 62 weeks (n = 6)[25]. This was compared with a greater decline of 77.6 meters in a group receiving placebo for 24 weeks followed by 38 weeks of 50 or 30 mg/kg/week eteplirsen (n = 4). Two subjects receiving 30 mg/kg were non-ambulant by week 24 and were excluded from the analysis. No clinically significant treatment-related AEs, serious AEs or discontinuations were reported up to week 62 and, while one patient had a transient elevation of urine protein, this was not evident following a 24-hour urine protein test, produced no clinical symptoms and did not result in treatment interruption. Based on the data currently available, there is proof of mechanism for antisense oligonucleotide-induced exon skipping to enable dystrophin expression in patients with DMD, without inducing an antidystrophin immune response. These results increase the likelihood of this approach being clinically applicable.

This study was initiated as a dose finding study, including a clinically heterogeneous cohort of subjects with DMD, in different stages of the disease. The natural course of this disease is however characterized by a non-linear pattern of evolution challenging the choice of outcome measure to assess clinical efficacy in clinical trials for DMD. Since the start of this study, increasing evidence has been obtained on the reliability, validity sensitivity and clinical meaningfulness of the 6MWT confirming its status of a clinical meaningful outcome measure in ambulatory DMD trials [7,24-26].

Natural history studies reporting on longitudinal observations of 6MWD in boys with DMD/BMD have improved our insights in this global measure of function, highlighting the effect of age and stage of disease on the rate of disease progression as measured by the 6MWD.[5-8,27] In one study an average 57 meter decrease from baseline was observed over 52 weeks [28] . Further studies demonstrated an average decline of 26 meters over 12 months, and 38 meters and 129 meters over 1 year and 2 years respectively [27]. A more recent study of 113 boys reported a mean decline of 23 meters in the first year of observation and a

further 65 meters in the second year. When split by age, 6MWD for boys below 7 years old remained stable with a slight increase in the first and second years; however, distance walked for boys aged 7 years or older declined by approximately 42 meters and 80 meters respectively [6]. Finally, a decline in of -59 m over a 48 week time frame was reported recently in DMD boys older than 7, and baseline age of >7 years was confirmed as a predictor of decline in a multicenter study [7,25]

All except one of the subjects in our study were older than 7 years at the start of the extension phase and would be expected to show declining 6MWD during the study period based on these longitudinal observations. However, there appeared to be a general improvement in the 6MWD over 12 weeks during the extension phase of our study[18], which was maintained in some subjects up. These increases exceed by far the minimal clinically important differences (MCID) in 6MWD estimated to be approx. 30 m [24] and represent a clear deviation from natural history data [6,7,27]

It should be noted that the mean value could be considered skewed, as it includes subjects who were unable to attempt the 6MWD test and has values of 0 meters included in the summary statistics. Consequently, the median is considered a more representative measure, and this increased by 20 meters (range –263 to 201) from the extension baseline.

Classification of subjects by their ambulatory disease status at extension baseline demonstrated notable differences in the 6MWD over 141 weeks. Six of the seven subjects classified as being in a stable disease state (i.e. in plateau phase) were able to walk further than at baseline at all subsequent visits, whereas those who were classified as in decline and able to complete the 6MWD at extension baseline (n = 3) generally continued to decline, with the exception of one subject who remained stable, walking further at some post baseline visits. All subjects in decline exhibited a baseline 6MWD below 300 m and percent predicted values below 55%, reflecting a more advanced stage of disease. This may indicate that a critical amount of residual muscle fibers is required to prevent further loss of ambulation with this therapeutic strategy. In these subjects long term assessment of upper limb, cardiac and respiratory function is needed to evaluate a potential disease modifying effect of treatment..

A recent study compared the performance of boys with DMD relative to the typical performance of healthy peers by calculating a percentage predicted value to account for normal growth and development[29]. An age- and height-based equation fitted to normative data by Geiger et al.[22] was used to convert the 6MWD to a percentage predicted value in the boys with DMD. This method demonstrated that boys with DMD aged 4–7 years maintained a stable 6MWD approximately 80% of that of their typically developing peers. In boys with DMD older than 7 years, there was a variable decline in percentage predicted 6MWD [29]. Data from a contemporaneous cohort of steroid treated boys. confirmed a decline in %predicted 6MWD after the age of 7 and reported an average decline of -8 % after one year and -21 % after two years in DMD boys in a comparable age range [27]. A similar magnitude of change in percent predicted 6MWD (9%) was reported recently in a multicenter study in DMD boys older than 7 over 48 weeks' time [7]. Using similar methodology, our data show that seven of the subjects in plateau phase had percentage predicted values of >70% by

week 93, and six of these subjects showed an increase in percentage predicted values, which is unexpected in this age range. Furthermore, a baseline value of 55% predicted 6MWD has been identified as a threshold value for risk of a more rapid deterioration in the following year [7,25]. It should be noted that in this study 4 subjects were below this threshold value at baseline, from which one remained remarkably stable despite his age and baseline 6MWD. Two other subjects presented with baseline 6MWD close to this threshold value and did improve significantly both in absolute and percent predicted values over time.

The small sample size and the absence of a control group however clearly hamper the evaluation of a treatment effect, although, as highlighted above, natural history studies suggest that a decline in the 6MWD would be expected [5-8,27].

Other functional tests remained largely stable, although a reduction in function was noted in subjects classified as in decline. Again, in the context of an expected deterioration in motor function[7], this stability may be regarded as a positive treatment effect, but the absence of a control group limits definitive conclusions. Although not documented using validated questionnaires, subjective improvements in activities of daily life were reported in some subjects, such as walking longer distances, taking the stairs and acquiring new motor skills such as jumping, rope skipping and cycling after the age of 9 which is unusual for DMD boys of this age yet on chronic steroids for several years. Improved participation in sports activities with peers was noted as well. Muscle strength data were highly variable and inconclusive over the first 141 weeks of the extension phase.

Concerns have been raised that improving exercise capacity could be detrimental to the cardiac muscle by increasing the load to the dystrophic cardiac muscle. In this limited sample, cardiac parameters remained stable after 144 week of treatment. Similarly, respiratory parameters remained relatively stable over the course of the study, which may indicate a deviation from natural history and warrants further evaluation.

While this study provides important long-term data on drisapersen, its limitations must be acknowledged. As the study was small, open label in design, and lacking a placebo control group, functional outcomes need to be interpreted with caution. However, a strength of the study is the long duration of follow-up, and the availability of natural history results to allow comparison is advantageous[27].

In conclusion, sc injection of antisense oligonucleotide drisapersen, 6 mg/kg, was well tolerated over 144 weeks, although possible renal effects, thrombocytopenia, and local injection-site reactions warrant continued monitoring. Improvements in the 6MWD observed at 12 weeks were sustained at 141 weeks in six of ten subjects who were able to complete the test at extension baseline. Although this is a small scale, uncontrolled, open-label study, the outcomes are encouraging, as deterioration in muscle strength and function has been shown even over a shorter observation period in the DMD patient population[5-8,27,30]. The extension phase is ongoing and confirmatory studies are underway, which will help define the safety and efficacy of exon-51 skipping.

Table 1. Demographic and dosing data for all subjects by disease status classification (extension study baseline), and use of ACE inhibitors and glucocorticosteroids during the extension phase.

	Extension baseline					Week 141			
	Exon deletion	Age (years)	Height (cm)	Weight (kg)	Body mass index (kg/m^2)	Age (years)	Height (cm)	Weight (kg)	Body mass index (kg/m^2)
Subjects in plateau phase at extension baseline									
Subject 1	52	10.9	124.0	27.8	18.1	13.6	128.5	29.4	17.8
Subject 2	45–50	8.0	119.7	25.5	17.8	10.7	128.9	32.2	19.4
Subject 4	48–50	10.3	122.0	25.8	17.3	13.0	125.6	30.5	19.3
Subject 5	45–50	9.2	117.9	24.4	17.6	11.9	121.8	26.1	17.6
Subject 9	48–50	7.5	114.0	24.6	18.9	10.2	118.5	29.0	20.7
Subject 11	45–50	5.9	97.5	14.6	15.4	8.6	107.0	19.1	16.7
Subject 12	45–50	9.9	114.5	27.8	21.2	12.6	124.5	34.9	22.5
Subjects who were in decline at extension baseline									
Subject 3	52	11.8	141.0	61.0	30.7	14.5	149.5	65.5	29.3
Subject 6	48–50	9.6	132.2	30.9	17.7	12.3	137.8	37.9	20.0
Subject 7	48–50	11.4	135.9	38.1	20.6	14.1	136.0	40.7	22.0
Subject 8	48–50	14.3	141.0	36.8	18.5	17.0	144.0	45.0	21.7
Subject 10	45–50	12.0	125.5	28.3	18.0	14.7	128.0	32.6	19.9
Concomitant medication use during the extension phase*									
ACE inhibitors									
Enalapril maleate, n (%)	3 (25.0) (subj 8, 10, 12)								
Glucocorticosteroids									
Deflazacort, n (%)	8 (66.7)								
Prednisolone, n (%)	4 (33.3)								

*****Safety population.

[†]Dose for Subject 3 was capped at 300 mg starting from week 54

ACE: angiotensin-converting enzyme.

Table 2. Treatment-related* AEs that occurred in more than two subjects during the 144-week extension phase.

Preferred term	Number (%) of subjects reporting event[†]
Injection site	
Induration	12 (100)
Erythema	12 (100)
Haematoma	12 (100)
Pain	9 (75)
Discolouration	8 (67)
Pruritus	5 (42)
Inflammation	3 (25)
Renal and urinary disorders	
Proteinuria	12 (100)
Albuminuria	11 (92)
Investigations	
α1-microglobulin urine increased	12 (100)
Glutamate dehydrogenase increased	8 (67)
Cystatin C increased (serum)	8 (67)
Urinary sediment abnormal	5 (42)
Laboratory test abnormal[‡]	5 (42)
γ-glutamyl transferase increased	3 (25)
Headache	4 (33)
Thrombocytopenia	5 (42)
Dry skin	4 (33)
Vomiting	3 (25)

*Includes adverse events classified as possibly, probably, or definitely related to treatment.

[†]If a subject had more than one event with the same preferred term, the subject was counted only once for that term.

[‡]Term relates to elevated monocyte chemotactic protein-1.

AE: adverse event.

Table 3. Summary of the 6MWD test by visit over 141 weeks (intent-to-treat population): subjects able to complete the 6MWD test at extension baseline and change from extension study baseline in the 6MWD test for all subjects.

		Distance walked (meters)		Change from extension study baseline in distance walked (meters)	
All subjects (N = 12)*	Number of subjects*	Mean (SD)	Median (range)	Mean (SD)	Median (range)
Original study baseline	10	401.8 (72.96)	394 (300–545)		
Extension study baseline	10	383.9 (121.22)	362 (243–647)	-	-
Week 12	10	419.1 (122.12)	423 (237–675)	35.2 (28.69)	39 (−6 to 69)
Week 24	10	420.7 (126.46)	460 (184–644)	36.8 (59.78)	37 (−59 to 115)
Week 36	10	408.9 (140.29)	439 (187–675)	25.0 (53.84)	21 (−56 to 114)
Week 48	10	412.5 (166.22)	463 (146–688)	28.6 (79.74)	46 (−110 to 127)
Week 60	10	410.1 (195.42)	483 (75–694)	26.2 (114.99)	62 (−185 to 147)
Week 72	10	381.6 (228.42)	470 (0–688)	−2.3 (144.25)	44 (−263 to 135)
Week 80	10	394.6 (228.68)	463 (0–690)	10.7 (156.23)	56 (−263 to 183)
Week 93	10	395.0 (233.35)	467 (0–700)	11.1 (157.19)	63 (−263 to 190)
Week 105	10	389.8 (224.76)	475 (0–642)	5.9 (160.83)	63 (–263 to 169)
Week 117	10	396.8 (231.75)	494 (0–695)	12.9 (163.52)	64 (–263 to 188)
Week 129	10	383.3 (233.40)	478 (0–691)	-0.6 (164.75)	59 (–263 to 192)
Week 141	10	375.7 (224.73)	464 (0–651)	-8.2 (160.91)	20 (–263 to 201)

Missing values replaced by zero for subjects who became unable to complete the test at later visits.

*Two subjects were not able to complete the 6MWD test at extension baseline; two subjects did not complete the 6MWD test at week 60.

6MWD: 6-minute walk distance; SD: standard deviation.

Table 4. Change from extension study baseline in the 6MWD test for subjects~~,~~ by disease status at extension baseline (intent-to-treat population): subjects able to complete the 6MWD test at extension baseline.

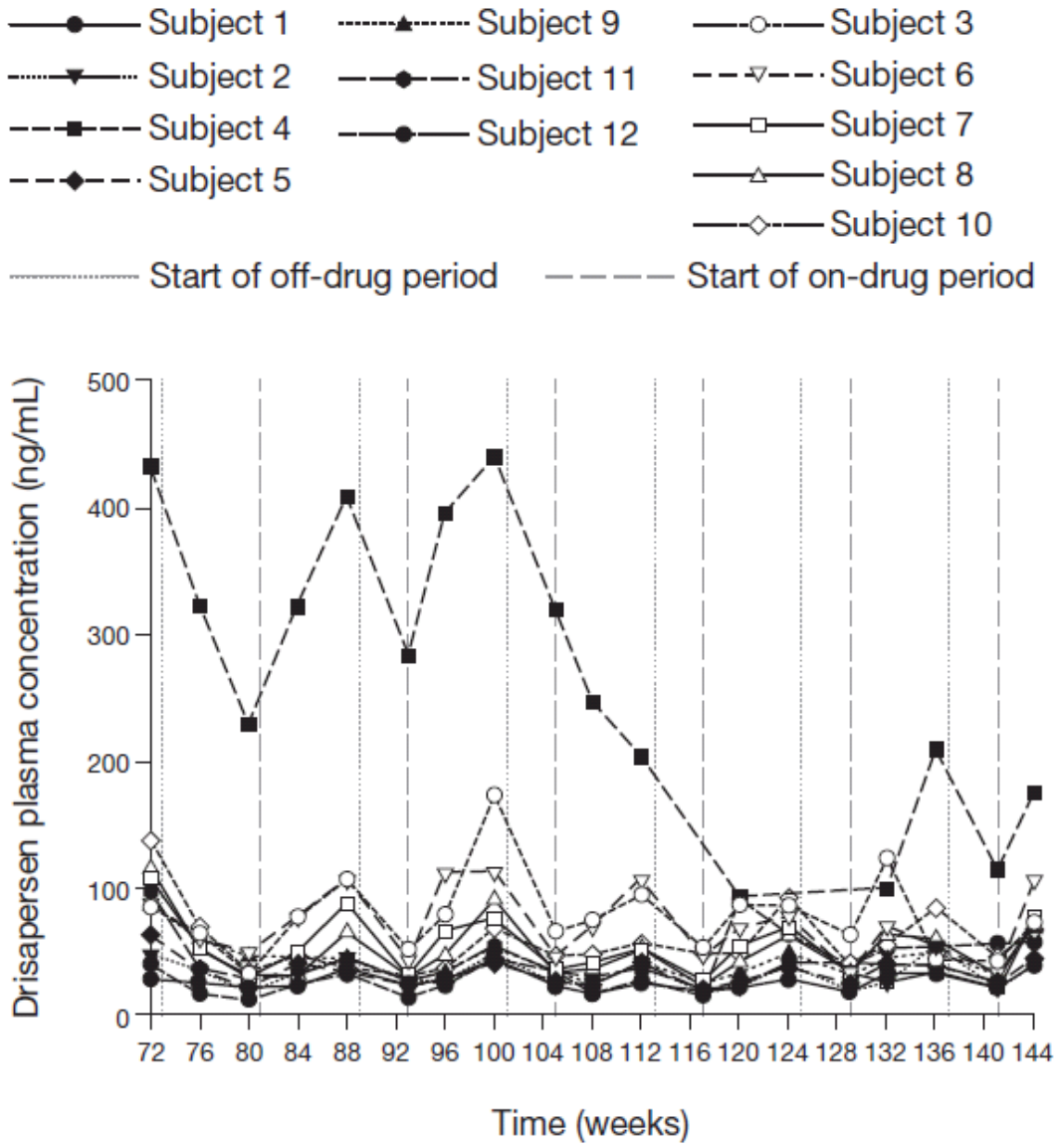
	Number of subjects	Change from extension study baseline in distance walked (meters)	
Extension study baseline	7	Mean (SD)	Median (range)
Week 12	7	41.9 (28.40)	50 (−6 to 69)
Week 48	7	63.1 (47.24)	79 (−15 to 127)
Week 93	7	88.1 (82.67)	95 (−67 to 190)
Week 105	7	76.4 (102.59)	96 (–115 to 169)
Week 117	7	87.0 (100.37)	128 (–116 to 188)
Week 129	7	76.6 (110.85)	104 (–150 to 192)
Week 141	7	65.9 (110.86)	109 (–138 to 201)
Extension study baseline	3	–	–
Week 12	3	19.7 (27.68)	16 (−6 to 49)
Week 48	3	−52.0 (89.44)	−97 (−110 to 51)
Week 93	3	−168.7 (146.41)	−243 (−263 to 0)
Week 105	3	–158.7 (163.70)	–243 (–263 to 30)
Week 117	3	–160.0 (161.39)	–243 (–263 to 26)
Week 129	3	–180.7 (125.68)	–243 (–263 to –36)
Week 141	3	–181.0 (125.11)	–243 (–263 to –37)

Missing values replaced by zero for subjects who became unable to complete the test at later visits.

*Two subjects were not able to complete the 6MWD test at extension baseline; two subjects did not complete the 6MWD test at week 60.

6MWD: 6-minute walk distance; SD, standard deviation.

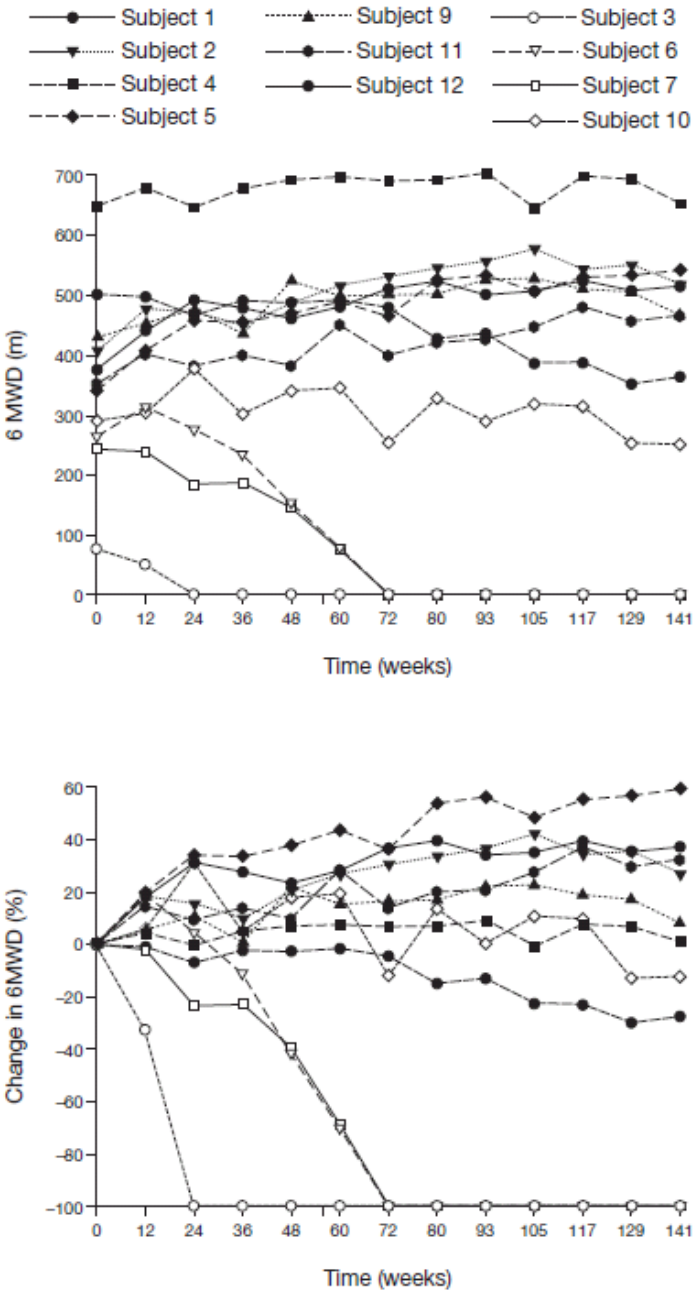
Figure 1. Individual drisapersen trough concentrations from weeks 72 to 144.



Subject 4 had an additional treatment break from weeks 101 to 128 inclusive.

Subjects who were in plateau phase at extension baseline have closed symbols; subjects who were in decline at extension baseline have open symbols.

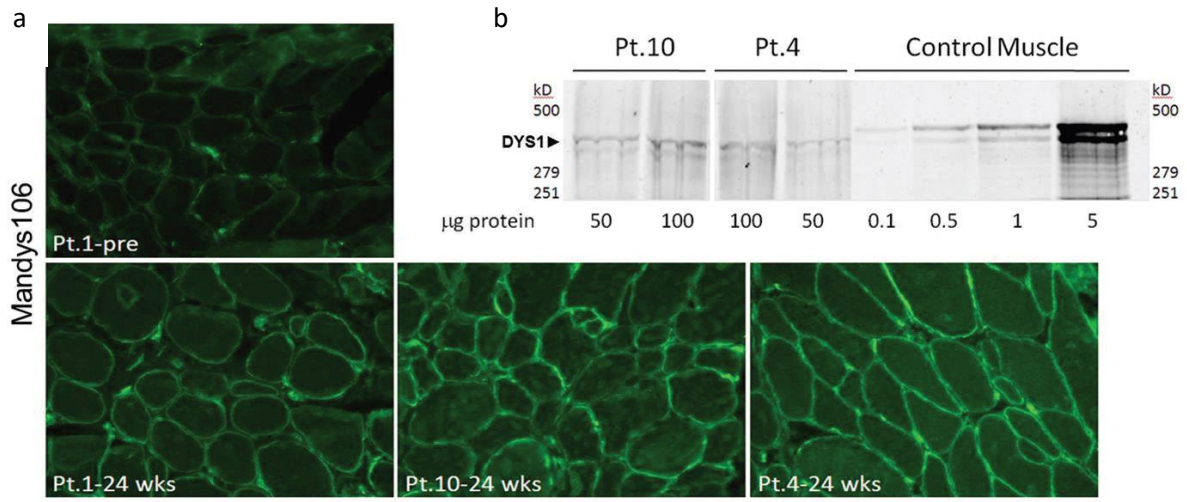
Figure 2. Change from extension study baseline in 6MWD, by visit over 141 weeks.



Data shown are for all subjects who took the test,regardless of whether the 6MWD test was completed: Subject 3 was unable to complete the 6MWD test at extension study baseline and at week 12. Subject 6 and 7 did not complete 6MWT at week 60. One subject (subject 8) was non-ambulant at study entry and did not participate in any 6MWD tests and is not shown here. Subjects who were in plateau phase at extension baseline have closed symbols; subjects who were in decline at extension baseline have open symbols.

6MWD: 6-minute walk distance.

Figure 3. Dystrophin expression in muscle biopsy cross-sections following treatment with drisapersen for 24 weeks. Samples were analyzed using (a) immuno-fluorescence and (b) Western blot analysis [17,18].



DYS1: NCL-DYS1 dystrophin monoclonal antibody; kD: kilodalton; MANDYS106: MANDYS106 dystrophin antibody; Pt: patient; wks: weeks.

Supporting Table S1a. Summary of the change from extension study baseline to each visit over 141 weeks for subjects in plateau phase at extension baseline, for timed functional tests (intent-to-treat population).

	Number of subjects (n = 7)	10-meter walk/run (seconds)		Rising from floor (seconds)		Four-stair climb (seconds)	
		Mean (SD)	Median	Mean (SD)	Median	Mean (SD)	Median
Week 8	7	0.05 (0.309)	0.090	0.20 (0.454)	0.430	–0.11 (0.318)	–0.090
Week 12	7	0.05 (0.392)	–0.050	–0.03 (0.374)	0.140	–0.18 (0.477)	–0.230
Week 16	7	0.40 (0.600)	0.480	0.01 (0.210)	0.070	–0.10 (0.420)	–0.140
Week 20	7	0.34 (0.630)	0.210	0.30 (0.302)	0.220	–0.20 (0.637)	–0.200
Week 24	7	0.24 (0.232)	0.240	–0.04 (0.300)	–0.060	–0.07 (0.381)	–0.040
Week 28	7	0.20 (0.500)	–0.050	0.26 (0.483)	0.280	–0.14 (0.580)	–0.140
Week 36	7	–0.01 (0.450)	0.050	0.03 (0.364)	0.110	–0.07 (0.462)	–0.210
Week 48	7	0.21 (0.375)	0.200	0.10 (0.380)	0.030	–0.12 (0.580)	–0.180
Week 60	7	0.01 (0.393)	–0.020	0.12 (0.320)	0.150	–0.03 (0.744)	–0.110
Week 72	7	0.22 (0.451)	0.180	0.34 (0.680)	0.430	0.02 (0.745)	–0.040
Week 80	7	0.29 (0.583)	0.270	0.49 (1.154)	0.440	0.26 (0.803)	0
Week 93	7	0.14 (0.468)	0.060	0.15 (0.764)	0.110	0.19 (0.939)	0.130
Week 105	7	0.30 (0.738)	0.210	0.86 (1.453)	0.270	0.39 (1.543)	–0.100
Week 117	7	0.43 (0.721)	0.240	1.06 (1.976)	0.320	0.24 (1.251)	–0.140
Week 129	7	0.58 (0.714)	0.360	4.45 (10.037)	0.270	0.49 (1.417)	0.230
Week 141	7	0.68 (0.938)	0.290	1.61 (3.13)	0.720	0.60 (1.460)	0.530

SD: standard deviation.

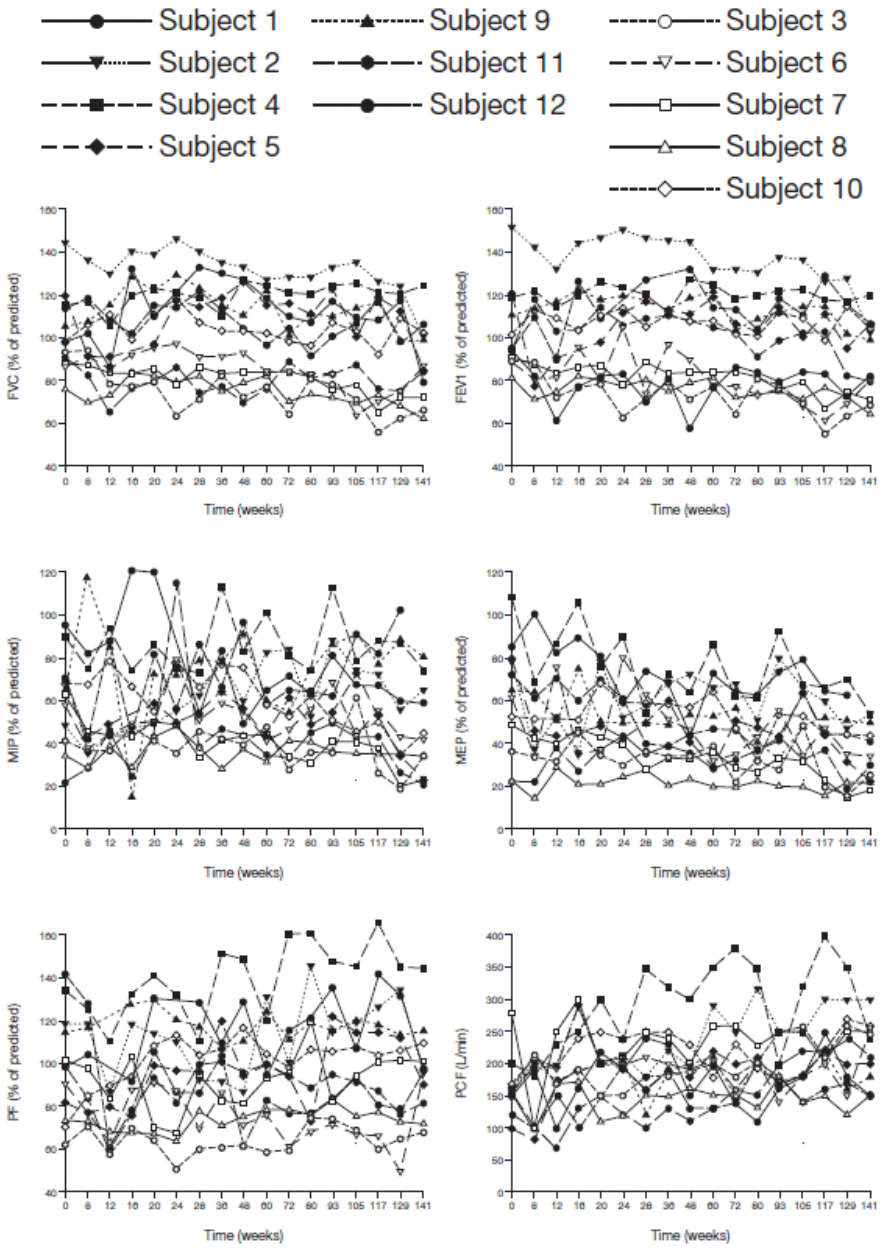
Supporting Table S1b. Summary of the change from extension study baseline to each visit over 141 weeks for subjects in decline at extension baseline, for timed functional tests (intent-to-treat population).

	Number of subjects (n = 5)	10-meter walk/run (seconds)		Rising from floor (seconds)		Four-stair climb (seconds)	
		Mean (SD)	Median	Mean (SD)	Median	Mean (SD)	Median
Week 8	4*	0.14 (2.115)	0.865	1.57 (4.427)	1.570	–2.16 (2.633)	–1.775
Week 12	4*	0.65 (0.519)	0.745	–0.76 (0.983)	–0.775	–2.63 (3.044)	–2.110
Week 16	4*	0.97 (0.539)	1.175	0.22 (1.761)	0.215	–0.93 (2.763)	–0.855
Week 20	3*	1.05 (0.943)	1.290	3.45 (6.280)	3.450	–1.04 (1.774)	–2.000
Week 24	3*‡	0.78 (0.467)	0.820	–0.22 (2.496)	–0.215	–0.57 (4.250)	–0.565
Week 28	3*	2.17 (0.969)	2.630	1.58 (0.933)	1.580	–1.70 (3.043)	–3.120
Week 36	3*‡	1.73 (0.900)	1.730	3.59 (0.693)	3.590	–1.40 (2.906)	–1.395
Week 48	3†‡	2.24 (1.233)	1.620	1.67	1.670	–1.84 (1.146)	–1.840
Week 60	3†	3.11 (1.533)	3.290	7.48	7.480	–0.43 (0.477)	–0.680
Week 72	2†	5.14 (3.019)	5.135	11.60	11.600	0.11 (2.864)	0.105
Week 80	2†	8.95 (10.006)	8.945	6.79	6.790	0.53 (1.386)	0.530
Week 93	1†‡	2.94	2.940	12.97	12.970	2.77 (0.707)	2.770
Week 105	1	3.63	3.630	18.67	18.670	7.98	7.98
Week 117	1∞	4.84	4.840	–	–	5.07	5.07
Week 129	1∞	3.94	3.940	–	–	9.57	9.57
Week 141	1∞	5.54	5.540	–	–	9.57	9.57

*n = 2 for rising from floor; †n = 1 for rising from floor; ‡ n = 2 for four-stair climb; ∞n = 0 for rising from floor.

SD: standard deviation.

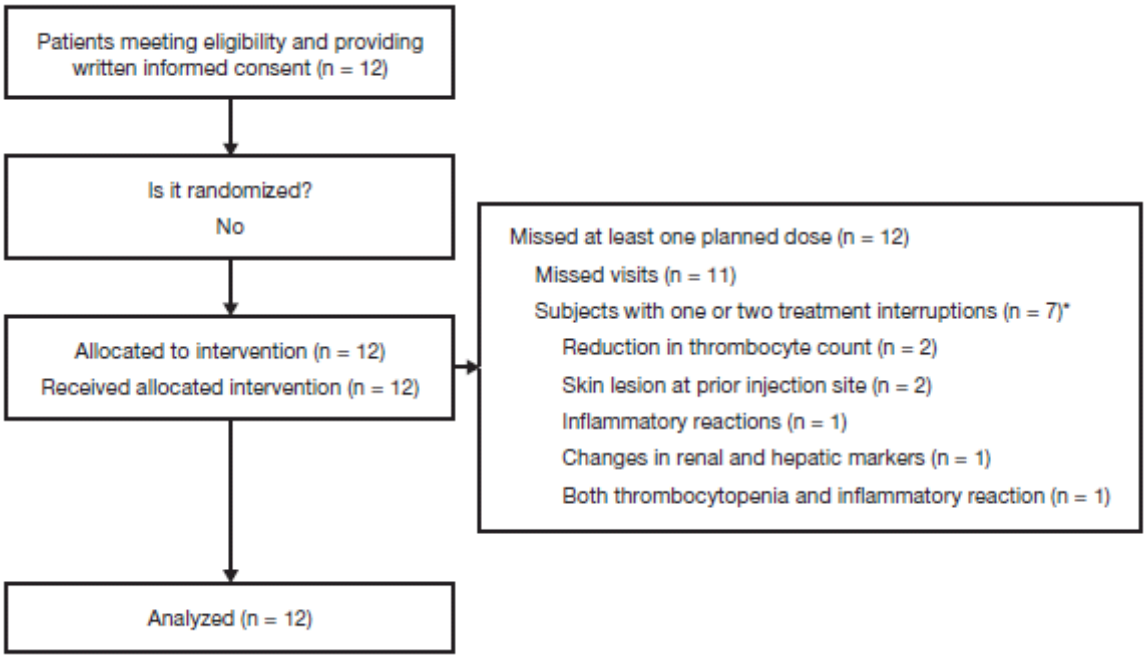
Supporting Figure S1. Individual profiles for spirometry parameters over 141 weeks



FEV_1: forced expiratory volume in 1 second; FVC: forced vital capacity; MEP: maximal expiratory pressure; MIP: maximal inspiratory pressure; PCF: peak cough flow; PF: peak flow

Only '% predicted' parameters and PCF are shown. Subjects who were in plateau phase at extension baseline have closed symbols; subjects who were in decline at extension baseline have open symbols.

Supporting Figure S2. Patient flow diagram



*Excluding planned washout and off-drug periods

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Chapter III — Part B

The Six Minute Walk Test as outcome measure for the ambulant stage of DMD

III.B.1

TEST–RETEST RELIABILITY AND DEVELOPMENTAL EVOLUTION OF THE 6-MIN WALK TEST IN CAUCASIAN BOYS AGED 5–12 YEARS

Published as:

Test–retest reliability and developmental evolution of the 6-min walk test in Caucasian boys aged 5–12 years

Nathalie Goemans, Katrijn Klingels, Marleen van den Hauwe, Anneleen Van Orshoven, Sofie Vanpraet, Hilde Feys, Gunnar Buyse

Neuromuscular disorders : NMD 2013;**23**(1):19-24)

III.B.2

SIX-MINUTE WALK TEST: REFERENCE VALUES AND PREDICTION EQUATION IN HEALTHY BOYS AGED 5 TO 12 YEARS

Manuscript Submitted:

Six-minute walk test: reference values and prediction equation in healthy boys aged 5 to12 years

Nathalie Goemans [1*], Katrijn Klingels [2], Marleen van den Hauwe [1,] Stefanie Boons [2], Liese Verstraete [2], Charlotte Peeters [2], Hilde Feys [2], Gunnar Buyse [1]

ABSTRACT

OBJECTIVE: This study aimed to (1) generate normative data in healthy boys aged 5-12 years for the 6-minute walk test (6MWT), an outcome measure currently used in clinical trials in Duchenne muscular dystrophy (DMD), (2) to describe the relation with anthropometric variables and myometry, and (3) to compare our data with published equations.

METHODS: The 6MWT was conducted in 442 boys according to a standardized protocol, as currently used in clinical trials in DMD. Maximal voluntary isometric contractions for knee flexion and extension were recorded with a hand-held myometer.

RESULTS: The 6MWD increased significantly with age, from 478.0 ±44.1m at age 5, to 650.0 ±76.8m at age 12, with the steepest increase between 5 and 8 years. Age-and height related percentile curves of the 6MWD were developed. Correlations with anthropometric variables were fair to good (age r=0.60, height r=0.57, weight r=0.44). Myometric variables (knee flexors and extensors) showed correlations of 0.46 and 0.50 respectively. When dividing into two age categories (5-8 years, 9-12 years), these magnitudes of correlations only applied to the younger age group. Additionally, predicted values were calculated according to available reference equations (Geiger and Ben Saad), indicating an overestimation by those equations. Finally, the Geiger equation was refitted to our population.

CONCLUSION: The percentile curves according to age and height provide a useful tool in the assessment of ambulatory capacity in boys aged 5 to 12 years. Significant correlations with anthropometric variables and myometry were only found in the 5-8 years age group. The Geiger prediction equation, currently used to assess ambulatory capacity in DMD was refitted to obtain a more accurate prediction model based on a large sample with a homogenous distribution across the age categories 5 to 12 years and applying the methodology as currently used in clinical trials in DMD.

INTRODUCTION

The 6-minute walk test (6MWT), a measure of function and endurance originating from the cardiorespiratory field, assesses the distance a subject is able to walk in six minutes at a normal pace (6-minute walking distance, 6MWD)[1]. This measure reflects the physical capacity and walking function at a submaximal level and has been accepted as a clinically meaningful outcome measure by the regulatory authorities in registration-directed clinical trials in neuromuscular and neurometabolic disorders[2].The 6MWT, slightly modified from the original American Thoracic Society guidelines, has been validated in Duchenne Muscular Dystrophy (DMD), a severe X linked progressive disorder, and is currently used as (primary) endpoint in therapeutic trials in ambulatory boys with DMD[3] Moreover, the correlation of 6MWT with the Pediatric Outcomes Data Collection Instrument (PODCI), a Quality of Life (QoL) instrument score, has been reported recently in DMD [4] confirming its applicability to detect clinically meaningful changes in ambulant DMD.

The evolution of the 6MWD in DMD is characterized by a specific pattern: despite of the degenerative character of this disease, recent studies showed an increment in the 6MWD up to approximately the age of 7 followed by a decline which becomes precipitous around the age of 12 years [5-8]. In order to account for maturational influences, it has been suggested to convert raw data of the 6MWD into percent predicted values based on normative data to describe the natural evolution or the impact of interventions in this disease against the background of growth and development [9]. This method is commonly applied for other outcome measures such as respiratory function [10,11].

Different studies have reported normative data and prediction equations for the 6MWT in healthy children from different ethnic, environmental and geographical backgrounds [12-20]. However, data on 6MWT are scarce in young boys of Western European descent in the age range and according to the procedures currently used in clinical trials in DMD. Moreover, study methods and testing procedures differ from one study to another, with different track lengths and testing instructions or pooling of data from both genders, hampering further comparison [12-20]. [19,21-27] Furthermore, recent studies indicated that published equations may over- or underestimate 6MWD when applied to other populations [12,15,18,19], highlighting that further research is warranted to validate the existing equations in large samples of typically developing children, applying standardized procedures currently used in DMD trials.

Predictive factors for the 6MWD have been explored such as age and anthropometric variables, underscoring the influence of age and height on this outcome measure [12-20]. However, the impact of other variables such as muscle strength has not yet been reported in children.

To address those issues, we investigated the 6MWD in typically developing young boys. In a previous study, we reported on the reliability of this outcome measure and the developmental evolution of the

6MWT in narrow age subcategories in boys between age 5 and 12 years, an age range of particular interest for clinical trials in ambulant DMD [28]. In this current study, the data set was further expanded to a larger cohort in order to generate reference values and percentile curves for the 6MWT in typically developing boys of this age. A second aim was to investigate the relation of this functional capacity measure with age, anthropometric variables and underlying muscle strength. Finally, we evaluated the applicability of published reference equations to our population.

METHODS

Participants

Typically developing boys aged 5-12 years were recruited from five randomly selected local primary schools in Belgium between January and May 2012. Children had to be able to understand and fully comply with the assessments. A questionnaire to identify health related problems was completed by the parents a few days before testing. Children with known chronic cardiac, respiratory, neurological or musculoskeletal disorders were excluded. Participants were sampled across eight age subcategories with one year interval between 5.0 and 13.0 year and six height subcategories with 10 cm interval from 105 cm on. The final sample included also 90 boys from our previously reported reliability study recruited based on the same in- and exclusion criteria and who completed the 6MWT according to the same procedure [28]

Ethics statement

Ethical approval was obtained from the institutional committee of the University Hospitals Leuven and the institutional boards of the participating schools. Parents of all children gave their written informed consent.

Test Procedure

The participants' weight and height were determined using standardized anthropometric methods before the testing. The 6MWT was conducted according to a standardized protocol, as described by McDonald et al.[3]. The test was performed in a flat, straight corridor. Each boy walked for six minutes counterclockwise at his preferred pace along a 25 m tape line, with cones placed at each end of the course. Evaluators gave a standardized demonstration prior to the test. Subsequently, one

practice trial over one track length was done to ensure that the child understood the instructions. During the test, each boy was followed by a 'safety chaser' giving limited standardized encouragements. Testing was performed by three physiotherapists experienced with the test procedure.

Myometry

Knee flexion and extension strength was measured in both legs using a calibrated MicroFET2 handheld myometer and expressed in Newton (N).Testing was performed in a standardized starting position, sitting with hips and knee in 90° of flexion and no feet contact with the floor. The investigator fixated the femur above the knee and placed the handheld myometer at the posterior part of the calcaneus for knee flexion and at the anterior part of the shin just proximally to the ankle joint for knee extension [29]. The highest value of three maximum isometric contractions was recorded in knee flexors and extensors bilaterally by using the ` 'make' technique which requires the patient to exert a maximal isometric contraction while the examiner holds the dynamometer in a fixed position.
To rule out the influence of left-right differences, summed scores for left and right leg were calculated,

Statistical analysis

Descriptive statistics were applied for the different variables within the total sample and within each age and height group. Data were tested for normality by Shapiro-Wilk tests and graphically checked for symmetry.
To construct percentile curves, the following percentiles of 6MWT were estimated for different values of age and height using a quantile regression analysis: 5%, 10%, 25%, 50%, 75%, 90% and 95%. The interior algorithm was used to estimate the regression parameters. The explanatory variables age and height were included using restricted cubic splines so that no assumptions had to be made regarding the type of association with the results of the 6MWT. Correlation analysis between 6MWD and age, anthropometric, and myometric variables was performed in the subgroup of 352 subjects in whom myometric data were available. Pearson product-moment correlation coefficients (r) were calculated for the total group and for two age categories, from 5 to 8 and 9 to 12 years. Correlation coefficients of >0.70 were considered as high, between 0.50-0.70 good, between 0.30-0.50 fair and of <0.30 weak or no association [30].
Measured 6MWDs were compared with the distances predicted based on the published reference equations by Geiger et al. in Austria [14] (6MWD (m) = 196.72 + 39.81 x age (years) - 1.36 x age^2 (years) +138.28 x height (m)) and Ben Saad et al. in North-Africa [12] (6MWD (m) = 4.63 x height (cm) - 3.53 x weight (kg) + 10.42 x age (years) + 56.32) using parametric paired t-tests and scatterplots.

Finally, a refitted Geiger model was obtained by applying the Geiger model to 100 bootstrap samples and averaging the regression coefficients across all bootstrap samples to obtain the final estimate of the regression coefficients.

All analyses were performed using SAS software, version 9.2 and SAS Enterprise Guide.

RESULTS

Participant characteristics

In the current study, 368 medical questionnaires were filled out correctly and returned by the parents. Sixteen children were excluded based on one or more exclusion criteria (chronic cardiovascular, respiratory or motor disorders). A total of 442 boys, including the 90 subjects of the first study [28], were included in the analysis. All boys were of Western European descent. Anthropometric data, age, 6MWD and velocity per age and height subcategory are reported in Table 1. Mean age, height and weight of the total group were 9.0 ± 2.3 years, 135 ± 14.16 cm and 31.5 ± 9.63 kg respectively.

6MWD and velocity

Descriptive data of 6MWD and velocity are given in Table 1.The overall mean 6MWD was 582.2 ± 88.2 m. Mean 6MWD increased between the age of 5 and 12 from 478.0 ± 44.1m to 650.0 ± 76.8m. In parallel, velocity increased from 79.7 ± 7.4 m/min at 5 years to 108.3 ± 12.8 m/min at 12 years. The steepest increase was observed between the age of 5 and 8 years (from 478.0 m to 604.3 m). Beyond this age, the 6MWD tended to stabilize (from 604.3 m to 650.0 m). In the height subcategories, distance increased significantly across the board with an increase of mean 6MWD from 468.6 ± 46.5 to 651.2 ± 88 in the smallest boys up to 651.2 ± 88.3 in the tallest boys with velocities of respectively 78.1 ± 7.8 m/min to 108.5 ± 14.7 m/min. A mild flattening was seen from a height of 140 cm.

Percentile curves according to age and height

Percentiles 5%, 10%, 25%, 50%, 75%, 90% and 95% of 6MWT were estimated for different values of age and of height and are shown in Figures 1 and 2.

Myometry

Myometry data were obtained in 352 subjects. The mean muscle strength ± SD for the total group for the knee flexors and extensors was 189.2 ± 53.9N and 346.8 ± 112.7N, respectively (Table 2). Muscle strength increased with age for both knee flexors and extensors. Myometric variables were also calculated for the two age cohorts of 5 to 8 and 9 to 12 years.

Correlations of 6MWD with age anthropometric and myometric variables

Correlations between 6MWD and predictive variables were calculated for the total group and for the two age subcategories (5 to 8 and 9 to 12 years) (Table 3). For the total group, height, and weight were significantly correlated with the 6MWD ($r = 0.57$; $r = 0.44$ respectively, $p < 0.0001$). Age showed the highest correlation with the 6MWD ($r = 0.60$; $p < 0.0001$).

The 6MWD showed a fair to good correlation with knee flexion ($r= 0.46$; $p<0.05$), and knee extension ($r=0.50$; $p<0.05$). Inspection of the correlation coefficients in the two age subcategories revealed a clear difference between both groups. In the younger children, significantly higher correlations were found between all variables and 6MWD, compared to the older children. Anthropometric factors showed a good correlation with the 6MWD in the young age category, with coefficients above 0.50 while in the older age group, no to weak associations were found. The same trend was observed for the myometric variables, showing fair to good correlations in the younger age category ($r=0.46$ - 0.52) and weak or no association with 6MWD in the older age group.

6MWD reference equation

Table 4 compares our results to mean values of previous 6MWT studies in healthy children from different countries and ethnicities. Overall, data measured in Austrian [14] and North African boys [12] were higher in comparison with the present study. In contrast, measured data in children from U.S, U.K and South America were clearly lower [15,16,20].

A comparison with previously published reference equations was performed by calculating predicted values for our measured data based on the reference equations of Geiger et al.[14] and Ben Saad et al. [12]. Mean differences between our actual measured values and the predicted values by Geiger et al.[14] and Ben Saad et al. [12] were -33.7 m (95% CI 27.3 m - 40.1 m) and -76.1 m (95% CI 69.5 m - 82.6 m) respectively, indicating a significant difference (both $p < 0.0001$).

Comparison with Geiger prediction model

Figure 3 illustrates the observed 6MWD versus the predicted values based on the Geiger equation. This application resulted in a systematic overestimation with an associated R^2 value of 0.17.Therefore the Geiger model was refitted for our population with resulting R^2 value of 0.41. The following refitted Geiger equation was obtained:

6MWD = 86.795 + 74.547 x age (years) – 3.018 x age^2 (years) +63.204 x height (m).

Figure 4 shows the predicted versus observed 6MWT results for the refitted Geiger equation, indicating a better distribution around the identity line.

DISCUSSION

This study established normative data for the 6MWT, provided age- and height specific centile curves and investigated its correlation with age, anthropometric and myometric variables in a large cohort of 442 healthy boys aged 5-12 years, an age group of particular interest in relation to studies in ambulant DMD boys. In addition, a comparison with previously published reference data and prediction equations was made and the commonly used Geiger equation was refitted for this population. To our knowledge, this is the largest data set reporting on 6MWT in typically developing boys of this age range, using the methodology and track length currently applied in DMD studies [3].

Our findings confirmed previous reports of a significant improvement in 6MWD with increasing age [16,28]. The strongest increase was found between the age of 5 and 8 years, which is in accordance to previous reports from Austrian [14] and British children [16]. After the age of eight years, 6MWD tends to stabilize despite a further evolution of the anthropometric variables [12,14,16], which may possibly be explained by the developmental maturity of the human gait pattern and muscle activation patterns at this age. Moreover, the velocity of the walking pattern has a known physiological limit from approximately 5 km/h [31,32]. At higher velocity there is a disproportionate increase in energy expenditure, indicating that in older children where a full maturation of gait pattern is achieved, a switch to a running pattern is more economical from an energy standpoint and required to further increase velocity. It should however be noted that DMD subjects are typically unable to run due to their muscle weakness and generally show a decline in 6MWD after the age of 8.

A steady increase was noticed over the height subcategories, followed by a flattening of the curve from height 135 cm on, which is in accordance with the data of Lammers et al[16]. Both age- and height specific centiles curves were constructed, which could provide a user-friendly method in the prediction of 6MWD in boys of this age range. We were particularly interested in investigating the relation of the 6MWD with height and to provide height-specific references in addition to the age specific normative data, as those could be useful in DMD boys, known to have a stunted growth compared to their peers, which is aggravated by the chronic use of corticosteroids.

Height-specific reference centiles have been published based on data from 805 boys and 610 girls from Chinese ethnicity (aged 7-16 years) [17]. Comparison of the reported mean 6MWD and inspection of the centile curves for boys indicated slightly higher values for the Chinese population compared to our sample. This difference could be explained by differences in methodology, with the use of a longer track length in the Chinese study and /or by ethnic differences as suggested by other authors [12,15,18].

A second aim was to investigate the correlation of age, anthropometric variables and leg strength with the 6MWD. The highest correlation was found for age and height with correlation coefficients of 0.60 and 0.57 respectively, which confirms previously reported findings

[12,14,16,17,20]. A fair to good correlation was found between 6MWD and muscle strength in knee flexors and extensors. However, these magnitude of correlations appeared mainly driven by the age group 5 to 8 years and disappeared in the older age group. Discordance between muscle strength and function tests has been reported by other authors [33]. Finally, our data indicated that additional factors to age, anthropometric and myometric variables influence the 6MWD, which requires further investigations, especially in older children. Genetic predisposition, motor abilities, physical fitness and training as well as motivation of the individual child during a self-paced test may further impact on the measured 6MWD.

In addition, we aimed to compare our normative data with previous reports. Literature review revealed eight studies [12-18,20] that reported measured values and/or prediction equations for the 6MWT in healthy children from different ethnic, environmental and geographical backgrounds. Our 6MWD data showed consistently lower values than the measured values of Geiger et al.[14] and Ben Saad et al.[12] Adversely, measured 6MWDs in North American [15],British [16]], and South American boys [20] were lower than our measured values. However, a direct comparison of our findings to the reference values reported should be done cautiously since different testing procedures were applied and pooling of data for age and gender was done differently across studies [12-14,16,17,20]. Differences in testing procedures may contribute to the variability in distances walked in six minutes, such as influencing the motivation of the child with the use of a measuring wheel [14] or interfering with the test procedure by measuring additional variables [16]. The influence of track length on a child's 6MWD is also not clear from previous reports [1,15]. Furthermore, the pooling of data from both gender [16,20] may possibly explain the lower achieved distances in those studies, although reports on the influence of gender are conflicting [14-17,20].

The sample sizes were highly variable as well, with some studies reporting data from very limited samples [15]. Finally, the different ethnical backgrounds as well as anthropometric differences may further explain some of the variability across the different studies [12,15,18].

This study was set up to collect data on 6MWT in typically developing children as a reference for DMD boys. In this progressive disease of childhood, muscle loss and functional decline occur against a background of growth and development, resulting in the observation of an increase in 6MWD in younger children with DMD despite progressive impairment [5-8]. Converting raw 6MWD values into percentage predicted values based on a reference equation derived from age- and height matched peers seems the most straightforward method to account for those maturational influences [9]. Henricson et al.[9] concluded that the equation of Geiger et al.[14] was the most appropriate for their American DMD boys because of the large age range, Caucasian ethnicity and absence of heart rate as an independent variable. This equation has been derived from data collected from 280 healthy Austrian boys, unequally distributed between age 3 and18 years, with the largest sample in the age category 12 to 15 years, using a slightly different testing procedure (use of a measuring wheel, running allowed in

the younger ones, 20m track). We questioned whether this equation would be the most accurate for our sample as well.

When implementing the data of our study into the Geiger equation [14], the mean predicted value was higher than the originally measured value. This overestimation was observed in all age categories. The same observation was made for the North African reference equation [12]. As the Geiger equation has yet been implemented in DMD studies, we refitted this equation to our population to obtain a more accurate prediction model based on a larger sample with a homogenous distribution across age categories and applying a methodology as currently used in clinical trials in DMD.

This study is prone to several possible limitations. First, the data were obtained by a team of three different testers. Nonetheless, to limit possible inter-rater variation, all evaluators were well trained and followed a standardized operational protocol. Secondly, this study did not investigate parameters of endurance such as heart rate at baseline and post exercise, a variable reported to correlate with 6MWD [17,20]. However, this study was set up to explore variables that could be useful in relation to DMD. In this disease a higher baseline heart rate and stunted reactions of heart rate to exercise have been reported [3], impeding the use of those variables in equations derived from healthy boys. Finally, our data were limited to elementary school boys aged 5 to 12 years. Further studies are required to generate data in pubertal and adolescent boys, especially since loss of ambulation has shifted to an older age in the contemporary natural history of DMD boys under chronic steroid treatment [34]

Despite these limitations, this study was the first to establish normative data of the 6MWT in a large cohort (N = 442) of healthy boys (5-12 years) and this in relation to age and height, using the same methodology as currently used in DMD studies [3].The normal values were measured in narrow age subcategories of one year with each category containing minimum 48 children. This study provided age- and height specific centile curves which might be clinically useful to judge the performance of DMD boys, the evolution of the DMD disease and the response to therapeutic intervention. This study confirmed previous reports on correlations between 6MWD with age and anthropometric variables in young children. It was however the first study to explore relations between myometric variables and the 6MWT in a large cohort of healthy subjects. Our study indicated that those variables do influence the 6MWD, especially up to the age of 9, however further research is required to identify additional factors influencing the 6MWT, especially in the older age group. Finally, the Geiger prediction equation, which use has been advocated for DMD studies, was refitted to this large sample of boys aged 5 to 12 years.

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Table 1: Participants characteristics, six-minute walk distance and velocity according to age and height categories (Mean values ± standard deviation).

		N	Age (y)	Height (cm)	Weight (kg)	Distance (m)	Velocity (m/min)
Age	**5 years**	57	5.6 ± 0.3	115±5.69	20.7 ± 2.5	478.0 ± 44.1	79.7 ± 7.4
	6 years	52	6.5 ± 0.3	121 ± 6.13	23.6 ± 3.2	516.1 ± 61.8	86.0 ± 10.3
	7 years	56	7.5 ± 0.3	127 ± 4.41	25.8 ± 3.4	559.2 ± 65.4	93.2 ± 10.9
	8 years	55	8.5 ± 0.3	133 ± 5.93	29.0 ± 4.2	604.3 ± 72.0	100.7 ± 12.0
	9 years	60	9.5 ± 0.3	139 ± 5.61	32.8 ± 6.0	595.7 ± 69.0	99.3 ± 11.5
	10 years	53	10.5 ± 0.3	144 ± 7.28	35.5 ± 6.2	633.1 ± 70.0	105.5 ± 11.6
	11 years	61	11.4 ± 0.3	148 ± 6.73	39.5 ± 7.8	625.9 ± 83.0	104.3 ± 13.8
	12 years	48	12.5 ± 0.3	154 ± 6.74	46.7 ± 7.3	650.0 ± 76.8	108.3 ± 12.8
Height	**105-114 cm**	43	5.7 ± 0.4	111 ± 2.43	19.4 ± 1.9	468.6 ± 46.5	78.1 ± 7.8
	115-124 cm	70	6.6 ± 0.9	120 ± 2.83	23.1 ± 1.9	529.0 ± 65.6	88.2 ± 10.9
	125-134 cm	104	8.0 ± 1.1	129 ± 3.09	27.3 ± 3.6	576.5 ± 67.8	96.1 ± 11.3
	135-144 cm	100	9.8 ± 1.2	140 ± 2.83	32.7 ± 4.2	605.3 ± 73.3	100.9 ± 12.2
	145-154 cm	89	11.2 ± 0.7	149 ± 2.64	40.6 ± 6.5	631.6 ± 80.2	105.3 ± 13.4
	>155 cm	36	12.1 ± 0.7	159 ± 4.65	49.3 ± 7.1	651.2 ± 88.3	108.5 ± 14.7
TOTAL		442	9.0 ± 2.3	135 ± 14.16	31.5 ± 9.63	582.2 ± 88.2	97.0 ± 14.7

Table 2: Mean values (standard deviation) of myometric variables for the total group and according to eight and two age categories.

	Total group	Eight age categories								Two age categories	
	5-12 years	**5 years**	**6 years**	**7 years**	**8 years**	**9 years**	**10 years**	**11 years**	**12 years**	**5-8 years**	**9-12 years**
N	352	42	42	43	45	46	44	48	42	172	180
					Myometric variables						
Flexors	189.2	127.0	143.0	168.4	182.0	194.1	219.4	214.7	260.2	155.6	221.2
(N)	(53.9)	(27.1)	(28.3)	(30.5)	(25.0)	(32.5)	(45.5)	(45.9)	(48.8)	(34.9)	(49.2)
Extensors	346.8	221.1	250.3	301.6	328.5	366.8	417.4	405.5	472.3	276.5	414.1
(N)	(112.7)	(39.3)	(55.0)	(55.0)	(65.4)	(97.2)	(87.1)	(97.3)	(121.4)	(65.8)	(107.0)

Table 3: Correlation coefficients between six-minute walk distance and anthropometric and myometric variables for the total group and for two age categories with statistical comparison.

	Total group	**Two age categories**		**z-value**	**p-value**
	5-12 years	**5-8 years**	**9-12 years**		
N	352	172	180		
Age	0.60*	0.65*	0.16*	5.71	p<0.0001
Weight (kg)	0.44*	0.50*	-0.02	5.29	p<0.0001
Height (cm)	0.57*	0.60*	0.09	5.61	p<0.0001
Flexors (N)	0.46*	0.46*	0.08	3.88	p=0.0001
Extensors (N)	0.50*	0.52*	0.16*	3.86	p=0.0001

*p<0.05

Table 4: Measured 6MWD of the present study and comparison with reported and predicted 6MWD.

	5 years	6 years	7 years	8 years	9 years	10 years	11 years	12 years
Measured 6MWD of Present study (m) Belgium	478.0 ± 44.1	516.1 ± 61.8	559.2 ± 65.4	604.3 ± 72.0	595.7 ± 69.0	633.1 ± 70.0	625.9 ± 83.0	650.0 ± 76.8
Predicted 6MWD by Geiger et al. (14) (m)	527.6 ± 11.1	556.9 ± 12.1	586.9 ± 7.7	611.9 ± 9.1	635.4 ± 8.5	655.6 ± 11.2	670.2 ± 9.6	685.5 ± 9.7
Predicted 6MWD by Ben Saad et al. (12) (m)	566.3 ± 20.6	595.9 ± 21.1	624.3 ± 17.2	652.7 ± 18.3	677.0 ± 18.2	703.4 ± 25.4	718.8 ± 23.0	730.5 ± 29.6
Measured 6MWD of Geiger et al (14) (m) Austria	536. 5 ± 95.6		577.8 ± 56.1 (6-8 y)			672.8 ± 61.6 (9-11 y)		697.8 ± 74.7 (12-14y)
Measured 6MWD of Ben Saad et al. (12) (m) North Africa		543 ± 33 (6-7 y)		667 ± 55 (8-9y)		715 ± 31 (10-11 y)		725 ± 68 (12-13y)
Measured 6MWD of Klepper et al (15) (m) United States			534.5 ± 60.3 (7-8 y)		515.8 ± 81.4	497.9 ± 74.0	534.9 ± 88.9	
Measured 6MWD of Lammers et al (16)* (m) United Kingdom	420 ± 39	463 ± 40	488 ± 35	483 ± 40	496 ± 53	506 ± 45	512 ± 41	
Measured 6MWD of Priesnitz et al (20)* (m) South America		508.3 ± 54.0	550.2 ± 61.6	556.7 ± 67.2	594.2 ± 60.6	602.4 ± 61.1	608.0 ± 54.3	618.1 ± 51.4

* All measured 6MWD are reported for males, except Lammers et al. and Priesnitz et al. who reported mean distances for males and females together.

Figure 1: Plot of estimated percentiles of six-minute walk test versus age

Percentiles 5%, 10%, 25%, 50%, 75%, 90% and 95% of 6MWT were estimated for different values of age.

Figure 2: Plot of estimated percentiles of six-minute walk test versus height

Percentiles 5%, 10%, 25%, 50%, 75%, 90% and 95% of 6MWT were estimated for different values of height.

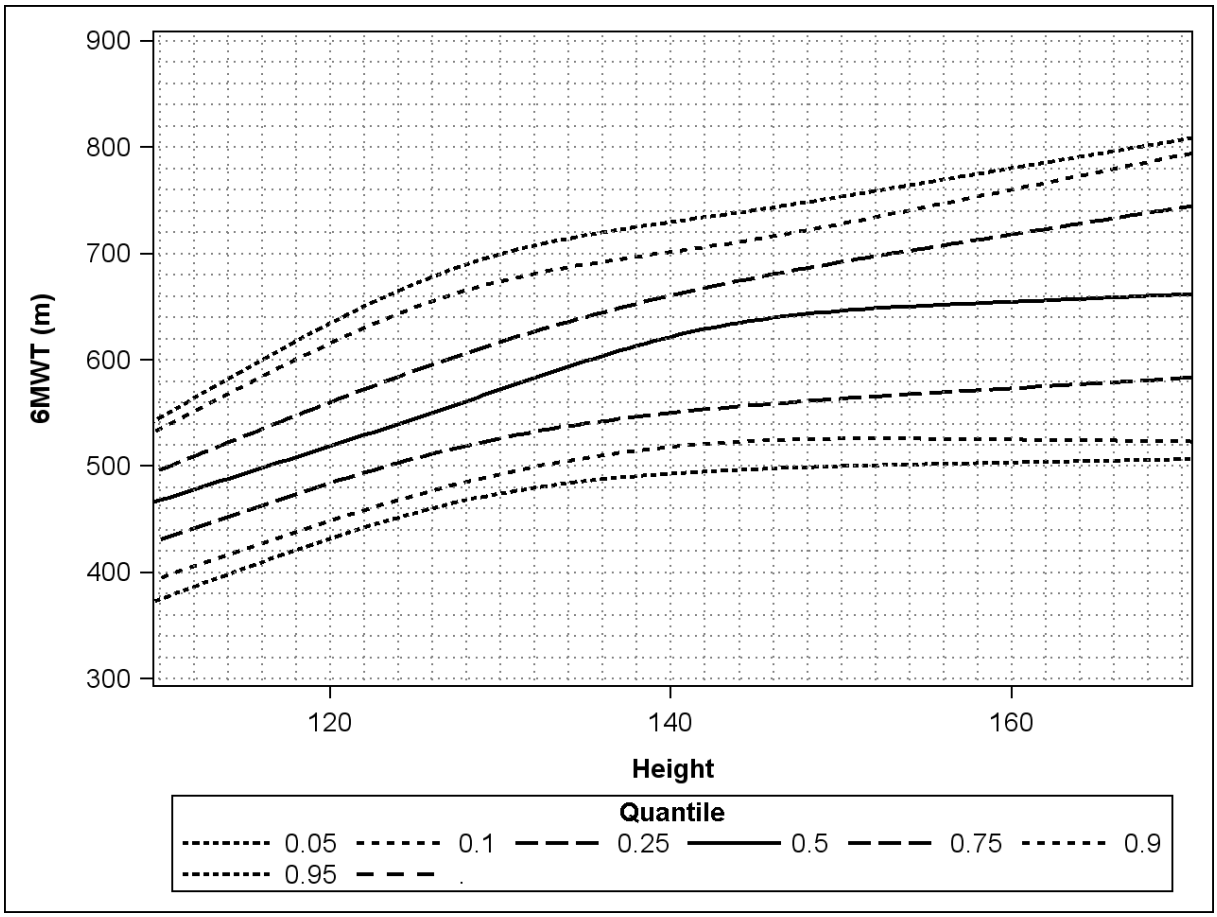


Figure 3: Predicted versus observed six-minute walk test data for the Geiger equation

The observed 6MWT data were plotted against the predicted data based on the Geiger equation applied to our study sample.

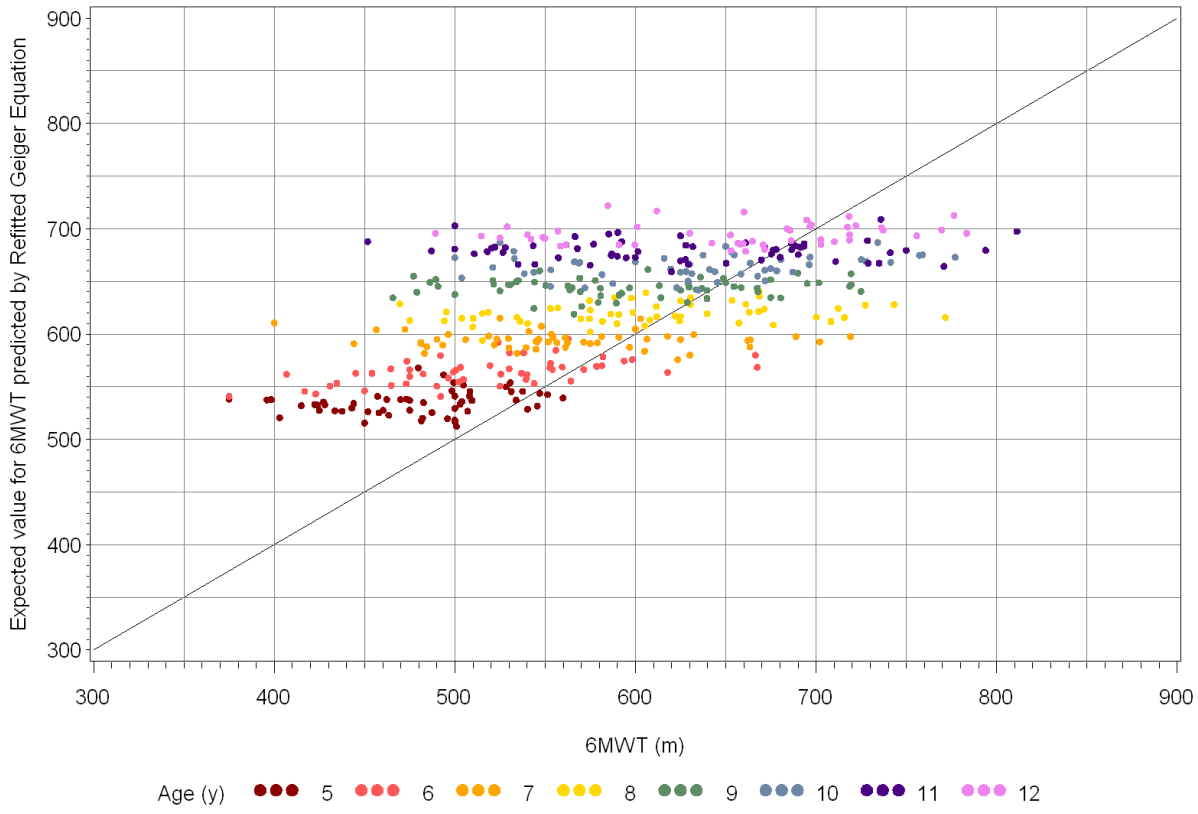
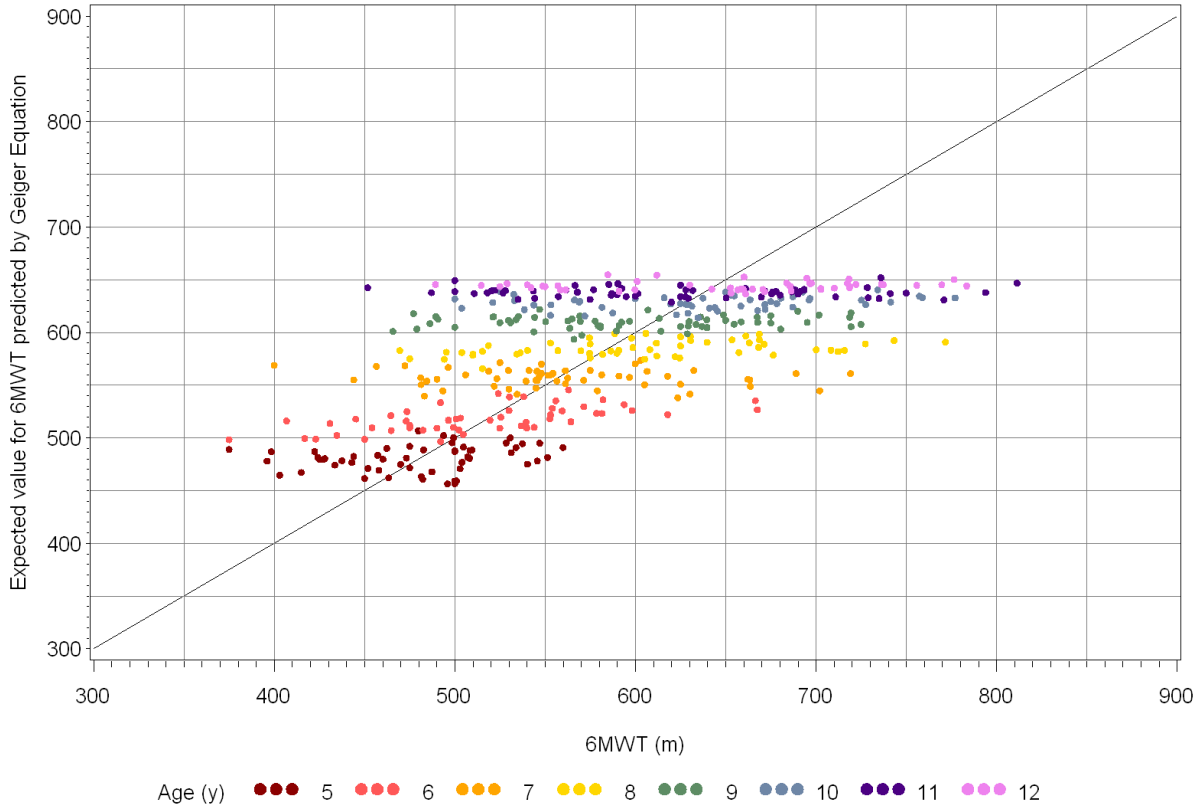


Figure 4: Predicted versus observed six-minute walk test results for the refitted Geiger equation

The observed 6MWT data were plotted against the predicted data based on the refitted Geiger equation applied to our study sample.



III.B.3

AMBULATORY CAPACITY AND DISEASE PROGRESSION AS MEASURED BY THE 6-MINUTE-WALK-DISTANCE IN DUCHENNE MUSCULAR DYSTROPHY SUBJECTS ON DAILY CORTICOSTEROIDS

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Ambulatory capacity and disease progression as measured by the 6-minute-walk-distance in Duchenne muscular dystrophy subjects on daily corticosteroids

Nathalie Goemans, Marleen van den Hauwe, Rosamund Wilson, Annelies van Impe,
Katrijn Klingels, Gunnar Buyse

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CHAPTER IV

GENERAL DISCUSSION

Duchenne muscular dystrophy (DMD) is a lethal inherited neuromuscular disorder, arising from mutations in the DMD gene and characterized by progressive skeletal and cardiac muscle deterioration leading to an early death. The complexity of this degenerative disease and its multisystem involvement have been described in Chapter 1.

Coordinated clinical management, improvement in cardiac and respiratory care together with the chronic use of glucocorticosteroids, have delayed disease progression and have significantly improved longevity, with DMD patients currently surviving into their third or fourth decade. However, despite improvements in care, there is currently no causative treatment that targets the underlying cause of the muscle fragility and degradation. Therefore, patients with DMD will spend the greater part of their life in a stage of very severe muscle weakness with a major impact on functional performance and, eventually, a need for respiratory support.

Notwithstanding encouraging preclinical results in gene transfer and stem cell approaches, the size and the complex structure of the dystrophin gene, together with technical and immunological issues, have impeded the clinical development of these approaches targeting the gene defect. This has fueled the research of therapeutic strategies that act downstream of the gene mutation, targeting the expression of the protein product dystrophin.

Oligonucleotide-based splicing-modulation, aiming to restore the open reading frame and the production of a truncated but functional protein by skipping targeted exons, is a promising approach. Local clinical proof of concept has been obtained, demonstrating localized novel dystrophin expression in DMD patients after a single intramuscular injection [109,111]. However, the therapeutic applicability of this exon skipping strategy would require a systemic approach to allow body-wide production of novel dystrophin.

The development and clinical translation of new potential therapies have highlighted the urgent need for sensitive, reliable, age- and stage-specific assessment tools to allow efficacy assessments of therapeutic interventions. Moreover, to meet the regulatory requirements for approval of new therapeutic compounds for specific diseases, these outcome measures should be able to detect clinical meaningful changes in patients' daily life.

The aim of this doctoral project was (1) to contribute to the clinical development of the exon skipping strategy as a therapeutic approach for DMD, and (2) to improve our understanding of age- and stage-specific clinical outcome measures of disease evolution to reliably measure the effect of interventions in DMD.

In the following general discussion we will summarize the main findings of our studies and discuss their significance for the development of new treatments for DMD. We will place these findings in the context of the current status of the clinical development of antisense oligonucleotides as a

potential therapeutic strategy for DMD, and discuss issues and challenges for the clinical application of this approach. We will further discuss the relevance of our studies on the 6 minute walk test (6MWT) both in healthy and in DMD boys for the methodology of clinical trials in DMD. Finally, we will identify and discuss domains for future research.

A. EXON SKIPPING STRATEGY FOR DMD

1. The systemic administration of antisense oligonucleotides as a therapeutic approach for DMD: proof of concept

The first part of this doctoral work investigated the potential of systemic administration of the 2OMePS antisense oligonucleotide PRO 051/drisapersen (developed by Prosensa and GSK), targeting exon 51 of the DMD gene, as a novel therapeutic approach for a genetic subgroup of DMD.

A first study (*See Chapter III A.1.)* investigated the safety, tolerability, pharmacokinetics and molecular and clinical effects of five weekly subcutaneous administration of PRO 051/drisapersen in an open label, dose escalating phase I/IIa study in twelve DMD patients carrying mutations correctable by exon 51 skipping. Pharmacokinetic studies indicated a favorable profile for PRO 051/drisapersen, with rapid absorption and distribution to the tissue and a mean terminal half-life of 29 days. When dosed at 6 mg/kg for 12 weeks, the most common adverse events were related to transient and variable changes in renal parameters s and to local administration site reactions. PRO 051/drisapersen induced detectable, specific exon 51 skipping at ≥2 mg/kg and novel dystrophin expression was observed in in a dose-dependent manner in 60–100% of muscle fibers in post-treatment biopsies of 10/12 patients. After 12 weeks of treatment, a mean improvement of 35.2 (±28.7) meters was observed in the distance walked in 6 minutes, with an increase above 65 meters in 3 patients, in clear contrast with the expected decline in six-minute walk distance (6MWD) observed in the natural history of DMD patients of this age, indicating possible functional improvement. In conclusion, this first ever systemic administration of an antisense oligonucleotide in DMD was well tolerated, without serious adverse events or discontinuations, showed a molecular efficacy in a dose-dependent manner in muscle distant from injection site and indicated possible functional improvement as measured by changes in the 6MWD after 12 weeks of treatment. However, data interpretation is limited by the fact that this study was not controlled, small and of short duration.

2. Long-term systemic administration of an antisense oligonucleotide targeting exon 51 of the DMD gene: safety, tolerability and efficacy data

As a treatment strategy with AON implies life-long repeated administration, therapeutic applicability of this approach requires proof of safety, tolerability and efficacy of long-term chronic systemic administration.

To this extent, we have further investigated the safety and efficacy of the long-term systemic administration of PRO 051/drisapersen in subjects with DMD in an ongoing open label extension study following on the aforementioned original dose finding study (See Chapter III A 2). PRO051/drisapersen, at a dose of 6 mg/kg, was administered weekly subcutaneously up till week 72, and further intermittently for 8 weeks on- and 4 weeks off-treatment. Overall, this compound was well tolerated over a treatment course of 144 weeks. Most adverse events were mild, most commonly related to local injection-site reactions, variable changes in renal parameters (raised urinary α1-microglobulin, and proteinuria), and a reduced thrombocyte count, all known class effects of phosphotioate antisense oligonucleotides. Although the local injection site reactions did not impact on the subjects' daily life and did not cause any major discomfort, fibrosclerotic reactions were persistent and are of clinical concern, as chronic long-term subcutaneous administration may lead to exhaustion of suitable injection sites. In general, renal parameters and thrombocyte counts changed back towards normal values during the intermittent drug-free periods. The improvements in the 6-minute walk distance, observed at 12 weeks, were sustained at 141 weeks. Six of the 10 subjects showed absence of deterioration or even an improvement from the extension baseline. Awaiting results of ongoing research on the quantitative assessment of induced novel dystrophin expression, interpretation of induced dystrophin expression in this study cohort is not yet available. For a small, uncontrolled study, the outcomes were encouraging, as natural history studies have shown a progressive decline in 6-minute walk distance over shorter observation periods in this age group.

3. Current status of the exon skipping approach for DMD

Since the start of this doctoral project, further progress has been made in the clinical development of the exon skipping strategy as a potential therapeutic approach for DMD. Several clinical trials targeting exon 51 are currently ongoing. Furthermore, AONs applicable to other deletions of the DMD gene have entered the phase of clinical development

3a. Skipping Exon 51

Several clinical trials are currently ongoing for two distinct compounds targeting exon 51: PRO051/drisapersen, a 2O-methyl phosphorothioate AON (2OmePS) delivered subcutaneously, developed by GSK/Prosensa, and eteplirsen, a phosphorodiamidate morpholino oligomer AON (PMO), developed by AVI Biopharma/Sarepta, which is delivered intravenously.

1. 2-O Me PS antisense oligonucleotide (PRO051/Drisapersen):

The dose escalation phase I/IIb and its open label extension study assessing safety, tolerability and pharmacokinetics of the first ever systemic administration of PRO051/drisapersen have been reported in this doctoral work. Subsequently, worldwide registration studies have been initiated. A double blind controlled dosing regimen study has been completed, demonstrating a statistically significant difference in 6MWT in the continuous treatment arm compared to placebo at 24 weeks, and an encouraging trend differentiating from placebo at 48 weeks (ClinicalTrials.gov trial n° NCT01153932)

Furthermore, a phase III double blind placebo controlled trial assessing its safety and efficacy in DMD in a 48 week study has recently been completed, the full results of which are awaited. (ClinicalTrials.gov trial n° NCT01254019).

Finally, additional studies assessing dosing as well as safety and tolerability of systemic administration of this compound both in ambulant and non-ambulant DMD are currently ongoing (ClinicalTrials.gov trial n° NCT01803412, NCT01462292). *(Table 1*)

	Title	ClinicalTrials.gov
DMD114044	A phase III,randomized,double blind,placebo-controlled clinical study to assess the efficacy and safety of GSK2402968 in subjects with Duchenne muscular dystrophy	NCT01254019
DMD114117	A phase II, double blind, exploratory parallel-group placebo controlled clinical study to assess two dosing regimens of GSK2402968 for efficacy, safety, tolerability and pharamcokinetics in ambulant subjects with Duchenne muscular dystrophy	NCT01153932
DMD114349	An open-label extension study of the long-term safety, tolerability and efficacy of GSK2402968 in subjects with Duchenne muscular dystrophy.	NCT01480245
DMD114876	An exploratory study to assess two doses of GSK2402968 in the treatment of ambulant boys with Duchenne muscular dystrophy	NCT01462292
DMD115501	An open label extension study of the long-term safety, tolerability and efficacy of drisapersen (GSK2402968h) in US subjects with Duchenne muscular Dystrophy	NCT01803412

2. PMO antisense oligonucleotide Eteplirsen/AVI14658

The IV administration of eteplirsen, an antisense oligonucleotide with PMO backbone chemistry targeting exon 51, has been tested in a dose escalation study with doses up to 20 mg/kg for 12 weeks, indicating a variable molecular effect with exon skipping and protein expression in the higher dose groups [110]. A study comparing the administration of 30 or 50 mg/kg eteplirsen weekly to placebo has reported molecular response and encouraging clinical effects as measured by the 6MWT after 48 weeks [129]. However, the small numbers in the different arms and the exclusion of subjects that lost ambulation from the analysis of the clinical efficacy data warrant a careful interpretation of the results.

The differences in the backbone chemistries of the nucleotides result in a different pharmacokinetic profile of the two AONs, which may translate in a different efficacy and safety profile. The negatively charged 2-O-MePS AON binds to plasma proteins, thus prolonging plasma half-life (29 days) and plasma exposure, hence improving muscle tissue uptake. In contrast, the uncharged PMO AON

exhibits a short plasma half-life (2 to 6 hours) with a high renal clearance, leading to peak levels in the kidney and requiring high doses to achieve the same muscle tissue exposure. PMOs are reported to have reduced sequence specificity compared to 2OMePS AONs, increasing the risk of off-target RNA effects [130]. The class-related toxicity profile of 2OMePS AONs has been well-documented in the past decade, based on the experience with these compounds for various applications in more than 3000 subjects [131]. Local site reactions, complement activation, reduction in thrombocyte count and prolongation of aPTT are side effects reported in clinical trials with 2-O-MePS AONs for various indications.

No long-term safety data are currently available for PMO AONs. Weekly administration of PMO AON eteplirsen in 12 DMD patients for 48 weeks was reported to be well tolerated [132]

The uncharged backbone chemistry may confer a better safety profile to these class of AONs, but may hamper efficient delivery to the muscles considering the short half-life and short plasma exposure with high renal clearance.

3b. Expanding exon skipping approach to other exons of the DMD gene

New 2'OMePS AONs, targeting other exons of the DMD gene, are under clinical development. We are currently contributing to the conduct of several multicentric international studies to assess the safety, tolerability, effect and pharmacokinetics of the subcutaneous and iv administration of 2'OMePS AONs targeting exon 44 (PRO 044, ClinicalTrials.gov trial n° NCT01037309, exon 45 (PRO 045, ClinicalTrials.gov trial n° NCT01826474) and exon 53 (PRO 053 Eudract n° 2011-005042-35) in phase I/II open label escalating dose pilot studies in patients with Duchenne muscular dystrophy.

4. Issues and challenges of exon skipping

While being a promising approach with encouraging results in phase I/IIa and phase IIb trials in DMD patients, splicing modulation with AON warrants careful considerations. Further research is required to demonstrate efficacy, safety and tolerability on the long term with this AON treatment. The mutation-specific approach and the issues associated with the conduct of clinical trials for a rare and degenerative disorder in a pediatric population, impose important challenges on the clinical development of these compounds.

4a. Efficacy and safety of the exon skipping approach

The exon skipping strategy is a disease-modifying approach aiming to convert a severe DMD phenotype into the substantially milder Becker phenotype. The result of exon skipping, leading to a

expression of a truncated dystrophin protein, is anticipated based on the clinical and genetic description of Becker patients, and the occurrence of asymptomatic subjects carrying intragenic deletions in the DMD gene [133]. It is not yet known how much restitution of dystrophin is required for clinical beneficial effects. Observations in subjects with X-linked cardiomyopathy, an allelic variant to DMD, have demonstrated that dystrophin protein levels between 29% and 57% of control muscle are sufficient to avoid muscular dystrophy [134]. In the *mdx* mice, levels of approximately 15 to 20% are needed for a normalization of the phenotype [135,136]. It is anticipated that even lower levels of dystrophin could have a beneficial effect on the disease course by protecting the muscle membrane from exercise-induced damage and by restoring the linking and signaling function of this protein, hence delaying decline. However, further studies are required to assess the expression of the novel dystrophin BMD-like isoforms and their correlation with clinical relevant measurements after chronic administration. In addition, the outcome of the exon skipping approach will depend on the stability and expression levels of dystrophin mRNA, as has been highlighted in a recent publication [137].

AON tissue uptake and the levels of exon skipping after systemic administration differ across different muscles and organs, as evidenced by preclinical studies in various animal models. In healthy animals, AON uptake in muscles is limited, with preferential accumulation in the liver and the kidneys. However, in dystrophic mice (*mdx* mice and *mdx/utr*-/+mice*),* the leaky muscle membrane favors uptake, resulting in a muscle uptake of up to 90% of the systemically delivered AON [104,130]. In this context, further research is needed to define whether restoration of the muscle membrane impermeability as a result of novel dystrophin expression, will impact on the chronic uptake of AON. Preclinical data indicate poor exon skipping levels in the cardiac muscle after systemic administration of AON, probably due to the lower uptake of AON by the less leaky cardiac muscle fibers [138]. This fuels the concerns that the exon skipping approach could have a deleterious effect on the cardiac muscle due to exercise–overload as a result of improved skeletal muscle strength. However, further data in the *mdx* mouse have indicated that prolonged administration did substantially increase the cardiac uptake, anticipating beneficial molecular effects at cardiac level on the long-term administration [104]. Continued careful monitoring of the evolution of the cardiac disease is warranted to address this question.

The identification of possible modifications to the chemical structure of AONs, which could further improve tissue uptake and reduce possible toxic effect of AON, is another field of future studies. Additionally, efforts aiming at improving the delivery of AON to muscle are ongoing, such as the use of viral vectors, cell-penetrating or-targeting peptides, and nano particles. While these strategies have the potential to improve the effect of AONs, they will also pose additional technical and immunogenic challenges [129].

The risk of cell-mediated immunogenicity of novel dystrophin isoforms has been questioned as a possible issue in the exon skipping approach. However, as exon skipping resulting in dystrophin positive revertant fibres occurs naturally, albeit at a very low frequency, immune tolerance of the novel dystrophin isoform is hypothesized. In our study, no dystrophin antibodies could be detected after 144 weeks of administration.

Finally, assessing potential toxic effects on other target organs such as kidney and liver is hampered by the limitations inherent to the measurements currently applied to monitor hepatic and renal function, which are not reliable in DMD. Raised AST and ALT from muscular origin compromise the interpretation of the levels of these enzymes currently used as marker of hepatic toxicity. Monitoring renal safety is hindered by the low creatinine levels both in plasma and urine of DMD patients. Additional research is needed to improve our insights or to develop new tools for the assessment of renal and hepatic function in DMD, independently from markers influenced by the dystrophic process.

4b. Mutation-specific approach applicable to a subset of DMD population

Exon skipping is a mutation-specific approach, thus requiring multiple sequence-specific AONs to correct the reading frame of the entire range of deletions described in patients with DMD. This approach is also limited to mutations anticipated to generate a novel, BMD-like dystrophin isoform with preserved anchoring actin and cystein rich domains, and is thus only applicable to mutations affecting the central rod domain. Nevertheless, due to the clustering of mutations in several 'hot spot' regions, a panel of AONs targeting eight exons would be sufficient to theoretically correct approximately 70% of all described deletions. Of these, exon 51 skipping would benefit the largest subgroup (approximately 13 % of all mutations), whereas AON targeting exons lower in ranking would only benefit for a small subgroup of patients.[108]. Furthermore, additional data are required to evaluate whether efficacy from one sequence can be extrapolated to another as the response to exon skipping and the efficacy of the novel dystrophin transcript may not be similar for different targeted mutations within the DMD gene.

In contrast to their efficacy, which is linked to the specific nucleotide sequence of the AON, the general safety and pharmacokinetic characteristics of this class of compound are sequence-independent and well-documented, supporting the extrapolation between sequences. Off-target RNA interactions however need to be excluded preclinically for each sequence by screening against the human genome database to avoid a coincidental full-length antisense hits elsewhere in the genome.

A major challenge for the clinical development of AON targeting genetic subpopulations of DMD is the limited number of patients available to investigate the safety and the efficacy of each individual sequence with conventionally designed trials as required for approval by regulatory authorities. This issue highlights the need for an innovative and more flexible approach in the clinical development and registration of therapeutic compounds applicable to small subpopulations of patients. Collaborative efforts from scientists, clinicians, patients representatives, industry and regulatory authorities are ongoing to discuss guidelines for the conduct of clinical trials in DMD [129], suggesting smart design of trial protocols, the optimization of data collection from specific patient cohorts, a cautious extrapolation of safety and pharmacokinetic data from analogous AONs, and the use of natural history data from contemporaneous age- and stage- matched DMD patients as a background against which efficacy data could be interpreted.

Finally one of the key issues in the development of new therapeutic compounds is the identification of outcome measures to assess the efficacy of interventions in DMD. These outcome measures should be robust, valid, reliable and able to capture clinically meaningful changes in the time frame of a clinical trial. Additionally, longitudinal data of these outcome measures collected in cohorts of subjects with DMD treated with corticosteroids, are eagerly needed to inform on the evolution of the modified natural history of this disease since the instauration of corticosteroid treatment as standard of care.

B. OUTCOME MEASURES FOR THE AMBULATORY STAGE OF DMD

The clinical characteristics of DMD, with a predictable non-linear decline with age, and the limited duration inherent to clinical trials, have highlighted the need for different outcome measures across the different stages of the disease. These outcome measures should capture disease aspects sensitive to changes in this particular stage of the disease, and this within the time windows achievable in human clinical trials. As most of the clinical trials in DMD focus on the ambulant stage of the disease, aiming to intervene before irreversible muscle damage has occurred, there is an urgent need for ambulation-related outcome measure to assess disease status and evolution in this particular stage of the disease.

The 6MWT is a global measure of function and endurance reflecting a subject's physical capability and walking function at a submaximal level. Its acceptance by regulatory authorities as a clinical meaningful endpoint in registration-directed clinical trials in neuromuscular and neurometabolic disorders [126] has raised interest and favored further research of the 6MWT as an outcome measure in ambulant DMD. A modified 6MWT has been described and validated in DMD [127] and has been chosen as primary endpoint for clinical trials in DMD. At the start of this doctoral work, insights in this modified 6MWT in healthy young boys in the age group of particular interest in relation to clinical trials in ambulant DMD, as well as data on this modified 6MWT in ambulant DMD boys were scarce.

The second part of this project aimed to investigate the 6MWT as a clinically meaningful, relevant and suitable outcome measure for therapeutic trials in DMD

1. The test-retest reliability and the developmental evolution of the 6MWT

In a first study (*See* Chapter III B.1) we investigated the test-retest reliability, the developmental evolution, and the correlation with anthropometric variables of the 6MWT in young healthy Caucasian boys aged 5 to 12 years. Our findings demonstrate the reliability of the 6MWT, even in young children from the age of 5, and the increment of the 6MWD within small age categories. Finally, our findings indicate that a shorter, 3 minute version of the test is also reliable, already from the age of 5 years old and onwards.

2. Normative data for the 6MWT

In a second study (*See* Chapter III B.2) the data set was expanded to contribute to the generation of normative data for the 6MWT in a young healthy male population, which is of particular interest in relation to natural history studies and clinical trials in DMD. Growth and maturational influences on the 6MWT were confirmed and age-and height-related percentile curves of the 6MWD were developed. These percentile curves provide an important tool in the assessment of the ambulatory capacity in boys with variable diseases and more specifically in DMD patients. While significant correlations were found with anthropometric variables and myometry in the 5-8 years age group, further study is needed to define the additional factors influencing 6MWT results, especially in the older boys (9-12 years). Finally, the Geiger prediction equation, currently used to asses ambulatory capacity in DMD in comparison to typically developing healthy peers, was refitted to obtain a more accurate prediction model based on a large sample with a homogenous distribution across the age categories 5 to 12 years and applying the 6MWT methodology currently used in clinical trials in DMD [139].

3. Ambulatory capacity and disease evolution in DMD as assessed by the 6MWT

Despite the absence of a causative treatment for DMD, improvements in clinical care have significantly impacted on the course of the disease. Therefore, historical series no longer accurately describe the current natural history of DMD patients treated with standard of care. To address this issue, we conducted a third study (See Chapter III B. 3) which contributed to the understanding of the modified natural history of DMD in the context of corticosteroid treatment. Our results provide insight in the 6MWT from a homogenous cohort of corticosteroid treated DMD patients receiving the same treatment and care. Additionally, we generated data on % predicted values in corticosteroid treated DMD boys, which are of particular interest for the design of clinical trials in ambulatory DMD boys. Importantly, our natural history data from contemporaneous DMD patients provide a background against which efficacy data from age and stage matched study subjects can be interpreted in the context of trials for subsets of DMD, where the eligible pool of patients is too limited for a conventional trial design.

This study confirmed the age-dependent biphasic pattern of the evolution of the 6MWD, and contributed to the understanding of the variability of this outcome measure. These data are in line with recent reports on longitudinal data in ambulant DMD from Italy and the US [139,140], confirming the non-linear pattern of decline over time in DMD. Improvements may be observed in the age range younger than 7 years, partially due to maturational influences and cognitive development with improved attention, with an additional beneficial effect of corticosteroid treatment. These increases in 6MWD are followed by a temporarily tendency for plateauing, which will finally end in a steep, "cliff"-like drop in 6MWD often seen once DMD boys have reached 6MWD values below the 300m. These data inform on the selection of age- and stage-based inclusion criteria for clinical trials in ambulant DMD.

Data from a placebo arm of an interventional multicentric study have further confirmed the applicability of the 6MWT as outcome measure in ambulant DMD trials, and have identified the minimal clinical important difference (MCID) for the 6MWT. Using a dimension-based statistic approach, they confirm the clinical meaningfulness of 6MWD changes in the range of approximately 30m for boys with DMD. Additionally, an anchor-based approach demonstrated that the MCID varied according to the baseline 6MWD, indicating that at lower levels of ability, even smaller changes in 6MWD correlated with meaningful changes in the Pediatric Outcomes Data Collection Instrument (PODCI), a patient-reported outcome tool [141].

Despite the homogenous steroid treatment regimen and standard of care, a marked variability was observed in the 6MWD, as expressed by the large SD. This underscores the heterogeneity in ambulatory capacities and the rate of progression of disease in DMD boys in the age range currently selected for clinical trials. This variability has to be taken into account in the design and the interpretation of clinical trials. Random sampling examples from this cohort demonstrated that small samples, used as internal control arms in clinical studies, may be unrepresentative. This fuels the argumentation to use natural history data from large contemporary cohorts as controls for studies where the eligible subject pool is particularly small.

C. GENERAL CONCLUSION AND FURTHER RESEARCH

This doctoral work has contributed to the development of new therapies for DMD by investigating the first ever systemic application of an AON in DMD as a potential disease modifying treatment approach for a genetic subgroup of DMD patients. The systemic administration of an AON, targeting exon 51 of the DMD gene resulted in encouraging molecular and clinical effects, currently further investigated in confirmatory studies worldwide. The long-term safety and clinical effects of chronic administration were further investigated in an ongoing open label extension study. Side effects were manageable and in line with the known class effects of these compounds. Our results help to outline the areas that warrant further monitoring on long-term chronic administration.

Although efficacy data have to be interpreted with caution in the absence of a placebo group, the 6MWD of the patients participating in this open label extension study demonstrated encouraging results in comparison with contemporaneous DMD patients treated according to the same standards of care. This approach has opened promising perspectives, however, future research in the therapeutic application of AON in DMD is needed to assess the long-term efficacy of this approach and to address possible safety and tolerability issues of chronic use. This also includes research to improve our insight in hepatic, renal and inflammatory parameters in contemporaneous steroid-treated DMD patients, for which data are lacking, as well as the exploration of novel DMD appropriate tools to monitor renal and hepatic safety, unaffected by the dystrophic process.

Major progresses in therapeutic strategies moving into the clinical stage of development have highlighted the urgent need for robust outcome measures to assess disease progression and effects of interventions. The second part of this doctoral work has contributed to improving the methodology and design of clinical trials in DMD by providing substantial information on the 6MWT, a global measure of ambulatory capacity, which use is currently advocated as primary endpoint in trials for DMD. Our results improve our understanding of this outcome measure both in healthy young boys in an age range of particular interest for clinical trials in ambulant DMD, and yield insight in the modified natural history of DMD patients treated with current standard of care. These results are of importance both for the design and for the interpretation of results of future trials.

Future research should aim at addressing the issues of appropriate outcome measures for non-ambulant DMD and very young DMD patients. We are currently contributing to the development of new tools for the assessment of upper limb function in DMD in an international collaborative effort, and are taking the lead in the development of a DMD-specific patient-reported outcome measure

(PROM) to assess upper limb function [142]. These new tools will provide more sensitive instruments to assess the disease evolution and the efficacy of intervention in non-ambulatory stages of the disease.

Finally, it should be highlighted that the splicing modulation approach aims at modifying the DMD phenotype by restoring the production of a partially functional and truncated dystrophin protein. Data from intermediate type DMD and Becker populations are informative on the variability in severity of this phenotype, indicating that, even if this disease modifying approach is proven successful, a multidisciplinary approach and research aiming at improving guidelines for the care of this progressive multisystem disorder, will remain mandatory to further optimize the health and the quality of life of subjects with DMD/BMD.

SUMMARY

Duchenne muscular dystrophy (DMD) is an inherited muscle disorder affecting 1/3500 to 1/5000 live newborn males worldwide and leading to a progressive muscle weakness. Due to a genetic defect, these boys lack a protein called dystrophin, required for the normal structure and function of muscle fibers. Boys with DMD first start having difficulties with walking at toddler age, and, as their fragile muscle fibers become more and more damaged, eventually become wheelchair-bound around their teens. As they lose muscle function in their arms, they become more invalidated, and, eventually, weakness of the respiratory muscles and the failure of the heart function limit the survival to an age of around 20 to 30 years old without treatment.

Over the past decades, the care for DMD patients has improved. Advances in the treatment for heart muscle disease, new ways to support the breathing function of the respiratory muscles, and the use of corticosteroids to slow down the muscle damage have improved the quality of life, and even the survival, of these young men. However, as no treatment is able to correct the cause of the disease, namely the absence of dystrophin, current therapy cannot prevent this progressive muscle destruction, which eventually leads to death.

In a hope to halt the disease, recent research have studied strategies to correct the underlying genetic problem. Although it has been shown it may be possible to correct the genetic error responsible for DMD in laboratory models, these techniques require the change of the genetic information in all the muscle cells of the body, and are currently not feasible in patients. Therefore, rather than replacing the genetic error, research now focuses on strategies that can help the muscle cells to bypass that error in the DMD gene, and produce a more or less functional form of dystrophin in spite of the gene defect.

This approach does not target a subject's DNA, but interferes at the RNA level. These agents, known as antisense oligonucleotides (AONs), specifically bind to and mask the region of the genetic error, causing the cell to 'skip' or ignore the mistake. This leads to the production of a dystrophin protein which lacks a small portion, but is nevertheless functional, rather than a faulty protein. The potential of such an 'exon skipping' strategy has already yielded promising results in animal models and human cells, and we had previously shown that the local administration of AONs could restore dystrophin presence in muscles of patients with DMD.

In this doctoral work, we investigated the safety, tolerability, and clinical effects of the first ever systemic administration of a specific AON, PRO051, in a subset of patients with DMD. We demonstrated that treatment with this AON for DMD was well tolerated, without serious adverse events or discontinuations. The injection of AON led to a dose-dependent increase in dystrophin in a muscle at distance of the injection site, and, after 12 weeks of treatment, we had indication of a possible improvement in muscle function as measured by the walking distance of these patients. Although these results rise the hope for new treatment options, the number of patients in this study was still small, and the follow-up was limited in time.

The long-term safety and efficacy of systemic administration of PRO051 was further investigated in a still ongoing extension study. Overall, this compound was well tolerated over a total duration of 144 weeks. Most adverse events were mild and most commonly related to local injection-site reactions, or changes in parameters of liver and kidney function, and a reduction in blood platelets, all of which seemed to reverse after discontinuation of the drug.

The improvements in the 6-minute walking distance, observed at 12 weeks, were sustained at 141 weeks. In six of the 10 subjects, the normal decline in muscle function as measured by the walking distance could be halted, or even improved.

To better study the effect of these major advances in potential treatments in patients, reliable measurements of muscle function in boys with DMD are needed. In this doctoral work we investigated the 6 minute walking test (6MWT) as a measure of functionality, both in healthy typically developing boys and in boys with DMD. Our results help to improve our insights in this global measure of function, currently advocated as the primary endpoint in clinical trials in patients with DMD who can still walk.

We demonstrated the reliability of this test, and described how the 6MWT results change with age and height. Additionally, our findings indicated that a shorter (3 minute) version of the test is also reliable, already from the age of 5 years old onwards. Our data give much-needed information on the functional capacity of boys with DMD treated with the current standard of care, and led to a model that can be used to predict muscle function for a specific patient more accurately. Taken together, these observations are crucial to interpret the normal evolution of DMD patients, as well as the effect of new therapeutic interventions.

To conclude, this doctoral work has contributed to the development of new therapies for DMD by investigating the first ever systemic application of a AON in DMD. The second part of this research work has contributed to improving the methodology and design of clinical trials in DMD by investigating ways to measure disease evolution as well as the effect of interventions in boys with DMD who can still walk. This research has generated new insights on the six minute walk test, a global measure of function, both in healthy typically developing boys and boys affected with DMD.

SAMENVATTING

Duchenne spierdystrofie (DSD) is een erfelijke spierziekte die bij ongeveer 1 op 3500 à 1/6000 jongens voorkomt. De oorzaak van de ziekte ligt in een genetisch defect dat voor het dystrophine eiwit codeert. Dit dystrofine-eiwit is noodzakelijk voor de stevigheid en voor de goede werking van spiervezels. Bij jongens met DSD zorgt de afwezigheid van dit eiwit ervoor dat hun spieren gaandeweg beschadigd geraken, met een progressieve en onomkeerbare spierzwakte tot gevolg, aanvankelijk vooral merkbaar ter hoogte van de onderste ledematen , maar met nadien ook aantasting van de bovenste ledematen, ademhaling- en hartspier.

De ziekte kenmerkt zich voor het eerst door motorische problemen op peuter-kleuter leeftijd: deze jongetjes lopen vaak iets later, hebben een iets ander looppatroon en kunnen niet echt rennen.
Door de voortschrijdende beschadiging van de spieren krijgen deze jongens het steeds moeilijker met stappen en worden ze in hun tienerjaren rolstoelafhankelijk. Ook de spierkracht in de bovenste ledematen neemt progressief af en uiteindelijk zal de verzwakking van de ademhalingsspieren en de hartspier de levensverwachting van jongens met DSD beperken tot 20 - 30 jaar .

De afgelopen jaren is de zorg voor DSD-patiënten sterk verbeterd. Een betere behandeling van hartfalen, een betere ondersteuning van de falende ademhalingsspieren door beademingstechnieken, en het gebruik van corticosteroiden die de spierafbraak vertragen, hebben niet alleen de levenskwaliteit van deze jongens en jongemannen verbeterd, maar hebben ook de levensverwachting doen toenemen tot 30-40 jaar. Desalniettemin zijn er nu nog geen behandelingen die de onderliggende oorzaak van de ziekte, de afwezigheid van het dystrofine-eiwit, kunnen aanpakken. Met de huidige behandelingen kan de spierafbraak dan ook in het beste geval vertraagd, maar niet tegengehouden worden.

Recent onderzoek richtte zich dan ook op pogingen om het defecte gen te herstellen, in de hoop de ziekte te kunnen stilleggen. Hoewel door deze strategie van genetische manipulatie wel gunstige resultaten behaald konden worden in laboratorium setting, is een dergelijke behandeling waarbij, om effectief te zijn, het DNA gewijzigd moet worden in alle spiercellen nog niet mogelijk bij patiënten.
In plaats van het genetische materiaal zelf te wijzigen, proberen nieuwe technieken nu ervoor te zorgen dat de spiercellen ondanks de genetische fout toch een min of meer functionele vorm van het dystrofine-eiwit kunnen aanmaken door deze fout als het ware te ‘verbergen’.

Antisense oligonucleotiden (AON) zijn moleculen die interfereren met de foute genetische informatie, en die ervoor zorgen dat de cel de fout 'overslaat'. In plaats van een foutief 'zinloos' eiwit wordt er dan een korter, maar toch deels functioneel, dystrofine-eiwit gevormd. Deze techniek van '*exon skipping*' gaf al gunstige resultaten bij proefdieren, en in een voorgaande studie hebben we kunnen aantonen dat een lokale injectie met dit AON de aanmaak van het dystrofine-eiwit in spieren van patiënten met DSD kon herstellen rond de plaats van de injectie.

Ons onderzoek, beschreven in deze thesis, is de eerste studie ooit waarin onderzocht wordt of een van deze veelbelovende AONs, namelijk PRO 051, ook veilig via algemene weg toegediend kan worden, en zo in het hele lichaam kan werken. We konden aantonen dat de toediening van PRO 051 veilig en zonder ernstige problemen verliep. Bovendien leidde deze behandeling tot een dosisafhankelijke stijging van het dystrofine-eiwit in de spieren van deze studie patiënten met DSD. Na 12 weken behandeling bleken de behandelde patiënten bovendien gemiddeld beter te scoren op een wandeltest, een test gebruikt om de spierfunctie te meten. Hoewel deze resultaten erg hoopgevend zijn, gaat het hier nog om een beperkt aantal patiënten, en een beperkte behandelingsduur, zodat verder onderzoek noodzakelijk blijft.
In een opvolgstudie van deze eerste studie konden we ook de langetermijneffect van de toediening van PRO 051 onderzoeken, tot een behandelingsduur van 144 weken. Ook op deze lange termijn bleef de behandeling zonder grote problemen verlopen. De nevenwerkingen beperkten zich vooral tot lokale reacties ter hoogte van de injectieplaats, al zagen we bij sommige patiënten ook veranderingen in bloedwaarden van de lever- en nierfunctie, en een daling van de bloedplaatjes. Deze afwijkingen herstelden zich echter over het algemeen bij het onderbreken van de toediening.
De goede resultaten van de wandelafstand-test die we na 12 weken vaststelden, bleven behouden na langdurige behandeling. In zes van de tien patiënten kon de normale achteruitgang van de spierfunctie gestopt worden, of verbeterde deze functie zelfs.

Om het effect van deze en andere nieuwe behandelingen te kunnen bestuderen, is het van groot belang om de spierfunctie van patiënten met DSD op een betrouwbare manier te kunnen meten. Bovendien is het ook nodig om goed te weten hoe deze metingen van spierfunctie evolueren bij patiënten die volgende de huidige standaardtherapie behandeld worden, om een goede referentiewaarde te hebben waartegen effecten van nieuwe medicatie kunnen getest worden.
In het tweede deel van dit doctoraatsonderzoek hebben we de 6 minuten wandeltest (6MWT) als referentietest voor spierfunctie onderzocht. We verzamelden gegevens van deze test zowel bij gezonde jongens als bij jongens met DSD, en onderzochten hoe deze waarden correleren met leeftijd en lichaamslengte alsook met spierkracht. We bevestigden ook de betrouwbaarheid van deze test,

en konden aantonen dat ook een kortere, 3 minuten durende test, betrouwbaar is vanaf de leeftijd van 5 jaar. We ontwikkelden een verbeterde formule om voor een individuele patiënt de te verwachten spierfunctie te kunnen berekenen.
Deze resultaten geven belangrijke informatie over het natuurlijke verloop van de ziekte bij patiënten die volgens de huidige standaarden behandeld worden, en bieden nieuwe mogelijkheden om klinische studies optimaal te plannen, en om de resultaten optimaal te kunnen interpreteren.

Samengevat onderzochten we in dit doctoraatswerk twee belangrijke aspecten die kunnen bijdragen tot een verbetering van de huidige en toekomstige zorg voor DSD-patiënten. We beschreven de eerste studie ooit die het effect van veralgemeende toediening van AON bestudeerde bij patiënten met DSD, zowel op korte als op lange termijn. Bovendien ontwikkelden we nieuwe inzichten over de 6MWT, een test die gebruikt wordt om spierfunctie te meten bij DSD-patiënten die nog kunnen stappen. Deze informatie kan helpen om toekomstige studies optimaal te plannen.

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CURRICULUM VITAE

Goemans Nathalie, M.D.

Personalia

D.O.B. 20 October 1955

Married to Bruno Vanassche, mother of Thomas, Hans, Paul, Vincent and Lucas Vanassche

Present position: Head of Clinic, Neuromuscular Reference Centre for Children, Dept Child Neurology, University Hospitals, Leuven, Belgium

Education and certificates

- Medical Doctor *Magna cum laude* (KUL juni 1980)
- Paediatrics (KUL juli 1985)
- Certificate Rehabilitation Medicine (1991)
- Certificate Child Neurology (juli 1993)
- Certification Human Participants Protection Education for Research Team course (2006)
- Certification Motor Function Measure (MFM) (2009)
- Certification stagemeester in de revalidatie wetenschappen (2011)
- Certification GCP (2011)

Working experience:

- 1980-1985: Resident, Dept of Pediatrics UZ Leuven
- 1985-1986: SHO Child Neurology UZ Leuven
- 1986-1987: Fellow in clinical neurophysiology Radboud ZH, Nijmegen University (Netherlands)
- 1987-1998: Staff, Dept of Pediatrics: Consultant Child Neurology UZ Leuven
- 1998- 2009: Staff, Dept of Pediatrics: GKM,
- 1998-current: Head Neuromusculair Referentie Centrum voor Kinderen UZ Leuven
- 2009 - current: Staff, Dept of Pediatrics, Chef de Clinique

Active Professional Memberships

- Scientific Board ABMM (Association Belge contre les Maladies Musculaires)
- DMD CARE CONSIDERATION PROJECT (Center for Disease Control CDC)

- Cooperative International Research Group for neuromuscular disorders (CINRG)
- ALADIN, a Dutch-Belgian collaborative project to promote and enhance management and research in Duchenne muscular Dystrophy
- World Muscle Society (WMS)
- Belgische vereniging Kinderneurologie
- Vlaamse Vereniging voor artsen in de Gehandicapten zorg
- Nederlandse Vereniging Kinderneurologie
- Belgisch –Nederlandse Neuromusculaire Studieclub
- European Pediatric Neurology Society
- Participant in several ENMC (European Neuromuscular Centre) international expert workshop & consortia (since 1998)
- Trainer international training courses on Neuromuscular Disorder (TREAT-NMD- ENMC workgroup)
- Clinical curator Belgian Registry of Neuromuscular Disorders
- Cost MC member: COST action BM1207 (*Networking towards clinical application of antisense-mediated exon skipping project*
- Partner BIO-IMAGE-NMD project, FP7 project (Framework Programme for Research, technological Development and Demonstration) supported by the European commission
- Partner: TREAT NMD ALLIANCE: work package Care Standards and Outcome measures

Research experience:

- <u>CINRG co principal investigator University Hospitals Leuven 1999-2002:</u>
    - CINRG CNMC 0599 *A multicenter randomised placebo controlled double blind study to assess efficacy and safety of glutamine and creatine monohydrate in Duchenne Mucular Dystrophy*
    - CINRG KUL0401 *An open label pilot study of Oxatomide in steroid naive Duchenne Muscular Dystrophy*
- <u>SNTII-1 co-principal investigator 2005-</u>:
    - *SNT-II-001 trial: Phase IIa randomized placebo-controlled double-blind clinical trial of SNT-MC17 in Duchenne muscular dystrophy + Extension*
- <u>KUleuven:B32220072794 2008- current</u>: Principal investigator:
    - *Etude de l'activité physique d'enfants atteints de maladies neuromusculaires par mesure cinématique,in collaboration with Hopital Universitaire de Lausanne*
- <u>Prosensa PRO 051- 02: 2008-current Principal investigator:</u>

- *A phase I/II open label escalating dose pilot study to assess the effect, safety, tolerability and pharmacokinetics of multiple subcutaneous doses of PRO051 in patients with Duchenne muscular dystrophy*

- Prosensa PRO 051- 02 Extension 2009-current: Principal investigator:
  - *A phase I/II open label pilot study to assess the long term effect, safety, tolerability and pharmacokinetics of repeated subcutaneous doses of PRO051 in patients with Duchenne muscular dystrophy*
- Prosensa PRO 044-CLIN 01 2010-current: Principal investigator:
  - *A phase I/II open label escalating dose pilot study to assess the effect, safety, tolerability and pharmacokinetics of multiple subcutaneous doses of PRO044 in patients with Duchenne muscular dystrophy*
- Prosensa PRO 045-CLIN 01 2013: Principal investigator Belgium:
  - *A phase I/II open label escalating dose pilot study to assess the effect, safety, tolerability and pharmacokinetics of multiple subcutaneous doses of PRO045 in patients with Duchenne muscular dystrophy*
- Prosensa PRO 053-CLIN 01 2013: Principal investigator Belgium:
  - *A phase I/II open label escalating dose pilot study to assess the effect, safety, tolerability and pharmacokinetics of multiple subcutaneous doses of PRO053 in patients with Duchenne muscular dystrophy*
- GSK DMD 114044 DEMAND III Principal investigator Belgium 2010-current:
  - *a phase III randomized placebo controlled clinical study to assess efficacy and safety of GSK 2402968 in subjects with Duchenne muscular dystrophy.*
- PTC 124- GD -007-DMD: 2008-2010 Principal investigator Benelux site.
  - *A phase 2B Efficacy and Safety study of PTC124 in subjects with nonsense mutation mediated Duchenne and Becker dystrophy**.+ Extension study***
- PTC124-GD-019-DMD Principal investigator Benelux
  - *An open label study for previously treated Ataluren (PTC124)patients with nonsense mutations dystrophinopathies*
- PTC124-GD-020-DMD Principal investigator Benelux
  - *A phase II double blind placebo controlled study to assess efficacy of Ataluren (PTC124)in patients with nonsense mutations dystrophinopathie*
- B40320095819 Principal investigator Leuven, (national multicentric study) 2010-2011

- *Clinical, neurophysiological and electrophysiological study of cognitive function in patients with Duchenne Muscular Dystrophy. Prinicpal investigator Leuven, (national multicentric study)*

- TRO19622 CL E Q 1275-1: Principal investigator Belgium, 2010-current
  - *Phase II, multicenter, randomized, adaptive, double-blind,placebo controlled study to assess safety and efficacy of olesoxime (TRO19622) in 3-25 year old Spinal Muscular Atrophy (SMA) patients*
- PRO-DMD-O1 Principal and coordinating investigator international multicenter study:
  - A Prospective Natural History Study of the Progression of Physical Impairment, Activity Limitation and Quality of Life in Duchenne Muscular Dystrophy (DMD)

- CINRG co principal investigator University Hospitals Leuven 1999-2002:
  - CINRG CNMC 0599 *A multicenter randomised placebo controlled double blind study to assess efficacy and safety of glutamine and creatine monohydrate in Duchenne Mucular Dystrophy*
  - CINRG KUL0401 *An open label pilot study of Oxatomide in steroid naive Duchenne Muscular Dystrophy*
- SNTII-1 co-principal investigator 2005-2008:
  - *SNT-II-001 trial: Phase IIa randomized placebo-controlled double-blind clinical trial of SNT-MC17 in Duchenne muscular dystrophy + Extension*
- Santhera SNT III-03 (DELOS study) 2010-current: co prinicpal investigator:
  - *A Phase III Double-Blind, Randomised, Placebo-Controlled Study of Efficacy, Safety and Tolerability of idebenone in 10-18 Year Old Patients with Duchenne Muscular Dystrophy*

Grant and Audit Reviewer for

- ABMM, AFM, TELETHON ITALY, Duchenne Parent Project, Zon MW
- AERES (Association pour l'évaluation de la recherche et la science)

Manuscript Reviewer

- Neuromuscular Disorder, European Journal of Paediatric Neurology, European Journal of Paediatrics

Invited lectures and oral presentations related to doctoral work

National platforms:

- **Leuvense Dagen Kindergeneeskunde** Leuven 14/15 mei 2009. De ontwikkeling van nieuwe therapieën in spierziekten: Ethische aspecten
- **Spring Meeting Belgian Society of Paediatric Neurology**, Gent 23 April 2010, First systemic delivery study with antisense compound PRO 051in Duchenne muscular Dystrophy
- **Symposium 10 th anniversary NMRC Belgium**, Woluwe 25 November 2010: Antisense Oligonucleotide Treatment Strategy in Duchenne Muscular Dystrophy
- **Belgische vereniging Kindergeneeskunde,** Luik April 2009**:** Evaluation tools in Neuromuscular disorders what to follow, what to measure
- **Duchenne Parent Project-Belgium -First congress -** Lombeek 07 Februari 2011**:** Nieuw therapeutische inzichten in Duchenne Spier Dystrophie
- **Huisartsenkring Lombeek 9 juni 2012**: Duchenne spierdystrophie: "genpleisters", fictie of realiteit?

International platforms:

2009

- **I st Asian International Congress on Duchenne Muscular Dystrophy**. Bangalore India 26-28 februari 2009: Clinical Trials in Duchenne Muscular Dystrophy
- **World Muscle Society XIV,** Geneva, 9-12 September 2009: First systemic delivery study with antisense compound PRO 051 in DMD
- **TREAT NMD /EMEA/FDA meeting on development of antisense oligonucleotide in DMD**, London 24-25 September 2009

2010

- **Duchenne Education Meeting,** Oxford 8/9 februay 2010: Care , management and new therapeutic strategies in Duchenne Muscular Dytsrophy (IL)
- **Duchenne and Becker Muscular Dystrophy, from gene to drug, Duchenne Parent Project,** Italy , Rome 13/14 februay 2010 Standards of care: a multidisciplinary approach.(IL)
- **American Academy of Neurology 2010, 62nd Annual Meeting** ,Toronto 12/17 April 2010 : A Phase I/IIa Systemic Study on Antisense Oligonucleotide Compound PRO051 in Patients with Duchenne Muscular Dystrophy (PP)
- **Joint Meeting Deutsch Geselshaf /Belgisch-Nederlands Spierclub,** 24 April 2010: Clinical trial in Duchenne Muscular Dystrophy : overview

- **XI World Congress of ICNC** , Cairo, May 2/7 2010. Antisense Oligonucleotide PRO 051 : results of first systemic delivery study in Duchenne Muscular Dystrophy
- **TREAT NMD-ENMC Spring Myology School St Petersburg,** Russia: 17/21 May 2010 Duchenne Muscular Dystrophy: new therapeutic developments
- **Combined ESN spring meeting and Metabolics.be meeting ,** Leuven, 19/21 May 2010 : Recent diagnostic and therapeutic developments in Neuromuscular Disorders (IL)
- **DEMAND II , La Hulpe, Belgium :** Clinical experience from Exon skipping trial PRO 051-02 20 May 2010
- **European Academy of Childhood Disability, Brussels** 26-29 May 2010**:** Clinical trials in Neuromuscular disorders: Defining optimal assessment tools.in Neuromuscular Disorders
- **15th International congress of the World Muscle Society ,** Kumamoto, Japan,12-15 october 2010**:** 24 week follow-up data from a Phase I/IIa extension study of PRO051/GSK2402968 in Subjects with Duchenne Muscular Dystrop
- **EMA meeting on clinical development of Antisense Oligonucleotide in DMD,** 24th November 2010 ,London. Clinical experience on safety and efficacy of long-term systemic administration of PRO-051
- **181st ENMC workshop** Standard of Care in DMD Naarden**,**The Netherlands 10-12 december 2010 **:** Summary of known trials ongoing and planned in DMD

2011

- **American Academy of Neurology's (AAN) 63rd Annual Meeting**, Honolulu, Hawaii April 9/16, 2011 Honolulu, Hawaii. 48 week follow-up data from a Phase I/IIa extension study of PRO051/GSK2402968 in Subjects with Duchenne Muscular Dystrophy (PP)
- **37 th Jahrestagung der Gesellschaft für Neuropädiatrie und 8 th Fortbildungsakademie**, Garmisch-Partenkrichen 07/10 April 2011, Garmisch-Partenkrichen : New therapeutic developments in Duchenne Muscular Dystrophy (IL)
- **9 th European Paediatric Neurology Society Congress** ,Dubrovnik, Croatia 12-15 May 2011Systemic administration of PRO051/GSK2402968:48 week follow up data *from a Phase I/IIa extension study of systemic PRO051/GSK2402968 in Duchenne muscular dystrophy (PP)*
- **16th International congress of the World Muscle Society** WMS 17-21 October 2011, Faro, Portugal::Antisense Oligonucleotide (AON) mediated exon skipping trials in Duchenne Muscular Dystrophy (DMD): current status, future prospects and challenges.
- **TREAT NMD International Conference** Geneva, 4-7 November 2011 Exon skipping with 2OMePS antisense oligonucleotides (AON) in Duchenne Muscular Dystrophy (DMD): current clinical trials and future perspectives

2012

- **92st Meeting Belgisch Nederlandse Neuromusculaire Studieclub**, Utrecht ,4 April 2012: Exon skipping with oligonucleotide GSK 2402968 in Duchenne Muscular Dystrophy: 96 week safety and efficacy data of an open label phase I/IIa study
- **Riding the Wave 2012**, Bond University Gold Coast, Australia, 5-6 October 2012, Update on clinical trial for emerging therapies in Duchenne Muscular Dystrophy
- **CARE-NMD workshop Care standards for Duchenne patients based on international consensus** ,Warszawa ,9 November 2012, Clinical trials and future therapies in DMD
- **Parent Project Conference,** Rome 17-19 February 2012: Exon skipping: current clinical trials and future perspectives
- **Congress of the Italian Society of Human Genetics SIGU,**Sorrento 21-23 November 2012: Clinical trials with 2OMePS AON in Duchenne Muscular Dystrophy
- **194th ENMC workshop on Exon skipping,** Naarden, 7-9 December 2012: Clinical Outcome measures and Natural History data in DMD.
- **Progress in Paediatric Neurology research conference**, Bueggen,Germany,14-15 December 2012: Clinical trials in DMD, current status and challenges

2013

- **CARE NMD,** Budapest, Hungary, 18-19 April 2013: Overview of new therapeutic strategies for DMD
- **Duchenne Parent Project Belgium Meeting**, Mol, Belgium, March 2013, Recent development in therapies for DMD
- **Duchenne Parent Project Meeting**, Leiden, Nederland, April 2013, Deelname aan klinische testen: ervaringen van ouders en kinderen
- **Outcome Measures in DMD: Duchenne Parent Project Meeting**, Rome, Italy, June 2013: 6MWT, Development of a PROM assessing upper limb function
- **TREAT NMD –EMA Meeting guidelines for the development of clinical research in Duchenne muscular dystrophy** , London, United Kingdom, June 2013: Natural History
- **10th EPNS congress**, Brussels, Belgium, September 2013: Normative data of the 6-minute walk test in healthy boys aged 5-12 years and correlations with anthropometric variables and myometry
- **10th EPNS congress**, Brussels, Belgium, September 2013: Gene-derived therapeutic approaches for childhood neuromuscular disease
- **18th International congress of the World Muscle Society,** Asilomar,California, October 2013: First systemic delivery study with antisense compound PRO 044 in DMD

- **TREAT-NMD Alliance meeting Oct-Nov 2013**, New Castle, UK, 30-31 October 2013: AON in DMD

Poster presentations in relation to doctoral work:

- **16th International congress of the World Muscle Society** WMS 17-21 October 2011, Faro 48-week follow-up data from a Phase I/IIa extension study of systemic PRO051/GSK2402968 in Duchenne muscular dystrophy: comparison with contemporaneous controls for 6-minute walking distance test. **Nathalie M Goemans**, Mar Tulinius, Marleen van den Hauwe, Anna-Karin Kroksmark, Gunnar Buyse, Rosamund J Wilson, Judith C van Deutekom, Sjef J de Kimpe, and Giles V Campion
- **16th International congress of the World Muscle Society** WMS 17-21 October 2011, Faro Disease progression observed in ambulation-based outcome measures over 48 weeks in patients with non-sense mutation Dystrophinopathies. E.Mercuri, K.Bushby, C.McDonald, **N.Goemans,** F.Muntoni, B.Darras,J.Barth, A.Reha
- **17th International congress of the World Muscle Society** WMS , Perth, Australia, 9-13 October 2012 Disease progressions as measured by the 6MWD in Duchenne Muscular Dystrophy; a single centre experience of 62 boys treated with daily corticosteroids**. Nathalie Goemans**, Marleen van den Hauwe, Annelies van Impe, Toine Mercier, Rosamund J Wilson, Gunnar Buyse
- **TREAT NMD International Conference**, Geneva, 4-7 November2011 Test-retest reliability and normative data of the 6-minute walk test in healthy boys aged 5-12 years **Nathalie Goemans** , Marleen van den Hauwe , Katrijn Klingels , Anneleen Van Orshoven , Sofie Vanpraet , Hilde Feys , Gunnar Buyse

Invited lectures and oral presentations – other topics (since 2009)

- **I st Asian International Congress on Duchenne Muscular Dystrophy**.26-28 febr 2009, Bangalore India: Coordinated Care and Management in Duchenne Muscular Dystrophy
- **I st Asian International Congress on Duchenne Muscular Dystrophy**.26-28 febr 2009, Bangalore India Physiotherapy in Duchenne Muscular Dystrophy
- **TREAT NMD Meeting** : Outcome Measures in SMA. Rome 20-21 April 2009 (IL)
- **Duchenne Parent Project,** Nijmegen, 19 september 2009 : Het leven zoals het is : achter de schermen van een multidisciplinair NMRC.
- **Congenital Muscular Dystrophy : Care consideration Project (CDC)** Brussels 14-16 november : Feeding and Nutritional aspects in Congenital Muscular Dystrophy.

- **Duchenne and Becker Muscular Dystrophy, from gene to drug, Duchenne Parent Project, Italy , Rome 13-14 february 2010** Standards of care: a multidisciplinary approach.
- **TREAT NMD-ENMC Spring Myology School St Petersburg, Russia: 17-21 May 2010** Duchenne Muscular Dystrophy: care and management,
- **181st ENMC workshop Naarden,10-12 december 2010 :** Current standard of care in DMD: weight and nutrition
- **Expert meeting on Transition and Adulthood in DMD**,17/18 June 2011, Amsterdam: Quality of Life and Participation in DMD adults living in residential setting.
- **3rd Endocrine in Duchenne conference**.1-2 december 2011, Toronto : Bone metabolism in DMD
- **Riding the Wave 2012**, Bond University Gold Coast, Australia, 5-6 October 2012: Care considerations in Congenital Muscular Dystrophy
- **Riding the Wave 2012**, Bond University Gold Coast, Australia, 5-6 October 2012: Update on clinical trial for emerging therapies in Spinal Muscular Atrophy
- **Duchenne Parent Project meeting:** 24-25 April 2012, Amsterdam :Outcome measures for upper limb function Amsterdam (IL)
- **Duchenne Parent Project meeting:** 19-21July 2012, Rome : The development of a PROM for upper limb function in DMD
- **Spring Meeting Belgian Society of Paediatric Neurology**, Luxemburg, 26 April 2013: Congenital Muscular Dystrophy: what's in a name?

LIST OF PUBLICATIONS

1. Mayhew A, Mazzone ES, Eagle M, Duong T, Ash M, Decostre V, Vandenhauwe M, Klingels K, Florence J, Main M, Bianco F, Henrikson E, Servais L, Campion G, Vroom E, Ricotti V, **Goemans N**, McDonald C, Mercuri E; Development of the Performance of the Upper Limb module for Duchenne muscular 1.dystrophy. Dev Med Child Neurol. 2013 Aug 1. doi: 10.1111/dmcn.12213.

2. Mazzone E, Bianco F, Main M, van den Hauwe M, Ash M, de Vries R, Fagoaga Mata J, Stein S, De Sanctis R, D'Amico A, Palermo C, Fanelli L, Scoto MC, Mayhew A, Eagle M, Vigo M, Febrer A, Korinthenberg R, de Visser M, Bushby K, Muntoni F, **Goemans N**, Sormani MP, Bertini E, Pane M, Mercuri E. Six minute walk test in type III spinal muscular atrophy: A 12month longitudinal study. Neuromuscul Disord. 2013 Aug;23(8):624-8. doi: 10.1016/j.nmd.2013.06.001. Epub 2013 Jul 1.

3. **Goemans N**, van den Hauwe M, Wilson R, van Impe A, Klingels K, Buyse G. Ambulatory capacity and disease progression as measured by the 6-minute-walk-distance in Duchenne muscular dystrophy subjects on daily corticosteroids.

Neuromuscul Disord. 2013 Aug;23(8):618-23. doi: 10.1016/j.nmd.2013.05.006. Epub 2013 Jun 13.

4. Malfait F, Symoens S, **Goemans N**, Gyftodimou Y, Holmberg E, López-González V, Mortier G, Nampoothiri S, Petersen MB, De Paepe A. Helical mutations in type I collagen that affect the processing of the amino-propeptide result in an Osteogenesis Imperfecta/Ehlers-Danlos Syndrome overlap syndrome Orphanet J Rare Dis. 2013 May 21;8:78. doi: 10.1186/1750-1172-8-78.

5. Jaeken J, Goubau C, Buyse GM, **Goemans N**, Levtchenko EN. Another cause of hyperglyceroluria: aquaporin 7 gene mutation. J Pediatr Gastroenterol Nutr. 2013 May 8. [Epub ahead of print] No abstract available.

6 **Goemans N**, Klingels K, van den Hauwe M, Van Orshoven A, Vanpraet S, Feys H, Buyse G. Test-retest reliability and developmental evolution of the 6-min walk test in Caucasian boys aged 5-12years. Neuromuscular disorders : NMD 2013;**23**(1):19-24.

7. Goubau C, Jaeken J, Levtchenko EN, Thys C, Di Michele M, Martens GA, Gerlo E, De Vos R, Buyse GM, **Goemans N**, Van Geet C, Freson K. Homozygosity for aquaporin 7 G264V in three unrelated

children with hyperglyceroluria and a mild platelet secretion defect. Genetics in medicine: official journal of the American College of Medical Genetics 2013;**15**(1):55-63.

8 Schrans DG, Abbott D, Peay HL, Pangalila RF, Vroom E, **Goemans N**, Vles JS, Aldenkamp AP, Hendriksen JG. Transition in Duchenne Muscular Dystrophy: An expert meeting report and description of transition needs in an emergent patient population: (Parent Project Muscular Dystrophy Transition Expert Meeting 17-18 June 2011, Amsterdam, The Netherlands). Neuromuscular disorders : NMD 2012.

9. Mercuri E, McDonald C, Mayhew A, Florence J, Mazzone E, Bianco F, Decostre V, Servais L, Ricotti V, **Goemans N**, Vroom E. International workshop on assessment of upper limb function in Duchenne Muscular Dystrophy: Rome, 15-16 February 2012. Neuromuscular disorders : NMD 2012;**22**(11):1025-8.

9. Ganea R, Jeannet PY, Paraschiv-Ionescu A, **Goemans N**M, Piot C, Van den Hauwe M, Aminian K. Gait assessment in children with duchenne muscular dystrophy during long-distance walking. Journal of child neurology 2012;**27**(1):30-8.

10. Buyse GM, **Goemans N**, van den Hauwe M, Meier T. Effects of glucocorticoids and idebenone on respiratory function in patients with duchenne muscular dystrophy. Pediatric pulmonology 2012.

11. Van Opstal N, Verlinden C, Myncke J, **Goemans N**, Moens P. The effect of Luque-Galveston fusion on curve, respiratory function and quality of life in Duchenne muscular dystrophy. Acta orthopaedica Belgica 2011;**77**(5):659-65.

12 Goemans NM, Tulinius M, van den Akker JT, Burm BE, Ekhart PF, Heuvelmans N, Holling T, Janson AA, Platenburg GJ, Sipkens JA, Sitsen JM, Aartsma-Rus A, van Ommen GJ, Buyse G, Darin N, Verschuuren JJ, Campion GV, de Kimpe SJ, van Deutekom JC. Systemic administration of PRO051 in Duchenne's muscular dystrophy. The New England journal of medicine 2011;**364**(16):1513-22.

13. Buyse GM, **Goemans N**, van den Hauwe M, Thijs D, de Groot IJ, Schara U, Ceulemans B, Meier T, Mertens L. Idebenone as a novel, therapeutic approach for Duchenne muscular dystrophy: results from a 12 month, double-blind, randomized placebo-controlled trial. Neuromuscular disorders : NMD 2011;**21**(6):396-405.

14 Wang CH, Bonnemann CG, Rutkowski A, Sejersen T, Bellini J, Battista V, Florence JM, Schara U, Schuler PM, Wahbi K, Aloysius A, Bash RO, Beroud C, Bertini E, Bushby K, Cohn RD, Connolly AM, Deconinck N, Desguerre I, Eagle M, Estournet-Mathiaud B, Ferreiro A, Fujak A, **Goemans N**,

Iannaccone ST, Jouinot P, Main M, Melacini P, Mueller-Felber W, Muntoni F, Nelson LL, Rahbek J, Quijano-Roy S, Sewry C, Storhaug K, Simonds A, Tseng B, Vajsar J, Vianello A, Zeller R. Consensus statement on standard of care for congenital muscular dystrophies. Journal of child neurology 2010;**25**(12):1559-81.

15 Regal L, Ebberink MS, **Goemans N**, Wanders RJ, De Meirleir L, Jaeken J, Schrooten M, Van Coster R, Waterham HR. Mutations in PEX10 are a cause of autosomal recessive ataxia. Annals of neurology 2010;**68**(2):259-63.

16 Casteels K, Fieuws S, van Helvoirt M, Verpoorten C, **Goemans N**, Coudyzer W, Loeckx D, de Zegher F. Metformin therapy to reduce weight gain and visceral adiposity in children and adolescents with neurogenic or myogenic motor deficit. Pediatric diabetes 2010;**11**(1):61-9.

17. Vandervelde L, Van den Bergh PY, Renders A, **Goemans N**, Thonnard JL. Relationships between motor impairments and activity limitations in patients with neuromuscular disorders. Journal of neurology, neurosurgery, and psychiatry 2009;**80**(3):326-32.

18. Vandervelde L, Van den Bergh PY, **Goemans N**, Thonnard JL. Activity limitations in patients with neuromuscular disorders: a responsiveness study of the ACTIVLIM questionnaire. Neuromuscular disorders : NMD 2009;**19**(2):99-103.

19. Jaeken J, Vleugels W, Regal L, Corchia C, **Goemans N**, Haeuptle MA, Foulquier F, Hennet T, Matthijs G, Dionisi-Vici C. RFT1-CDG: Deafness as a novel feature of congenital disorders of glycosylation. Journal of inherited metabolic disease 2009;**32 Suppl 1**:335-8.

20. Schessl J, **Goemans N**M, Magold AI, Zou Y, Hu Y, Kirschner J, Sciot R, Bonnemann CG. Predominant fiber atrophy and fiber type disproportion in early ullrich disease. Muscle & nerve 2008;**38**(3):1184-91.

21. Mertens L, Ganame J, Claus P, **Goemans N**, Thijs D, Eyskens B, Van Laere D, Bijnens B, D'Hooge J, Sutherland GR, Buyse G. Early regional myocardial dysfunction in young patients with Duchenne muscular dystrophy. Journal of the American Society of Echocardiography : official publication of the American Society of Echocardiography 2008;**21**(9):1049-54.

22. Labarque V, Freson K, Thys C, Wittevrongel C, Hoylaerts MF, De Vos R, **Goemans N**, Van Geet C. Increased Gs signalling in platelets and impaired collagen activation, due to a defect in the dystrophin gene, result in increased blood loss during spinal surgery. Human molecular genetics 2008;**17**(3):357-66.

23 van Deutekom JC, Janson AA, Ginjaar IB, Frankhuizen WS, Aartsma-Rus A, Bremmer-Bout M, den Dunnen JT, Koop K, van der Kooi AJ, **Goemans N**M, de Kimpe SJ, Ekhart PF, Venneker EH, Platenburg GJ, Verschuuren JJ, van Ommen GJ. Local dystrophin restoration with antisense oligonucleotide PRO051. The New England journal of medicine 2007;**357**(26):2677-86.

24. Vandervelde L, Van den Bergh PY, **Goemans N**, Thonnard JL. ACTIVLIM: a Rasch-built measure of activity limitations in children and adults with neuromuscular disorders. Neuromuscular disorders : NMD 2007;**17**(6):459-69.

25. Buyse GM, **Goemans N**, Henricson E, Jara A, van den Hauwe M, Leshner R, Florence JM, Mayhew JE, Escolar DM. CINRG pilot trial of oxatomide in steroid-naive Duchenne muscular dystrophy. European journal of paediatric neurology : EJPN : official journal of the European Paediatric Neurology Society 2007;**11**(6):337-40.

26. Verhoeven K, Claeys KG, Zuchner S, Schroder JM, Weis J, Ceuterick C, Jordanova A, Nelis E, De Vriendt E, Van Hul M, Seeman P, Mazanec R, Saifi GM, Szigeti K, Mancias P, Butler IJ, Kochanski A, Ryniewicz B, De Bleecker J, Van den Bergh P, Verellen C, Van Coster R, **Goemans N**, Auer-Grumbach M, Robberecht W, Milic Rasic V, Nevo Y, Tournev I, Guergueltcheva V, Roelens F, Vieregge P, Vinci P, Moreno MT, Christen HJ, Shy ME, Lupski JR, Vance JM, De Jonghe P, Timmerman V. MFN2 mutation distribution and genotype/phenotype correlation in Charcot-Marie-Tooth type 2. Brain : a journal of neurology 2006;**129**(Pt 8):2093-102.

27. Coen K, Pareyson D, Auer-Grumbach M, Buyse G, **Goemans N**, Claeys KG, Verpoorten N, Laura M, Scaioli V, Salmhofer W, Pieber TR, Nelis E, De Jonghe P, Timmerman V. Novel mutations in the HSN2 gene causing hereditary sensory and autonomic neuropathy type II. Neurology 2006;**66**(5):748-51.

28. Seneca S, **Goemans N**, Van Coster R, Givron P, Reybrouck T, Sciot R, Meulemans A, Smet J, Van Hove JL. A mitochondrial tRNA aspartate mutation causing isolated mitochondrial myopathy. American journal of medical genetics. Part A 2005;**137**(2):170-5.

29. Escolar DM, Buyse G, Henricson E, Leshner R, Florence J, Mayhew J, Tesi-Rocha C, Gorni K, Pasquali L, Patel KM, McCarter R, Huang J, Mayhew T, Bertorini T, Carlo J, Connolly AM, Clemens PR, **Goemans N**, Iannaccone ST, Igarashi M, Nevo Y, Pestronk A, Subramony SH, Vedanarayanan VV, Wessel H. CINRG randomized controlled trial of creatine and glutamine in Duchenne muscular dystrophy. Annals of neurology 2005;**58**(1):151-5.

30. Baker NL, Morgelin M, Peat R, **Goemans N**, North KN, Bateman JF, Lamande SR. Dominant collagen VI mutations are a common cause of Ullrich congenital muscular dystrophy. Human molecular genetics 2005;**14**(2):279-93.

31. Jungbluth H, Beggs A, Bonnemann C, Bushby K, Ceuterick-de Groote C, Estournet-Mathiaud B, **Goemans N**, Guicheney P, Lescure A, Lunardi J, Muntoni F, Quinlivan R, Sewry C, Straub V, Treves S, Ferreiro A. 111th ENMC International Workshop on Multi-minicore Disease. 2nd International MmD Workshop, 9-11 November 2002, Naarden, The Netherlands. Neuromuscular disorders : NMD 2004;**14**(11):754-66.

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