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Duchenne Muscular Dystrophy: Therapeutic Approaches to Restore Dystrophin

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1. Introduction

Duchenne muscular dystrophy (DMD) is a progressive neuromuscular disorder. The first symptoms involve the lower limbs and appear between the third and fifth year. Due to weakness of the knee and hip extensors, patients rise from a sitting position using the Gower's maneuver. Muscle weakness progresses to the shoulder girdle-, upper arm and trunk-muscles and patients loose ambulation before the age of 12 (Emery, 1993). Histological changes involve variation in fiber size with atrophic and hypertrophic fibers, degeneration and regeneration of the muscle fibers, infiltration of inflammatory cells and fibrosis. The fiber necrosis results in leakage of the enzyme creatine kinase (CK), resulting in very high serum CK levels in DMD patients (20,000 to 50,000 U/L compared to 80 to 250 U/L in unaffected individuals). These levels decline as patients get older and the overall muscle mass decreases progressively. The pathology is caused by mutations in the *DMD* gene, which was known to be on the X chromosome long before the responsible gene was cloned due to an X-linked recessive inheritance pattern. The protein product of the gene is a 427 KDa protein called dystrophin. In the early 80s several groups were collaborating on the regional cloning of the gene responsible for DMD (Burghes et al., 1987; Monaco et al., 1985) which happened to co-localize with the locus for Becker muscular dystrophy (BMD) (Kingston et al., 1984). This is a milder disease, where patients are diagnosed in adolescence or adulthood, remain ambulant longer and survival is generally only slightly decreased (Emery, 2002). After a couple of years Monaco and colleagues confirmed that deletions in the identified locus caused DMD (Monaco et al., 1985) and BMD (Hoffman et al., 1988). In the following years, the coding sequence of the gene was identified, which turned out to occupy a huge genomic region. The complete cDNA and protein product of the *DMD* gene were published in 1987 (Hoffman et al., 1987; Koenig et al., 1987). The cloning of the genomic and coding sequence of the *DMD* gene allowed the development of tools for the molecular diagnosis of DMD. Deletions of one or more exons were found to be most common (65% of patients) and mainly localized in two hotspot regions in the gene (exons 2-20 and 45-53). This led Chamberlain and colleagues to develop a multiplex PCR able to detect the most frequent mutations (Chamberlain et al., 1988). This technique has been used for years, but recently multiple ligation-dependent probe amplification (MLPA) has been developed that allows an exact characterization of exons involved in deletions and duplications (Janssen et al., 2005; Schwartz & Duno, 2004). For small mutations a more

labour-intensive method of PCR analysis of each exon, followed by direct sequencing is required (Spitali et al., 2009). The study of mutations and clinical features in DMD and BMD patients led to a deeper understanding of the disease, the gene and disease causing mutations. This made it possible to correlate genotype and phenotype and explain the discrepancy that mutations in one gene could lead to a severe DMD and a milder BMD phenotype. In 1989 two groups postulated that frame disrupting mutations were responsible for DMD while BMD was caused by frame maintaining mutations (Koenig et al., 1989; Monaco, 1989). This has been crucial for the development of certain potential therapies such as exon skipping and microdystrophins.

2. Dystrophin and the associated glycoprotein complex (DGC)

Dystrophin consists of 3685 amino acids and is a 427 kDa protein (Koenig et al., 1988). Dystrophin is composed of 4 domains, the first 240 N-terminal aminoacids define the actin-binding domain, which contains two actin-binding sites (Jarrett & Foster, 1995; Koenig & Kunkel, 1990). This domain is followed by a central rod shaped domain, consisting of 24 spectrin-like repeat units interrupted by 4 proline-rich hinge regions (Koenig & Kunkel, 1990). It has been demonstrated that an extra actin binding domain is present between repeats 11 and 17 (Rybakova et al., 1996) and that repeat 16 and 17 contain an nNOS binding site (Lai et al., 2009). The cysteine-rich domain encompasses aminoacids 3080 to 3360 and includes 15 cysteines, two EF hand motifs and a ZZ domain (Koenig et al., 1988) and binds to β-dystroglycan. Finally the C-terminal domain consists of the last 325 amino acids involved in protein-protein interactions. Dystrophin is part of the dystrophin-associated glycoprotein complex (DGC) (Figure 1). The cysteine-rich and C-terminal domains of dystrophin bind to several parts of the DGC, which can be divided into the dystroglycan complex, the sarcoglycan-sarcospan complex and the cytoplasmatic, dystrophin containing complex (Blake et al., 2002; Yoshida et al., 1994). In skeletal muscle the dystroglycan complex consists of α-dystroglycan and β-dystroglycan, which are both heavily glycosylated (Ibraghimov-Beskrovnaya et al., 1992). Dystrophin binds to β-dystroglycan, a transmembrane protein that binds to the extra-cellular α-dystroglycan; α-dystroglycan on its part binds to the extracellular matrix component laminin-2 (Hohenester et al., 1999; Rentschler et al., 1999; Suzuki et al., 1994). The sarcoglycan-sarcospan complex includes α-, β-, γ- and δ-sarcoglycan and sarcospan (Blake et al., 2002). The cytoplasmatic part of the DGC includes dystrophin itself, syntrophin and α-dystrobrevin, which binds to both dystrophin and syntrophin (Ahn et al., 1996). Alpha-syntrophin also binds to dystrophin and, additionally, it recruits the enzyme nNOS to the sarcolemma (Ahn & Kunkel, 1995; Brenman et al., 1995; Yoshida et al., 1995), although it has been recently demonstrated that the recruitment of nNOS by dystrophin repeats 16 and 17 is more important (Lai et al., 2009). Dystrophin also binds to and influences microtubules organization in the cytoplasm (Prins et al., 2009). In BMD patients, internally deleted dystrophins maintaining the N-terminal and C-terminal domains are able to bind to the DGC complex at the sarcolemma (Matsumura et al., 1994; Matsumura et al., 1993; Mirabella et al., 1998), while in DMD patients the absence of dystrophin results in the complete loss or decrease of other DGC proteins, and in the loss of nNOS at the sarcolemma (Brenman et al., 1995; Ervasti et al., 1990; Ohlendieck & Campbell, 1991). The function of the DGC is still largely unknown. However, since the complex forms a mechanical link between the cytoskeleton and the

extracellular matrix, it is assumed that the DGC has a function in maintaining sarcolemma stability during contraction (Matsumura & Campbell, 1994).

3. Pathology

Dystrophin loss leads to a high susceptibility of muscle fibers to injury after repeated eccentric contractions. This results in a chronic inflammation state, which provokes damage and necrosis. Muscle tissue is lost and replaced by fibrosis after exhausted cycles of damage and repair. Recent data suggest that stretched contractions activate reactive oxygen species (ROS) production, which causes opening of stretch-activated channels (SACs) and Ca2+ entry via src kinase activation induced by caveolin-3 (Allen et al., 2010). Oxidative stress may amplify the process inducing activation of the inflammatory transcription factor NF-κB, and thus functional impairment of force-generating capacity (Lawler, 2011).

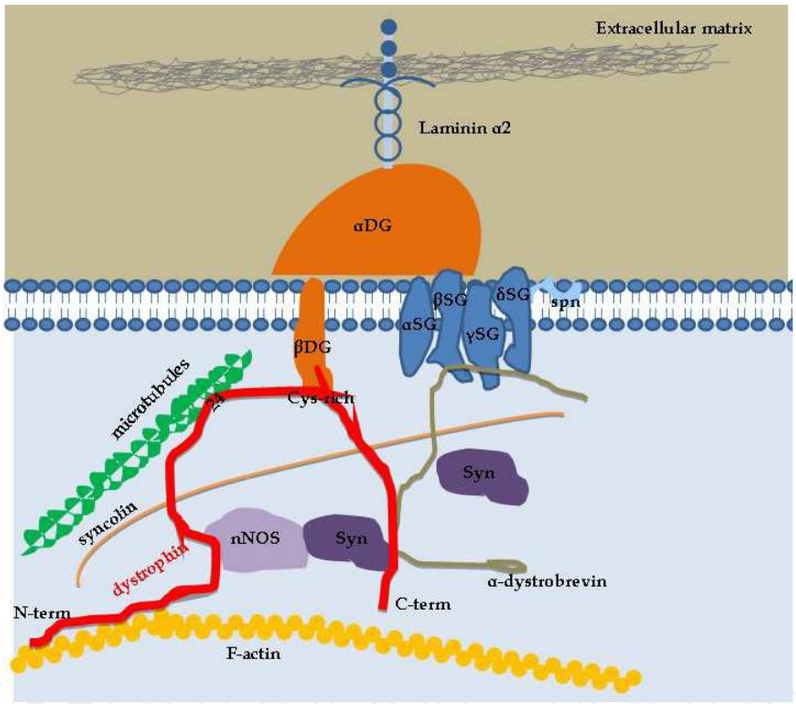


Fig. 1. Schematic representation of the dystrophin associated glycoprotein complex (DGC). αDG: α-dystroglycan; βDG: β-dystroglycan; αSG: α-sarcoglycan; βSG: β-sarcoglycan; γSG: γ-sarcoglycan; δSG: δ-sarcoglycan; spn: sarcospan; N-term: dystrophin aminoterminal domain; Cys-rich: dystrophin cystein rich domain; C-term: dystrophin carboxyterminal domain; nNOS: neuronal nitric oxide synthase; Syn: syntrophin.

4. Current treatment

There is currently no therapy for DMD. Nevertheless the lifespan and quality of life of DMD patients has significantly improved during the last 2 decades due to improved health care, especially assisted ventilation (Eagle et al., 2002). The mean age of death in the 1960s was 14.4 years, whereas for those ventilated since 1990 it was 25.3 years. The chances of survival to 25 years have increased from 0% in the 1960s to 4% in the 1970s and 12% in the 1980s, and

that the impact of nocturnal ventilation has further improved this chance to 53% for those ventilated since 1990. Another crucial step has been the use of corticosteroids (mainly prednisone (Moxley, III & Pandya, 2011) and deflazacort (Biggar et al., 2001)), which reduce the inflammatory response in patients' muscle and the accompanied damage and fibrosis, thus longer maintaining muscle quality. The benefit of corticosteroids has been clearly demonstrated for DMD patients in a double-blind randomized controlled trial in more than 100 boys (Mendell et al., 1989). Corticosteroids treatment extends the ambulation of patients for about 2 years and reduces scoliosis (King et al., 2007). The prolonged use of corticosteroids has however known side effects, which include weight gain, hypertension, bone demineralization, vertebral compression fractures and sometimes behavior disorders. Guidelines for DMD patients' management have been published in order to harmonize the standards of clinical practice (Bushby et al., 2010a; Bushby et al., 2010b).

There are numerous therapeutic approaches under development for DMD. Some aim at addressing specific issues of pathology such as Idebenone, or green tea extract to reduce oxidative stress (Dorchies et al., 2009; Nagy & Nagy, 1990), or myostatin inhibition to increase muscle mass (Bish et al., 2011; Dumonceaux et al., 2010), while others directly aim at dystrophin restoration. In this chapter we will focus on the latter.

5. Therapeutic approaches

5.1 Stop codon read-through

This approach has been developed to address nonsense mutations, which are responsible for 14% of DMD cases (Aartsma-Rus et al., 2009). The rationale is to use a compound that interacts with the translation machinery to incorporate an amino acid instead of terminating protein translation at the site of a premature stop codon. This will result in a protein that is – aside from a one amino acid change at the location of the stop mutation – completely normal (Aurino & Nigro, 2006; Kaufman, 1999; Linde & Kerem, 2008; Malik et al., 2010a). This approach can also induce read-through of real stop codons, but this is thought to be less efficient due to differences in sequence context and location of real vs. aberrant stop codons (Manuvakhova et al., 2000). So far, three compounds have been reported induce efficient read-through stop codons in the DMD mRNA.

5.1.1 Gentamicin

Gentamicin is an aminoglycoside antibiotic binding to the 40S ribosomal subunit when this recognizes a stop codon (Palmer et al., 1979; Singh et al., 1979; Yoshizawa et al., 1998). This causes the insertion of an amino acid at the stop codon position. It has first been shown that it can act on each type of stop codon without any preference in vitro (Howard et al., 2004). Gentamicin (and negamycin) can induce the read-through stop codon in the mdx mouse (Arakawa et al., 2003; Barton-Davis et al., 1999), the most used mouse model for DMD which carries a nonsense mutation in exon 23 (Danko et al., 1992). However, in another report gentamicin was unable to restore dystrophin expression in the same mouse model (Dunant et al., 2003). It was later revealed that there are a number of gentamicin isomers, which all have different read-through efficiencies, and that different gentamicin batches consist of different mixes of these isomers, which can explain these controversial results (Aartsma-Rus et al., 2010; Yoshizawa et al., 1998). Three different clinical trials have been undertaken in

DMD patients using gentamicin (Malik et al., 2010b; Politano et al., 2003; Wagner et al., 2001). In the last one Malik and colleagues used the most active gentamicin isomer and treated the patients for 6 months. For 3 out of 12 patients the number of dystrophin positive fibers increased as assessed by immune histochemical analysis, while the effect was less clear by western blot analysis, as dystrophin was already visible before treatment, possibly due to spontaneous read-through or exon skipping (see below). Since chronic gentamicin use, is known to result in reversible kidney toxicity and irreversible ototoxicity, long term treatment with gentamicin – which would be required for DMD patients – is not realistic.

5.1.2 Ataluren

Ataluren, also called PTC124, was identified via in vitro screening in a luciferase assay. It is more selective for premature stop codons than regular ones, and it can be taken orally unlike gentamicin, which is administered intravenously. Studies in the mdx mouse showed that dystrophin expression could be restored after subcutaneous ataluren treatment (Welch et al., 2007). The compound was first tested in healthy volunteers where it was well tolerated (Hirawat et al., 2007). Then different doses were tested in DMD patients and an increase in dystrophin was reported for 18/38 patients (http://www.drugs.com/clinical_trials/ptc-therapeutics-announces-additional-results-phase-2-study-ptc124-duchenne-muscular-dystrophy-2308.html). In a subsequent placebo-controlled phase IIb trial 174 DMD and BMD patients were treated with two doses or placebo for 48 weeks, and then all were treated with the high dose in an open label extension study (Finkel, 2010). Treatment was well tolerated, but the primary outcome – set at 30 meter increase compared to placebo treated patients in the six minute walk test (6MWT) - was not reached, and the extension study was put on hold. From the data released (http://ptct.client.shareholder.com/releasedetail.cfm?ReleaseID=518941) it could be inferred that the low dose worked better than the high dose. It has been postulated that ataluren efficiency works through a bell shaped curve, which could explain this finding. Dystrophin analysis is pending, and different analyses of subgroups of patients is currently ongoing, as well as studies to identify the most optimal dose. Recently (May 2011) Genzyme and PTC Therapeutics announced that they are planning a follow-up clinical study for DMD patients who previously participated in the clinical trials in the UK, Europe, Israel and Australia, starting December 2011. This will provide access to ataluren to patients who have been involved in earlier clinical trials, as the trial in the USA had already been reinititated.

It has been recently published that the results obtained with the in vitro luciferase screening used to identify ataluren may have been biased, as atluren derivatives can stabilize the luciferase enzyme, giving rise to a false positive (Auld et al., 2010; Auld et al., 2009). However, it has been shown that ataluren has at least some read-through potential (Du et al., 2008; Welch et al., 2007), though it is uncertain whether the levels of dystrophin restoration will be sufficient to restore muscle function.

5.1.3 RTC13

RTC13 is a new compound for stop codon read through. Promising preliminary data were presented at the 14th annual meeting of the American Society of Gene and Cell Therapy (http://www.cureduchenne.org/site/PageServer?pagename=research_index). In the mdx mouse model RTC13 was able to restore dystrophin expression as assessed by

western blot and immune-fluorescent analyses. Muscle fiber uptake was improved compared to the previous tested compounds. After 6 weeks of treatment mice showed improvement in muscle strength that was dependent on dystrophin recovery. Serum CK levels dropped and no toxicity was detected. Research to assess if RTC13 oral treatment is feasible is ongoing.

5.1.4 Nonsense mediated decay

A modifying factor which can play a role in the stop codon-read through is nonsense-mediated decay (NMD). This mechanism breaks down mRNAs that carry premature stop codons, thus resulting in less target mRNAs for stop codon read through compounds. It is known that NMD efficiency varies among individuals, for different stop codons, location within the mRNA, and sequence context. In a study of cystic fibrosis patient-derived cell cultures carrying premature stop mutations in their *CFTR* gene, it was shown that NMD was more active in patients who did not respond to gentamicin treatment than in patients who did respond to gentamicin patients (Linde et al., 2007). When NMD was blocked in non-responders' cells, they became more responsive to gentamicin treatment (Linde et al., 2007). It is anticipated that NMD influences other stop codon read through approaches.

5.2 Exon skipping

The idea of the exon skipping approach is based on the observation that the milder BMD phenotype is due to mutations in which the mRNA reading frame is maintained, while the more severe DMD phenotype is caused by frame disrupting mutations. The rationale is to restore the open reading frame. Most (~65%) of the DMD causing mutations are deletions (Aartsma-Rus et al., 2009). Scientists have tried to reframe these mutations by inducing the skipping of additional exons adjacent to the out of frame deletions during pre-mRNA splicing (Figure 2). The reframed mRNA will then allow translation into a smaller, partially functional, BMD-like dystrophin protein. Several groups have worked on this approach using antisense oligonucleotides (AONs) or snRNAs to induce the specific exon skipping in patients' cells in vitro and in several animal models.

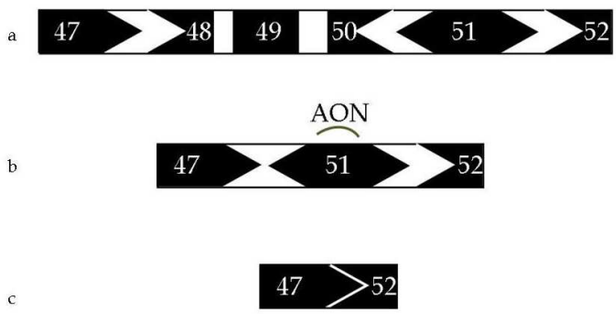


Fig. 2. a. Schematic representation of the DMD genomic region encompassing exons 47 to 52. b. Deletion of exons 48 to 50 leads to an out-of-frame mRNA which can be corrected into an in-frame deletion with the use of antisense oligonucleotides (c).

There are two AON chemistries used in clinical trials for DMD: 2-O-Methyl-Phosphorothioates (2OMePS) and phosphorodiamidate morpholino oligomers (PMOs).

5.2.1 2OMePS studies

2OMePS have a methyl group at the 2'-O position of the ribose, which increases the AON affinity for RNA and avoids RNase H activation of RNA:RNA hybrids (Dominski & Kole, 1993; Sproat et al., 1989). The PS modification is required to further increase the AON nuclease resistance, enhance cellular uptake and increase the serum half-life in vivo.

Proof of principle for the exon skipping approach and dystrophin restoration has been achieved in DMD derived cells and in murine cells (Aartsma-Rus et al., 2003; Errington et al., 2003; Mann et al., 2002; Van Deutekom et al., 2001). To test the feasibility of the approach in vivo the mdx mouse model was mainly used. The premature stop codon in exon 23 leads to a complete absence of dystrophin, and a mild dystrophic phenotype in mice, probably due to a better regenerative capacity. Mouse dystrophin exon 23 is an in frame exon, so it is possible to skip this exon, inducing an exon 22-exon 24 junction which preserves the reading frame. Intramuscular injection of AONs targeting the exon 23 donor splice site resulted in exon 23 skipping and dystrophin synthesis (Lu et al., 2003; Mann et al., 2002). This was accompanied by rescued sarcoglycan expression at the sarcolemma, improved titanic force, while no antibodies against the newly synthesized dystrophin were found in the serum. Gene expression profiling to evaluate AON efficacy and safety was tested in preclinical experiments. AONs were delivered using different carriers (PEI – F127 – Optison) to enhance muscle fibers uptake, or with recombinant adeno-associated virus (rAAV) expressing antisense sequences incorporated in a U7 snRNP gene. Exon skipping induced a shift towards wild type expression levels, which became statistically significant when high exon skipping levels were induced ('t Hoen et al., 2006). Since AON-PEI complexes worsened the muscle inflammation, while F127 and Optison did not enhance AON efficacy in vivo, following experiments were performed using naked AONs. These studies all tested local intramuscular injection, while whole body treatment is required for DMD. It is known that 2OMePS AONs have a favorable serum half-life, as the PS backbones binds to serum proteins with low affinity, which prevents renal filtration and excretion in urine. Normally, 2OMePS AONs are primarily taken up by liver and kidney and the uptake in muscle is poor. However, due to the dystrophic pathology of skeletal muscle in DMD patients, AON uptake is up to 10-fold higher, resulting in sufficient AON levels for exon skipping and dystrophin restoration in skeletal muscles in mdx mice after systemic AON administration (Heemskerk et al., 2009; Lu et al., 2005). In a comparison of intravenous, subcutaneous and intraperitoneal delivery, subcutaneous and intraperitoneal delivery showed the most preferable pharmacokinetic and pharmacodynamic profiles (lower uptake by liver and kidney), while slightly higher exon skipping levels were achieved by intravenous injections (Heemskerk et al., 2010). Based on these results and the relative easy and low invasiveness of subcutenaous injection, this delivery route was selected for systemic clinical trials.

5.2.1.1 Clinical trials with 2OMePS

Since the exon skipping is a mutation specific approach clinical experimentation started with the largest patient cohort that would potentially benefit of skipping a single exon, i.e. exon 51 (13% of all DMD patients (Aartsma-Rus et al., 2009)). Deep phenotypic screening of two BMD patients carrying deletions that could result from exon 51 skipping, showed that these dystrophins can be largely functional (Helderman-van den Enden AT et al., 2010). A first clinical trial was coordinated by Prosensa/GSK in collaboration with the Leiden

University Medical Center, using an AON targeting exon 51 (PRO051, currently called GSK2402968). This trial involved 4 patients who were each injected intramuscularly with 0.8 mg GSK2402968 in their tibialis anterior muscle. This induced specific exon 51 skipping and dystrophin recovery in 64-97% of muscle fibers at levels of 17-35% and 3-12% when quantified by immune-histochemical and western blot analysis, respectively) (Van Deutekom et al., 2007). After these encouraging results, the same AON was used in a subsequent Phase I/IIb clinical trial in which patients were subcutaneously injected and divided into 4 cohorts of 3 patients each based on the dose used (0.5 – 2 - 4 - 6 mg/kg). The AON was well tolerated, and patients showed a dose dependent dystrophin recovery in 60-100% of fibers (Goemans et al., 2011). Dystrophin amounts were quantified via fluorescent signal intensities in immune-histochemical analysis (4 to 11% dystrophin recovery) and via western blot analysis (2 to 20% dystrophin recovery). All patients were included in an open label extension study where they received weekly, subcutaneous treatments of the highest dose (6 mg/kg). The 6-minutes walk test was used as a functional outcome parameter and for most of the patients an improvement in walking distance was found after 3 months (Goemans et al., 2011). This trial did not have a placebo group, so these results, while promising, have to be interpreted with caution. A phase III double blind clinical trial in 180 DMD patients is ongoing to determine whether long term treatment with 6 mg/kg/week GSK2402968 is safe and effective ((http://clinicaltrials.gov/ct2/show/NCT01254019?term=duchenne&rank=4). Furthermore, a trial comparing weekly and biweekly dosing at 6 mg/kg is ongoing (http://clinicaltrials.gov/ct2/show/NCT01153932?term=duchenne&rank=11). Finally, a phase I double-blind, escalating dose, randomized, placebo-controlled study assessing pharmacokinetics, safety, and tolerability in non-ambulant DMD patients is ongoing (http://clinicaltrials.gov/ct2/show/NCT01128855?term=GSK2402968&rank=3) in which patients will receive different dosages (3 – 6 - 9 -12 mg/kg).

5.2.2 PMOs

PMOs contain a morpholino moiety instead of the ribose sugar and phosphoroamidate intersubunit linkages instead of phosphodiester bonds (Kurreck, 2003). PMOs have an affinity for RNA that is comparable to DNA oligos, are nuclease resistant and non-toxic (Summerton, 1999). Their backbone is uncharged, which makes them difficult to transfect in vitro, and as they do not bind serum proteins, their serum half-life is limited as they are filtered out by the kidneys. PMOs have been shown to induce exon skipping and dystrophin restoration in the mdx mouse after intramuscular (Gebski et al., 2003) and systemic injections (Alter et al., 2006; Malerba et al., 2011a; Wu et al., 2010). Upon direct comparison with 2OMePS they were shown to be more effective in inducing exon 23 skipping in the mdx mouse (Heemskerk et al., 2009). However, in all studies exon skipping and dystrophin restoration was only observed in skeletal muscle and not in heart, or only at very low levels unless heroic doses (up to 3 g/kg!) or microbubbles to improve uptake in heart were used (Alter et al., 2009; Wu et al., 2010). It has become clear that repeated low dose injections are more effective than single high dosage injections (Malerba et al., 2011b; Malerba et al., 2009), probably because of the fast PMOs clearance from the body by the kidneys (Heemskerk et al., 2010). Survival studies show that high doses of PMOs could correct the pathology and were well tolerated (Wu et al., 2011b). PMOs have also been used to restore dystrophin expression in the canine model of Duchenne, the golden retriever muscular dystrophy model (GRMD). GRMD dogs carry a splice site mutation in intron 6 leading exon 7

skipping. The skipping of exons 6 and 8 produces an in-frame exon-exon junction. Three dogs have been treated with an equimolar mixture of 3 morpholinos (2 targeting exon 6 and 1 targeting exon 8 in a cumulative dose of 120-200 mg/kg). PMOs were injected 5 to 11 times at weekly or biweekly intervals and tissue examination was performed 2 weeks after the last injection. Dystrophin restoration was achieved showing that exon skipping represents a possible choice also for complex mutations for which more than one exon needs to be skipped (Yokota et al., 2009).

5.2.2.1 Clinical trials with PMOs

Before starting clinical studies optimization of PMOs targeting exon 51 was done in cells and in the hDMD mouse (Arechavala-Gomeza et al., 2007). Clinical studies in DMD patients using PMOs targeting exon 51 (AVI-4658) were performed in the UK by the MDEX consortium in collaboration with AVI Biopharma. Local intramuscular injection in the extensor digitorum longus (EDL) muscle induced exon skipping and dystrophin restoration. After baseline correction for the controlateral, saline injected muscle, 44 to 79% of dystrophin positive fibers were observed (Kinali et al., 2009) at 22-32% of wild type levels (controlateral muscle showed dystrophin levels between 4 and 14%). This led to a Phase I/IIb clinical trial in which 19 patients received 12 weekly intravenous doses of PMO. Patients were divided in cohorts based on six different doses (0.5 – 1 – 2 – 4 - 10 - 20 mg/kg). PMOs were not toxic and well tolerated. The 2 high dosage cohorts showed an increase in the fluorescent intensity per fiber and dystrophin was restored in 7/19 patients. Three patients responded very well showing up to 55% of dystrophin positive fibers with an increase above 10% in mean fluorescence intensity per fiber (Cirak et al., 2011). Based on the varying response it was concluded that dosing was not yet optimal. In a subsequent study recently initiated, weekly intravenous doses of 30 mg/kg and 50 mg/kg for 24 weeks are tested (http://clinicaltrials.gov/ct2/show/NCT01396239?term=AVI-4658&rank=1).

It is difficult to compare results for the experiments performed with the PMOs and 2OMePS, as they were performed by different groups and different analyses were used to quantify dystrophin. In a direct comparison using equal molar amounts of PMO and 2OMePS in the mdx mouse, PMOs targeting exon 23 showed higher exon skipping percentages and higher dystrophin rescue. However, experiments performed in the hDMD mouse model, carrying a copy of the complete human *DMD* gene, there was no clear difference between 2OMePS and PMO AONs targeting exon 44, 45, 46 and 51 upon intramuscular injection (Heemskerk et al., 2009). Differences in the systemic trials for GSK2402968 and AVI-4658 are probably also due to the different pharmacokinetic and pharmacodynamic properties of the AONs. PMOs are extremely stable, but due to their uncharged nature they are filtered out by the kidney and their serum half-life is ~1 hour, so the time for tissue uptake is limited. The 2OMePS AONs by contrast bind serum proteins due to the PS backbone. This prevents renal clearance and increases their serum half-life to weeks. This may underlie the different staining patterns observed between PMO trials (patchy) and 2OMePS trials (more homogeneous).

5.2.3 AON chemistry development

While results in clinical trials are encouraging, ways to improve delivery to muscle tissues, allowing lower AON dosages would be preferred. Many approaches have been tested. Cell penetrating peptides (Jearawiriyapaisarn et al., 2008; Jearawiriyapaisarn et al., 2010; Wu et

al., 2008; Yin et al., 2008), muscle targeting peptides connected to cell penetrating peptides (Yin et al., 2009) and guanidine analogs (Hu et al., 2010; Wu et al., 2009) showed the most promising results in the mdx mouse and in the hDMD mouse model (Wu et al., 2011a). Notably, pPMOs (containing arginine-rich peptides covalently bound to the morpholino AONs) have shown great potential in the mdx mouse, inducing high levels of exon skipping in skeletal muscles and heart and high levels of dystrophin rescue. Promising results using pPMOs have also been achieved in the severe mdx-utrophin$^{-/-}$ mouse model, which is defective for dystrophin and its homologue utrophin gene. Normally these mice do not survive beyond 3 months, but survival was increased to over a year after pPMO treatment (Goyenvalle et al., 2010). Unfortunately, preliminary tests in non-human primates showed mild tubular degeneration in the kidney after 4 weekly injections of 9 mg/kg of pPMOs (Moulton & Moulton, 2010). Additional peptide conjugates will hopefully be less toxic (Yin et al., 2011).

5.2.4 Antisense snRNP mediated exon skipping

Due to AON turnover and clearance, life-long treatment would be required. An alternative approach uses viral vectors expressing antisense sequences incorporated in a small nuclear ribonucleoprotein (snRNP). Adeno-associated viral vectors (AAVs) have been used to deliver the modified snRNPs as they have the best capacity to infect the muscle tissue. Different serotypes have been investigated and two molecular strategies which make use of modified U7 and U1 snRNAs have been developed. These snRNAs ensure an efficiently nuclear localization of the antisense construct, specific exon skipping and sustained dystrophin rescue in the mdx mouse model (Denti et al., 2006a; Denti et al., 2006b; Goyenvalle et al., 2004). Optimization for human exons using splicing enhancers has been also performed (Goyenvalle et al., 2009). Preclinical studies show the long-term benefit of the approach for up to 1 year (Denti et al., 2008). However the clinical translation of this approach is complicated by immune response to the viral vector (see section 5.4). Thus immune-suppression will be required, especially for patients for which multiple injections to treat muscles or muscle groups will be required.

5.3 Gene editing

Gene editing is a process in which the endogenous mutated gene is modified to produce a functional dystrophin, either by correcting the DMD causing mutation or by introducing a second mutation which will rescue the effect of the first mutation. This approach has been developed using chimeric RNA-DNA oligonucleotides (RDOs or "chimeraplasts") which anneal to genomic DNA, inducing homologous recombination between the endogenous gene and the RDO, or activating the mismatch repair system. Proof of principle was demonstrated in the mdx mouse muscle (Rando et al., 2000), in muscle precursor cells in vitro and in vivo (Bertoni & Rando, 2002). It has been demonstrated that correction is more efficient when the RDOs target the coding strand (the non transcribed strand) (Bertoni et al., 2005). Unfortunately, the gene conversion efficiency is as yet too low for clinical application and systemic delivery of RDOs in larger animals needs to be optimized further.

Recently another group has pioneered the use of meganucleases to correct the effect of the genetic mutation. The rationale is to correct the reading frame by introducing a micro-

deletion or micro-insertion into the *DMD* gene. This is done by specific double strand breaks at the end of an exon which precedes a deletion or at the beginning of an exon following a deletion. Meganucleases can be engineered to specifically cut at a certain genomic position causing non-homologous end joining (NHEJ) or homologous recombination when a donor corrected sequence is present. During this process often small deletions or insertions occur, which can restore the reading frame. Proof of principle for this approach has been recently demonstrated in vitro and after local delivery in vivo, albeit at low levels (Chapdelaine et al., 2010). The challenge of this approach will be the delivery of the meganucleases and the limited recognition of target sequences of meganucleases. The recently developed TALE nuclease system (Miller et al., 2011) allows targeting of almost all human sequences, and may provide a better alternative.

5.4 Gene therapy

Gene therapy approaches focus on providing an exogenous functional copy of the mutated gene. Gene therapy approaches are divided into 2 groups based on the type of delivery method used (viral or non-viral vector mediated). Muscle is a difficult target tissue for viral delivery (see below). Furthermore, the huge size of the *DMD* gene and its 11 Kb long full length cDNA sequence (FLDYS) has been one of the bottlenecks in developing this strategy, until smaller dystrophin versions were developed to make them fit into viral vector capsids. These smaller dystrophin coding sequences, called mini-dystrophins (mDYS) and micro-dystrophins (µDys), were designed based on the observations that BMD patients with minor dystrophic phenotype can carry very large deletions (England et al., 1990).

5.4.1 Viral vector based gene therapy

5.4.1.1 Lentiviral vectors

Lentiviral vectors have been used to treat mdx mice locally restoring dystrophin expression at different efficiencies (Kobinger et al., 2003; Li et al., 2005). Kimura and colleagues showed that a lentivirus encoding µDys intramuscularly injected into 2 weeks old mdx^{4cv} mice could restore dystrophin in up to 400-1200 fibers in the tibialis anterior muscle. Mice were sacrificed at 4 different time points (4 weeks – 4 months – 1 year – 2 years) and results were comparable over time. The virus was capable of infecting satellite cells ensuring long-term treatment efficacy (Kimura et al., 2010). However, this may also pose a safety risk, since the transgene expression is ensured by the integration of the viral genome into the host genome. This process can cause neoplastic mutations due to the integration of the viral genome which mainly occurs close to promoter sequences (Maruggi et al., 2009). Systemic delivery of lentiviral vectors to muscle tissue is very challenging, as muscle is post-mitotic and fibers bundles are surrounded by layers of connective tissue that filter out most viruses (>30 nm).

5.4.1.2 Adeno-associated viral vectors

Due to their small size (20 nm) adeno-associated viral vectors (AAVs) are able to efficiently infect muscles. They have been more broadly used for gene therapy for muscle diseases. They do not integrate into the host genome, making them safer than lentiviruses. At least five of the many serotypes known, show a high tropism for muscle tissues (serotypes 1, 2, 6, 8 and 9). Due to the low vector capacity mDYS and µDys have been used and efficiently

delivered to skeletal muscle (Gregorevic et al., 2004) and heart. Very promising studies performed in the mdx mouse showed high dystrophin recovery with AAV1 (Wang et al., 2008) and AAV2 (Wang et al., 2000), while studies in the dog model with AAV2, AAV6 (Wang et al., 2007) and AAV8 (Ohshima et al., 2009) raised the issue of cytotoxic immune response against the viral capsid proteins. AAV8 has also been tested in non human primates to delivery human µDys and levels up to 80% were obtained. Unfortunately these levels dropped to 40%when antibodies against the viral vector were present before the injection (Rodino-Klapac et al., 2010).

A clinical study has been also carried out in 6 DMD patients (aged 5-11 years) who received an intramuscular injection into the biceps muscle of a recombinant AAV (rAAV) vector carrying a µDYS gene (Mendell et al., 2010). This µDYS encoded the amino-terminal actin binding domain (ABD), 5 rod repeat domains (R1, R2, R22, R23, and R24), 3 hinge domains (H1, H3 and H4), and the cysteine-rich (CR) domain of the human *DMD* gene. The human cytomegalovirus (CMV) immediate early promoter regulated transgene expression. Vector genomes were packaged in AAV2.5, a serotype 2 capsid variant that contains five AAV1 amino acids (one insertion and four substitutions) in the AAV2 VP1 background. AAV2.5 offers improved muscle transduction properties of AAV1 with minimal recognition by serum neutralizing antibodies. Dystrophin recovery was only very limited (a few fibers for 2 patients). Mendell and colleagues further showed the presence of T-cells recognizing dystrophin epitopes in the circulation of some of the patients. For one patient the recognized epitopes were present in the µDys and deleted in the patient *DMD* gene, so this perhaps was not unexpected. Interestingly, for 2 patients T-cells able to recognize dystrophin expressed in revertant fibers were identified before and after treatment. However, the continuous presence of revertant fibers suggests that the immunity against dystrophin in the blood, did not lead to an auto-immune response in the muscle tissue.

5.5 Cell therapy

Another approach to restore dystrophin expression is based on the use of stem cells with myogenic potential, which can help repair the muscle damage and also delivery a healthy (when donor cells are used) or corrected (when autologous cells are used) *DMD* gene.

Initial efforts focused on the transplantation of adult myoblasts able to fuse with resident damaged muscle fibers creating hybrid muscle fibers (Brussee et al., 1999; Gussoni et al., 1997). However, this approach turned out to be hindered by poor cell survival, inability of the cells to extravagate into the muscle from the circulation, and limited migration of the injected cells within the host muscle (Qu et al., 1998). Results of clinical studies were discouraging (Tremblay et al., 1993). To compensate for the poor migration within muscle, a multiple injection technique has been used (up to 250 injections per square cm) (Skuk et al., 2006), but this is only feasible for small superficial muscles.

Many adult stem cells have been tested for their ability to fuse with muscle fibers in the host dystrophic muscles in murine and canine models. Cells have been grown in vitro and then transplanted in vivo with different efficiencies. Characterization of these cells has been based on their adhesion properties in vitro or on their membrane markers. Muscle side-populations cells, bone-marrow-derived stem cells, muscle-derived stem cells, mesangioblasts, blood and muscle derived CD133+ stem cells and pericytes have been

identified (Asakura & Rudnicki, 2002; Benchaouir et al., 2007; Dezawa et al., 2005; Doherty et al., 1998; Gavina et al., 2006; Palumbo et al., 2004; Qu-Petersen et al., 2002). Among all, satellite cells, mesangioblasts and pericytes have shown the most promising characteristics.

Satellite cells are small progenitor cells that lie between the basement membrane and the sarcolemma of the muscle fibers. They are normally in a quiescent state but they can be activated to form new muscle fibers or to fuse with damaged ones upon muscle fiber injury. They are characterized by the expression of *pax3* and *pax7* and they have been shown to restore dystrophin expression after transplantation in dystrophic dog muscle (Montarras et al., 2005). Satellite cells have a great myogenic potential that is unfortunately lost when they are expanded in vitro. Encouraging results obtained in a mouse model led to a phase I clinical trial in DMD patients. Donor satellite cells were isolated from muscle biopsies from first-degree relatives of the affected children and were grown in culture (Daston et al., 1996; Seale et al., 2004). Dystrophin production in muscle fibers was very low (~1%) and no functional or clinical improvement in the children was observed (Peault et al., 2007).

Mesangioblasts express early but not late epithelial markers, they can transmigrate from blood vessels in tissues and they can differentiate in to muscle (Meregalli et al., 2010).

Autologous corrected and donor mesangioblasts have shown to recover dystrophin expression in dystrophic dogs, although some dogs died due to pneumonia which may be caused by accumulation of these cells in the lungs (Sampaolesi et al., 2006). At the moment mesangioblasts are tested in a clinical safety trial in DMD patients.

Pericytes share various markers with mesangioblasts, they can be isolated from skeletal muscle (Dellavalle et al., 2007) and also from non-muscular tissues (Crisan et al., 2008). Dellavalle and coworkers demonstrated that pericytes have high myogenic capacity when injected into SCID/mdx mice. It still needs to be determined whether transplanted pericytes can fully reconstitute the satellite cell niche as real functional stem cells (Morgan & Muntoni, 2007) and whether systemic delivery can be performed.

The main hurdles facing stem cell treatment for DMD are the abundance of muscle (up to 40% of the bodyweight in men), which, combined with the poor efficiency of delivery of cells to muscle tissue (generally (much) below 10%), creates the need for the transplantation of huge numbers of cells in order to generate clinical benefit.

Finally the use of donor stem cells would need constant immune suppression to avoid a specific immune response against the newly formed myofibers. This issue can be solved using autologous stem cells modified ex vivo. However, this process reduces the myogenic properties of cells using current culturing methods, and may impact the behavior of the cells once they are re-injected in the patients.

6. Conclusion

In the last 20-25 years we have seen how basic science findings have been translated into clinical research. Many therapeutic approaches have been developed in vitro, in preclinical animal models and some of them have advanced to the clinical stage. Among all therapeutic approaches the exon skipping is at the moment the most promising for clinical application in the near future.

7. References

't Hoen, P. A., van der Wees, C. G., Aartsma-Rus, A., Turk, R., Goyenvalle, A., Danos, O., Garcia, L., van Ommen, G. J., den Dunnen, J. T., & Van Deutekom, J. C. (April 2006). Gene expression profiling to monitor therapeutic and adverse effects of antisense therapies for Duchenne muscular dystrophy. *Pharmacogenomics.*, Vol.7,No.3, (April 2006), pp. 281-297, ISSN

Aartsma-Rus, A., den Dunnen, J. T., & van Ommen, G. J. (December 2010). New insights in gene-derived therapy: the example of Duchenne muscular dystrophy. *Ann.N.Y.Acad.Sci.*, Vol.1214,No.December 2010), pp. 199-212, ISSN

Aartsma-Rus, A., Fokkema, I., Verschuuren, J., Ginjaar, I., van, D. J., van Ommen, G. J., & den Dunnen, J. T. (March 2009). Theoretic applicability of antisense-mediated exon skipping for Duchenne muscular dystrophy mutations. *Hum.Mutat.*, Vol.30,No.3, (March 2009), pp. 293-299, ISSN

Aartsma-Rus, A., Janson, A. A., Kaman, W. E., Bremmer-Bout, M., den Dunnen, J. T., Baas, F., van Ommen, G. J., & Van Deutekom, J. C. (April 2003). Therapeutic antisense-induced exon skipping in cultured muscle cells from six different DMD patients. *Hum.Mol.Genet.*, Vol.12,No.8, (April 2003), pp. 907-914, ISSN

Ahn, A. H., Freener, C. A., Gussoni, E., Yoshida, M., Ozawa, E., & Kunkel, L. M. (February 1996). The three human syntrophin genes are expressed in diverse tissues, have distinct chromosomal locations, and each bind to dystrophin and its relatives. *J.Biol.Chem.*, Vol.271,No.5, (February 1996), pp. 2724-2730, ISSN

Ahn, A. H. and Kunkel, L. M. (February 1995). Syntrophin binds to an alternatively spliced exon of dystrophin. *J.Cell Biol.*, Vol.128,No.3, (February 1995), pp. 363-371, ISSN

Allen, D. G., Gervasio, O. L., Yeung, E. W., & Whitehead, N. P. (February 2010). Calcium and the damage pathways in muscular dystrophy. *Can.J.Physiol Pharmacol.*, Vol.88,No.2, (February 2010), pp. 83-91, ISSN

Alter, J., Lou, F., Rabinowitz, A., Yin, H., Rosenfeld, J., Wilton, S. D., Partridge, T. A., & Lu, Q. L. (February 2006). Systemic delivery of morpholino oligonucleotide restores dystrophin expression bodywide and improves dystrophic pathology. *Nat.Med.*, Vol.12,No.2, (February 2006), pp. 175-177, ISSN

Alter, J., Sennoga, C. A., Lopes, D. M., Eckersley, R. J., & Wells, D. J. (June 2009). Microbubble stability is a major determinant of the efficiency of ultrasound and microbubble mediated in vivo gene transfer. *Ultrasound Med.Biol.*, Vol.35,No.6, (June 2009), pp. 976-984, ISSN

Arakawa, M., Shiozuka, M., Nakayama, Y., Hara, T., Hamada, M., Kondo, S., Ikeda, D., Takahashi, Y., Sawa, R., Nonomura, Y., Sheykholeslami, K., Kondo, K., Kaga, K., Kitamura, T., Suzuki-Miyagoe, Y., Takeda, S., & Matsuda, R. (November 2003). Negamycin restores dystrophin expression in skeletal and cardiac muscles of mdx mice. *J.Biochem.*, Vol.134,No.5, (November 2003), pp. 751-758, ISSN

Arechavala-Gomeza, V., Graham, I. R., Popplewell, L. J., Adams, A. M., Aartsma-Rus, A., Kinali, M., Morgan, J. E., Van Deutekom, J. C., Wilton, S. D., Dickson, G., & Muntoni, F. (September 2007). Comparative analysis of antisense oligonucleotide sequences for targeted skipping of exon 51 during dystrophin pre-mRNA splicing

in human muscle. *Hum.Gene Ther.*, Vol.18,No.9, (September 2007), pp. 798-810, ISSN

Asakura, A. and Rudnicki, M. A. (November 2002). Side population cells from diverse adult tissues are capable of in vitro hematopoietic differentiation. *Exp.Hematol.*, Vol.30,No.11, (November 2002), pp. 1339-1345, ISSN

Auld, D. S., Lovell, S., Thorne, N., Lea, W. A., Maloney, D. J., Shen, M., Rai, G., Battaile, K. P., Thomas, C. J., Simeonov, A., Hanzlik, R. P., & Inglese, J. (March 2010). Molecular basis for the high-affinity binding and stabilization of firefly luciferase by PTC124. *Proc.Natl.Acad.Sci.U.S.A*, Vol.107,No.11, (March 2010), pp. 4878-4883, ISSN

Auld, D. S., Thorne, N., Maguire, W. F., & Inglese, J. (March 2009). Mechanism of PTC124 activity in cell-based luciferase assays of nonsense codon suppression. *Proc.Natl.Acad.Sci.U.S.A*, Vol.106,No.9, (March 2009), pp. 3585-3590, ISSN

Aurino, S. and Nigro, V. (June 2006). Readthrough strategies for stop codons in Duchenne muscular dystrophy. *Acta Myol.*, Vol.25,No.1, (June 2006), pp. 5-12, ISSN

Barton-Davis, E. R., Cordier, L., Shoturma, D. I., Leland, S. E., & Sweeney, H. L. (August 1999). Aminoglycoside antibiotics restore dystrophin function to skeletal muscles of mdx mice. *J.Clin.Invest*, Vol.104,No.4, (August 1999), pp. 375-381, ISSN

Benchaouir, R., Meregalli, M., Farini, A., D'Antona, G., Belicchi, M., Goyenvalle, A., Battistelli, M., Bresolin, N., Bottinelli, R., Garcia, L., & Torrente, Y. (December 2007). Restoration of human dystrophin following transplantation of exon-skipping-engineered DMD patient stem cells into dystrophic mice. *Cell Stem Cell*, Vol.1,No.6, (December 2007), pp. 646-657, ISSN

Bertoni, C., Morris, G. E., & Rando, T. A. (January 2005). Strand bias in oligonucleotide-mediated dystrophin gene editing. *Hum.Mol.Genet.*, Vol.14,No.2, (January 2005), pp. 221-233, ISSN

Bertoni, C. and Rando, T. A. (April 2002). Dystrophin gene repair in mdx muscle precursor cells in vitro and in vivo mediated by RNA-DNA chimeric oligonucleotides. *Hum.Gene Ther.*, Vol.13,No.6, (April 2002), pp. 707-718, ISSN

Biggar, W. D., Gingras, M., Fehlings, D. L., Harris, V. A., & Steele, C. A. (January 2001). Deflazacort treatment of Duchenne muscular dystrophy. *J.Pediatr.*, Vol.138,No.1, (January 2001), pp. 45-50, ISSN

Bish, L. T., Sleeper, M. M., Forbes, S. C., Morine, K., Reynolds, C., Singletary, G. E., Trafny, D., Pham, J., Bogan, J., Kornegay, J. N., Vandenborne, K., Walter, G. A., & Sweeney, H. L. (July 2011). Long-term systemic myostatin inhibition via liver-targeted gene transfer in Golden Retriever Muscular Dystrophy. *Hum.Gene Ther.*, Vol.July 2011), pp.

Blake, D. J., Weir, A., Newey, S. E., & Davies, K. E. (April 2002). Function and genetics of dystrophin and dystrophin-related proteins in muscle. *Physiol Rev.*, Vol.82,No.2, (April 2002), pp. 291-329, ISSN

Brenman, J. E., Chao, D. S., Xia, H., Aldape, K., & Bredt, D. S. (September 1995). Nitric oxide synthase complexed with dystrophin and absent from skeletal muscle sarcolemma in Duchenne muscular dystrophy. *Cell*, Vol.82,No.5, (September 1995), pp. 743-752, ISSN

Brussee, V., Tardif, F., Roy, B., Goulet, M., Sebille, A., & Tremblay, J. P. (June 1999). Successful myoblast transplantation in fibrotic muscles: no increased impairment by the connective tissue. *Transplantation*, Vol.67,No.12, (June 1999), pp. 1618-1622, ISSN

Burghes, A. H., Logan, C., Hu, X., Belfall, B., Worton, R. G., & Ray, P. N. (July 1987). A cDNA clone from the Duchenne/Becker muscular dystrophy gene. *Nature*, Vol.328,No.6129, (July 1987), pp. 434-437, ISSN

Bushby, K., Finkel, R., Birnkrant, D. J., Case, L. E., Clemens, P. R., Cripe, L., Kaul, A., Kinnett, K., McDonald, C., Pandya, S., Poysky, J., Shapiro, F., Tomezsko, J., & Constantin, C. (January 2010a). Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and pharmacological and psychosocial management. *Lancet Neurol.*, Vol.9,No.1, (January 2010a), pp. 77-93, ISSN

Bushby, K., Finkel, R., Birnkrant, D. J., Case, L. E., Clemens, P. R., Cripe, L., Kaul, A., Kinnett, K., McDonald, C., Pandya, S., Poysky, J., Shapiro, F., Tomezsko, J., & Constantin, C. (February 2010b). Diagnosis and management of Duchenne muscular dystrophy, part 2: implementation of multidisciplinary care. *Lancet Neurol.*, Vol.9,No.2, (February 2010b), pp. 177-189, ISSN

Chamberlain, J. S., Gibbs, R. A., Ranier, J. E., Nguyen, P. N., & Caskey, C. T. (December 1988). Deletion screening of the Duchenne muscular dystrophy locus via multiplex DNA amplification. *Nucleic Acids Res.*, Vol.16,No.23, (December 1988), pp. 11141-11156, ISSN

Chapdelaine, P., Pichavant, C., Rousseau, J., Paques, F., & Tremblay, J. P. (July 2010). Meganucleases can restore the reading frame of a mutated dystrophin. *Gene Ther.*, Vol.17,No.7, (July 2010), pp. 846-858, ISSN

Cirak, S., Arechavala-Gomeza, V., Guglieri, M., Feng, L., Torelli, S., Anthony, K., Abbs, S., Garralda, M. E., Bourke, J., Wells, D. J., Dickson, G., Wood, M. J., Wilton, S. D., Straub, V., Kole, R., Shrewsbury, S. B., Sewry, C., Morgan, J. E., Bushby, K., & Muntoni, F. (August 2011). Exon skipping and dystrophin restoration in patients with Duchenne muscular dystrophy after systemic phosphorodiamidate morpholino oligomer treatment: an open-label, phase 2, dose-escalation study. *Lancet*, Vol.378,No.9791, (August 2011), pp. 595-605, ISSN

Crisan, M., Yap, S., Casteilla, L., Chen, C. W., Corselli, M., Park, T. S., Andriolo, G., Sun, B., Zheng, B., Zhang, L., Norotte, C., Teng, P. N., Traas, J., Schugar, R., Deasy, B. M., Badylak, S., Buhring, H. J., Giacobino, J. P., Lazzari, L., Huard, J., & Peault, B. (September 2008). A perivascular origin for mesenchymal stem cells in multiple human organs. *Cell Stem Cell*, Vol.3,No.3, (September 2008), pp. 301-313, ISSN

Danko, I., Chapman, V., & Wolff, J. A. (July 1992). The frequency of revertants in mdx mouse genetic models for Duchenne muscular dystrophy. *Pediatr.Res.*, Vol.32,No.1, (July 1992), pp. 128-131, ISSN

Daston, G., Lamar, E., Olivier, M., & Goulding, M. (March 1996). Pax-3 is necessary for migration but not differentiation of limb muscle precursors in the mouse. *Development*, Vol.122,No.3, (March 1996), pp. 1017-1027, ISSN

Dellavalle, A., Sampaolesi, M., Tonlorenzi, R., Tagliafico, E., Sacchetti, B., Perani, L., Innocenzi, A., Galvez, B. G., Messina, G., Morosetti, R., Li, S., Belicchi, M., Peretti,

G., Chamberlain, J. S., Wright, W. E., Torrente, Y., Ferrari, S., Bianco, P., & Cossu, G. (March 2007). Pericytes of human skeletal muscle are myogenic precursors distinct from satellite cells. *Nat.Cell Biol.*, Vol.9,No.3, (March 2007), pp. 255-267, ISSN

Denti, M. A., Incitti, T., Sthandier, O., Nicoletti, C., De Angelis, F. G., Rizzuto, E., Auricchio, A., Musaro, A., & Bozzoni, I. (June 2008). Long-term benefit of adeno-associated virus/antisense-mediated exon skipping in dystrophic mice. *Hum.Gene Ther.*, Vol.19,No.6, (June 2008), pp. 601-608, ISSN

Denti, M. A., Rosa, A., D'Antona, G., Sthandier, O., De Angelis, F. G., Nicoletti, C., Allocca, M., Pansarasa, O., Parente, V., Musaro, A., Auricchio, A., Bottinelli, R., & Bozzoni, I. (March 2006a). Body-wide gene therapy of Duchenne muscular dystrophy in the mdx mouse model. *Proc.Natl.Acad.Sci.U.S.A*, Vol.103,No.10, (March 2006a), pp. 3758-3763, ISSN

Denti, M. A., Rosa, A., D'Antona, G., Sthandier, O., De Angelis, F. G., Nicoletti, C., Allocca, M., Pansarasa, O., Parente, V., Musaro, A., Auricchio, A., Bottinelli, R., & Bozzoni, I. (May 2006b). Chimeric adeno-associated virus/antisense U1 small nuclear RNA effectively rescues dystrophin synthesis and muscle function by local treatment of mdx mice. *Hum.Gene Ther.*, Vol.17,No.5, (May 2006b), pp. 565-574, ISSN

Dezawa, M., Ishikawa, H., Itokazu, Y., Yoshihara, T., Hoshino, M., Takeda, S., Ide, C., & Nabeshima, Y. (July 2005). Bone marrow stromal cells generate muscle cells and repair muscle degeneration. *Science*, Vol.309,No.5732, (July 2005), pp. 314-317, ISSN

Doherty, M. J., Ashton, B. A., Walsh, S., Beresford, J. N., Grant, M. E., & Canfield, A. E. (May 1998). Vascular pericytes express osteogenic potential in vitro and in vivo. *J.Bone Miner.Res.*, Vol.13,No.5, (May 1998), pp. 828-838, ISSN

Dominski, Z. and Kole, R. (September 1993). Restoration of correct splicing in thalassemic pre-mRNA by antisense oligonucleotides. *Proc.Natl.Acad.Sci.U.S.A*, Vol.90,No.18, (September 1993), pp. 8673-8677, ISSN

Dorchies, O. M., Wagner, S., Buetler, T. M., & Ruegg, U. T. (May 2009). Protection of dystrophic muscle cells with polyphenols from green tea correlates with improved glutathione balance and increased expression of 67LR, a receptor for (-)-epigallocatechin gallate. *Biofactors*, Vol.35,No.3, (May 2009), pp. 279-294, ISSN

Du, M., Liu, X., Welch, E. M., Hirawat, S., Peltz, S. W., & Bedwell, D. M. (February 2008). PTC124 is an orally bioavailable compound that promotes suppression of the human CFTR-G542X nonsense allele in a CF mouse model. *Proc.Natl.Acad.Sci.U.S.A*, Vol.105,No.6, (February 2008), pp. 2064-2069, ISSN

Dumonceaux, J., Marie, S., Beley, C., Trollet, C., Vignaud, A., Ferry, A., Butler-Browne, G., & Garcia, L. (May 2010). Combination of myostatin pathway interference and dystrophin rescue enhances tetanic and specific force in dystrophic mdx mice. *Mol.Ther.*, Vol.18,No.5, (May 2010), pp. 881-887, ISSN

Dunant, P., Walter, M. C., Karpati, G., & Lochmuller, H. (May 2003). Gentamicin fails to increase dystrophin expression in dystrophin-deficient muscle. *Muscle Nerve*, Vol.27,No.5, (May 2003), pp. 624-627, ISSN

Eagle, M., Baudouin, S. V., Chandler, C., Giddings, D. R., Bullock, R., & Bushby, K. (December 2002). Survival in Duchenne muscular dystrophy: improvements in life

expectancy since 1967 and the impact of home nocturnal ventilation. *Neuromuscul.Disord.*, Vol.12,No.10, (December 2002), pp. 926-929, ISSN

Emery, A. E. (July 1993). Duchenne muscular dystrophy--Meryon's disease. *Neuromuscul.Disord.*, Vol.3,No.4, (July 1993), pp. 263-266, ISSN

Emery, A. E. (February 2002). The muscular dystrophies. *Lancet*, Vol.359,No.9307, (February 2002), pp. 687-695, ISSN

England, S. B., Nicholson, L. V., Johnson, M. A., Forrest, S. M., Love, D. R., Zubrzycka-Gaarn, E. E., Bulman, D. E., Harris, J. B., & Davies, K. E. (January 1990). Very mild muscular dystrophy associated with the deletion of 46% of dystrophin. *Nature*, Vol.343,No.6254, (January 1990), pp. 180-182, ISSN

Errington, S. J., Mann, C. J., Fletcher, S., & Wilton, S. D. (June 2003). Target selection for antisense oligonucleotide induced exon skipping in the dystrophin gene. *J.Gene Med.*, Vol.5,No.6, (June 2003), pp. 518-527, ISSN

Ervasti, J. M., Ohlendieck, K., Kahl, S. D., Gaver, M. G., & Campbell, K. P. (May 1990). Deficiency of a glycoprotein component of the dystrophin complex in dystrophic muscle. *Nature*, Vol.345,No.6273, (May 1990), pp. 315-319, ISSN

Finkel, R. S. (September 2010). Read-through strategies for suppression of nonsense mutations in Duchenne/ Becker muscular dystrophy: aminoglycosides and ataluren (PTC124). *J.Child Neurol.*, Vol.25,No.9, (September 2010), pp. 1158-1164, ISSN

Gavina, M., Belicchi, M., Rossi, B., Ottoboni, L., Colombo, F., Meregalli, M., Battistelli, M., Forzenigo, L., Biondetti, P., Pisati, F., Parolini, D., Farini, A., Issekutz, A. C., Bresolin, N., Rustichelli, F., Constantin, G., & Torrente, Y. (October 2006). VCAM-1 expression on dystrophic muscle vessels has a critical role in the recruitment of human blood-derived CD133+ stem cells after intra-arterial transplantation. *Blood*, Vol.108,No.8, (October 2006), pp. 2857-2866, ISSN

Gebski, B. L., Mann, C. J., Fletcher, S., & Wilton, S. D. (August 2003). Morpholino antisense oligonucleotide induced dystrophin exon 23 skipping in mdx mouse muscle. *Hum.Mol.Genet.*, Vol.12,No.15, (August 2003), pp. 1801-1811, ISSN

Goemans, N. M., Tulinius, M., van den Akker, J. T., Burm, B. E., Ekhart, P. F., Heuvelmans, N., Holling, T., Janson, A. A., Platenburg, G. J., Sipkens, J. A., Sitsen, J. M., Aartsma-Rus, A., van Ommen, G. J., Buyse, G., Darin, N., Verschuuren, J. J., Campion, G. V., de Kimpe, S. J., & Van Deutekom, J. C. (April 2011). Systemic administration of PRO051 in Duchenne's muscular dystrophy. *N.Engl.J.Med.*, Vol.364,No.16, (April 2011), pp. 1513-1522, ISSN

Goyenvalle, A., Babbs, A., Powell, D., Kole, R., Fletcher, S., Wilton, S. D., & Davies, K. E. (January 2010). Prevention of dystrophic pathology in severely affected dystrophin/utrophin-deficient mice by morpholino-oligomer-mediated exon-skipping. *Mol.Ther.*, Vol.18,No.1, (January 2010), pp. 198-205, ISSN

Goyenvalle, A., Babbs, A., van Ommen, G. J., Garcia, L., & Davies, K. E. (July 2009). Enhanced exon-skipping induced by U7 snRNA carrying a splicing silencer sequence: Promising tool for DMD therapy. *Mol.Ther.*, Vol.17,No.7, (July 2009), pp. 1234-1240, ISSN

Goyenvalle, A., Vulin, A., Fougerousse, F., Leturcq, F., Kaplan, J. C., Garcia, L., & Danos, O. (December 2004). Rescue of dystrophic muscle through U7 snRNA-mediated exon skipping. *Science*, Vol.306,No.5702, (December 2004), pp. 1796-1799, ISSN

Gregorevic, P., Blankinship, M. J., Allen, J. M., Crawford, R. W., Meuse, L., Miller, D. G., Russell, D. W., & Chamberlain, J. S. (August 2004). Systemic delivery of genes to striated muscles using adeno-associated viral vectors. *Nat.Med.*, Vol.10,No.8, (August 2004), pp. 828-834, ISSN

Gussoni, E., Blau, H. M., & Kunkel, L. M. (September 1997). The fate of individual myoblasts after transplantation into muscles of DMD patients. *Nat.Med.*, Vol.3,No.9, (September 1997), pp. 970-977, ISSN

Heemskerk, H., de, W. C., van, K. P., Heuvelmans, N., Sabatelli, P., Rimessi, P., Braghetta, P., van Ommen, G. J., de, K. S., Ferlini, A., Aartsma-Rus, A., & Van Deutekom, J. C. (June 2010). Preclinical PK and PD studies on 2'-O-methyl-phosphorothioate RNA antisense oligonucleotides in the mdx mouse model. *Mol.Ther.*, Vol.18,No.6, (June 2010), pp. 1210-1217, ISSN

Heemskerk, H. A., de Winter, C. L., de Kimpe, S. J., van Kuik-Romeijn, P., Heuvelmans, N., Platenburg, G. J., van Ommen, G. J., Van Deutekom, J. C., & Aartsma-Rus, A. (March 2009). In vivo comparison of 2'-O-methyl phosphorothioate and morpholino antisense oligonucleotides for Duchenne muscular dystrophy exon skipping. *J.Gene Med.*, Vol.11,No.3, (March 2009), pp. 257-266, ISSN

Helderman-van den Enden AT, Straathof, C. S., Aartsma-Rus, A., den Dunnen, J. T., Verbist, B. M., Bakker, E., Verschuuren, J. J., & Ginjaar, H. B. (April 2010). Becker muscular dystrophy patients with deletions around exon 51; a promising outlook for exon skipping therapy in Duchenne patients. *Neuromuscul.Disord.*, Vol.20,No.4, (April 2010), pp. 251-254, ISSN

Hirawat, S., Welch, E. M., Elfring, G. L., Northcutt, V. J., Paushkin, S., Hwang, S., Leonard, E. M., Almstead, N. G., Ju, W., Peltz, S. W., & Miller, L. L. (April 2007). Safety, tolerability, and pharmacokinetics of PTC124, a nonaminoglycoside nonsense mutation suppressor, following single- and multiple-dose administration to healthy male and female adult volunteers. *J.Clin.Pharmacol.*, Vol.47,No.4, (April 2007), pp. 430-444, ISSN

Hoffman, E. P., Brown, R. H., Jr., & Kunkel, L. M. (December 1987). Dystrophin: the protein product of the Duchenne muscular dystrophy locus. *Cell*, Vol.51,No.6, (December 1987), pp. 919-928, ISSN

Hoffman, E. P., Fischbeck, K. H., Brown, R. H., Johnson, M., Medori, R., Loike, J. D., Harris, J. B., Waterston, R., Brooke, M., Specht, L., & . (May 1988). Characterization of dystrophin in muscle-biopsy specimens from patients with Duchenne's or Becker's muscular dystrophy. *N.Engl.J.Med.*, Vol.318,No.21, (May 1988), pp. 1363-1368, ISSN

Hohenester, E., Tisi, D., Talts, J. F., & Timpl, R. (November 1999). The crystal structure of a laminin G-like module reveals the molecular basis of alpha-dystroglycan binding to laminins, perlecan, and agrin. *Mol.Cell*, Vol.4,No.5, (November 1999), pp. 783-792, ISSN

Howard, M. T., Anderson, C. B., Fass, U., Khatri, S., Gesteland, R. F., Atkins, J. F., & Flanigan, K. M. (March 2004). Readthrough of dystrophin stop codon mutations

induced by aminoglycosides. *Ann.Neurol.*, Vol.55,No.3, (March 2004), pp. 422-426, ISSN

Hu, Y., Wu, B., Zillmer, A., Lu, P., Benrashid, E., Wang, M., Doran, T., Shaban, M., Wu, X., & Lu, Q. L. (April 2010). Guanine analogues enhance antisense oligonucleotide-induced exon skipping in dystrophin gene in vitro and in vivo. *Mol.Ther.*, Vol.18,No.4, (April 2010), pp. 812-818, ISSN

Ibraghimov-Beskrovnaya, O., Ervasti, J. M., Leveille, C. J., Slaughter, C. A., Sernett, S. W., & Campbell, K. P. (February 1992). Primary structure of dystrophin-associated glycoproteins linking dystrophin to the extracellular matrix. *Nature*, Vol.355,No.6362, (February 1992), pp. 696-702, ISSN

Janssen, B., Hartmann, C., Scholz, V., Jauch, A., & Zschocke, J. (February 2005). MLPA analysis for the detection of deletions, duplications and complex rearrangements in the dystrophin gene: potential and pitfalls. *Neurogenetics.*, Vol.6,No.1, (February 2005), pp. 29-35, ISSN

Jarrett, H. W. and Foster, J. L. (March 1995). Alternate binding of actin and calmodulin to multiple sites on dystrophin. *J.Biol.Chem.*, Vol.270,No.10, (March 1995), pp. 5578-5586, ISSN

Jearawiriyapaisarn, N., Moulton, H. M., Buckley, B., Roberts, J., Sazani, P., Fucharoen, S., Iversen, P. L., & Kole, R. (September 2008). Sustained dystrophin expression induced by peptide-conjugated morpholino oligomers in the muscles of mdx mice. *Mol.Ther.*, Vol.16,No.9, (September 2008), pp. 1624-1629, ISSN

Jearawiriyapaisarn, N., Moulton, H. M., Sazani, P., Kole, R., & Willis, M. S. (February 2010). Long-term improvement in mdx cardiomyopathy after therapy with peptide-conjugated morpholino oligomers. *Cardiovasc.Res.*, Vol.85,No.3, (February 2010), pp. 444-453, ISSN

Kaufman, R. J. (August 1999). Correction of genetic disease by making sense from nonsense. *J.Clin.Invest*, Vol.104,No.4, (August 1999), pp. 367-368, ISSN

Kimura, E., Li, S., Gregorevic, P., Fall, B. M., & Chamberlain, J. S. (January 2010). Dystrophin delivery to muscles of mdx mice using lentiviral vectors leads to myogenic progenitor targeting and stable gene expression. *Mol.Ther.*, Vol.18,No.1, (January 2010), pp. 206-213, ISSN

Kinali, M., Arechavala-Gomeza, V., Feng, L., Cirak, S., Hunt, D., Adkin, C., Guglieri, M., Ashton, E., Abbs, S., Nihoyannopoulos, P., Garralda, M. E., Rutherford, M., McCulley, C., Popplewell, L., Graham, I. R., Dickson, G., Wood, M. J., Wells, D. J., Wilton, S. D., Kole, R., Straub, V., Bushby, K., Sewry, C., Morgan, J. E., & Muntoni, F. (October 2009). Local restoration of dystrophin expression with the morpholino oligomer AVI-4658 in Duchenne muscular dystrophy: a single-blind, placebo-controlled, dose-escalation, proof-of-concept study. *Lancet Neurol.*, Vol.8,No.10, (October 2009), pp. 918-928, ISSN

King, W. M., Ruttencutter, R., Nagaraja, H. N., Matkovic, V., Landoll, J., Hoyle, C., Mendell, J. R., & Kissel, J. T. (May 2007). Orthopedic outcomes of long-term daily corticosteroid treatment in Duchenne muscular dystrophy. *Neurology*, Vol.68,No.19, (May 2007), pp. 1607-1613, ISSN

Kingston, H. M., Sarfarazi, M., Thomas, N. S., & Harper, P. S. (1984). Localisation of the Becker muscular dystrophy gene on the short arm of the X chromosome by linkage to cloned DNA sequences. *Hum.Genet.*, Vol.67,No.1, (1984), pp. 6-17, ISSN

Kobinger, G. P., Louboutin, J. P., Barton, E. R., Sweeney, H. L., & Wilson, J. M. (October 2003). Correction of the dystrophic phenotype by in vivo targeting of muscle progenitor cells. *Hum.Gene Ther.*, Vol.14,No.15, (October 2003), pp. 1441-1449, ISSN

Koenig, M., Beggs, A. H., Moyer, M., Scherpf, S., Heindrich, K., Bettecken, T., Meng, G., Muller, C. R., Lindlof, M., Kaariainen, H., & . (October 1989). The molecular basis for Duchenne versus Becker muscular dystrophy: correlation of severity with type of deletion. *Am.J.Hum.Genet.*, Vol.45,No.4, (October 1989), pp. 498-506, ISSN

Koenig, M., Hoffman, E. P., Bertelson, C. J., Monaco, A. P., Feener, C., & Kunkel, L. M. (July 1987). Complete cloning of the Duchenne muscular dystrophy (DMD) cDNA and preliminary genomic organization of the DMD gene in normal and affected individuals. *Cell*, Vol.50,No.3, (July 1987), pp. 509-517, ISSN

Koenig, M. and Kunkel, L. M. (March 1990). Detailed analysis of the repeat domain of dystrophin reveals four potential hinge segments that may confer flexibility. *J.Biol.Chem.*, Vol.265,No.8, (March 1990), pp. 4560-4566, ISSN

Koenig, M., Monaco, A. P., & Kunkel, L. M. (April 1988). The complete sequence of dystrophin predicts a rod-shaped cytoskeletal protein. *Cell*, Vol.53,No.2, (April 1988), pp. 219-228, ISSN

Kurreck, J. (April 2003). Antisense technologies. Improvement through novel chemical modifications. *Eur.J.Biochem.*, Vol.270,No.8, (April 2003), pp. 1628-1644, ISSN

Lai, Y., Thomas, G. D., Yue, Y., Yang, H. T., Li, D., Long, C., Judge, L., Bostick, B., Chamberlain, J. S., Terjung, R. L., & Duan, D. (March 2009). Dystrophins carrying spectrin-like repeats 16 and 17 anchor nNOS to the sarcolemma and enhance exercise performance in a mouse model of muscular dystrophy. *J.Clin.Invest*, Vol.119,No.3, (March 2009), pp. 624-635, ISSN

Lawler, J. M. (May 2011). Exacerbation of pathology by oxidative stress in respiratory and locomotor muscles with Duchenne muscular dystrophy. *J.Physiol*, Vol.589,No.Pt 9, (May 2011), pp. 2161-2170, ISSN

Li, S., Kimura, E., Fall, B. M., Reyes, M., Angello, J. C., Welikson, R., Hauschka, S. D., & Chamberlain, J. S. (July 2005). Stable transduction of myogenic cells with lentiviral vectors expressing a minidystrophin. *Gene Ther.*, Vol.12,No.14, (July 2005), pp. 1099-1108, ISSN

Linde, L., Boelz, S., Nissim-Rafinia, M., Oren, Y. S., Wilschanski, M., Yaacov, Y., Virgilis, D., Neu-Yilik, G., Kulozik, A. E., Kerem, E., & Kerem, B. (March 2007). Nonsense-mediated mRNA decay affects nonsense transcript levels and governs response of cystic fibrosis patients to gentamicin. *J.Clin.Invest*, Vol.117,No.3, (March 2007), pp. 683-692, ISSN

Linde, L. and Kerem, B. (November 2008). Introducing sense into nonsense in treatments of human genetic diseases. *Trends Genet.*, Vol.24,No.11, (November 2008), pp. 552-563, ISSN

Lu, Q. L., Mann, C. J., Lou, F., Bou-Gharios, G., Morris, G. E., Xue, S. A., Fletcher, S., Partridge, T. A., & Wilton, S. D. (August 2003). Functional amounts of dystrophin

produced by skipping the mutated exon in the mdx dystrophic mouse. *Nat.Med.*, Vol.9,No.8, (August 2003), pp. 1009-1014, ISSN

Lu, Q. L., Rabinowitz, A., Chen, Y. C., Yokota, T., Yin, H., Alter, J., Jadoon, A., Bou-Gharios, G., & Partridge, T. (January 2005). Systemic delivery of antisense oligoribonucleotide restores dystrophin expression in body-wide skeletal muscles. *Proc.Natl.Acad.Sci.U.S.A*, Vol.102,No.1, (January 2005), pp. 198-203, ISSN

Malerba, A., Boldrin, L., & Dickson, G. (August 2011a). Long-term systemic administration of unconjugated morpholino oligomers for therapeutic expression of dystrophin by exon skipping in skeletal muscle: implications for cardiac muscle integrity. *Nucleic Acid Ther.*, Vol.21,No.4, (August 2011a), pp. 293-298, ISSN

Malerba, A., Sharp, P. S., Graham, I. R., Arechavala-Gomeza, V., Foster, K., Muntoni, F., Wells, D. J., & Dickson, G. (February 2011b). Chronic systemic therapy with low-dose morpholino oligomers ameliorates the pathology and normalizes locomotor behavior in mdx mice. *Mol.Ther.*, Vol.19,No.2, (February 2011b), pp. 345-354, ISSN

Malerba, A., Thorogood, F. C., Dickson, G., & Graham, I. R. (September 2009). Dosing regimen has a significant impact on the efficiency of morpholino oligomer-induced exon skipping in mdx mice. *Hum.Gene Ther.*, Vol.20,No.9, (September 2009), pp. 955-965, ISSN

Malik, V., Rodino-Klapac, L. R., Viollet, L., & Mendell, J. R. (November 2010a). Aminoglycoside-induced mutation suppression (stop codon readthrough) as a therapeutic strategy for Duchenne muscular dystrophy. *Ther.Adv.Neurol.Disord.*, Vol.3,No.6, (November 2010a), pp. 379-389, ISSN

Malik, V., Rodino-Klapac, L. R., Viollet, L., Wall, C., King, W., Al-Dahhak, R., Lewis, S., Shilling, C. J., Kota, J., Serrano-Munuera, C., Hayes, J., Mahan, J. D., Campbell, K. J., Banwell, B., Dasouki, M., Watts, V., Sivakumar, K., Bien-Willner, R., Flanigan, K. M., Sahenk, Z., Barohn, R. J., Walker, C. M., & Mendell, J. R. (June 2010b). Gentamicin-induced readthrough of stop codons in Duchenne muscular dystrophy. *Ann.Neurol.*, Vol.67,No.6, (June 2010b), pp. 771-780, ISSN

Mann, C. J., Honeyman, K., McClorey, G., Fletcher, S., & Wilton, S. D. (November 2002). Improved antisense oligonucleotide induced exon skipping in the mdx mouse model of muscular dystrophy. *J.Gene Med.*, Vol.4,No.6, (November 2002), pp. 644-654, ISSN

Manuvakhova, M., Keeling, K., & Bedwell, D. M. (July 2000). Aminoglycoside antibiotics mediate context-dependent suppression of termination codons in a mammalian translation system. *RNA.*, Vol.6,No.7, (July 2000), pp. 1044-1055, ISSN

Maruggi, G., Porcellini, S., Facchini, G., Perna, S. K., Cattoglio, C., Sartori, D., Ambrosi, A., Schambach, A., Baum, C., Bonini, C., Bovolenta, C., Mavilio, F., & Recchia, A. (May 2009). Transcriptional enhancers induce insertional gene deregulation independently from the vector type and design. *Mol.Ther.*, Vol.17,No.5, (May 2009), pp. 851-856, ISSN

Matsumura, K., Burghes, A. H., Mora, M., Tome, F. M., Morandi, L., Cornello, F., Leturcq, F., Jeanpierre, M., Kaplan, J. C., Reinert, P., & . (January 1994). Immunohistochemical analysis of dystrophin-associated proteins in Becker/Duchenne muscular

dystrophy with huge in-frame deletions in the NH2-terminal and rod domains of dystrophin. *J.Clin.Invest*, Vol.93,No.1, (January 1994), pp. 99-105, ISSN

Matsumura, K. and Campbell, K. P. (January 1994). Dystrophin-glycoprotein complex: its role in the molecular pathogenesis of muscular dystrophies. *Muscle Nerve*, Vol.17,No.1, (January 1994), pp. 2-15, ISSN

Matsumura, K., Nonaka, I., Tome, F. M., Arahata, K., Collin, H., Leturcq, F., Recan, D., Kaplan, J. C., Fardeau, M., & Campbell, K. P. (August 1993). Mild deficiency of dystrophin-associated proteins in Becker muscular dystrophy patients having in-frame deletions in the rod domain of dystrophin. *Am.J.Hum.Genet.*, Vol.53,No.2, (August 1993), pp. 409-416, ISSN

Mendell, J. R., Campbell, K., Rodino-Klapac, L., Sahenk, Z., Shilling, C., Lewis, S., Bowles, D., Gray, S., Li, C., Galloway, G., Malik, V., Coley, B., Clark, K. R., Li, J., Xiao, X., Samulski, J., McPhee, S. W., Samulski, R. J., & Walker, C. M. (October 2010). Dystrophin immunity in Duchenne's muscular dystrophy. *N.Engl.J.Med.*, Vol.363,No.15, (October 2010), pp. 1429-1437, ISSN

Mendell, J. R., Moxley, R. T., Griggs, R. C., Brooke, M. H., Fenichel, G. M., Miller, J. P., King, W., Signore, L., Pandya, S., Florence, J., & . (June 1989). Randomized, double-blind six-month trial of prednisone in Duchenne's muscular dystrophy. *N.Engl.J.Med.*, Vol.320,No.24, (June 1989), pp. 1592-1597, ISSN

Meregalli, M., Farini, A., Parolini, D., Maciotta, S., & Torrente, Y. (August 2010). Stem cell therapies to treat muscular dystrophy: progress to date. *BioDrugs.*, Vol.24,No.4, (August 2010), pp. 237-247, ISSN

Miller, J. C., Tan, S., Qiao, G., Barlow, K. A., Wang, J., Xia, D. F., Meng, X., Paschon, D. E., Leung, E., Hinkley, S. J., Dulay, G. P., Hua, K. L., Ankoudinova, I., Cost, G. J., Urnov, F. D., Zhang, H. S., Holmes, M. C., Zhang, L., Gregory, P. D., & Rebar, E. J. (February 2011). A TALE nuclease architecture for efficient genome editing. *Nat.Biotechnol.*, Vol.29,No.2, (February 2011), pp. 143-148, ISSN

Mirabella, M., Galluzzi, G., Manfredi, G., Bertini, E., Ricci, E., De, L. R., Tonali, P., & Servidei, S. (August 1998). Giant dystrophin deletion associated with congenital cataract and mild muscular dystrophy. *Neurology*, Vol.51,No.2, (August 1998), pp. 592-595, ISSN

Monaco, A. P. (October 1989). Dystrophin, the protein product of the Duchenne/Becker muscular dystrophy gene. *Trends Biochem.Sci.*, Vol.14,No.10, (October 1989), pp. 412-415, ISSN

Monaco, A. P., Bertelson, C. J., Middlesworth, W., Colletti, C. A., Aldridge, J., Fischbeck, K. H., Bartlett, R., Pericak-Vance, M. A., Roses, A. D., & Kunkel, L. M. (August 1985). Detection of deletions spanning the Duchenne muscular dystrophy locus using a tightly linked DNA segment. *Nature*, Vol.316,No.6031, (August 1985), pp. 842-845, ISSN

Montarras, D., Morgan, J., Collins, C., Relaix, F., Zaffran, S., Cumano, A., Partridge, T., & Buckingham, M. (September 2005). Direct isolation of satellite cells for skeletal muscle regeneration. *Science*, Vol.309,No.5743, (September 2005), pp. 2064-2067, ISSN

Morgan, J. and Muntoni, F. (March 2007). Mural cells paint a new picture of muscle stem cells. *Nat.Cell Biol.*, Vol.9,No.3, (March 2007), pp. 249-251, ISSN

Moulton, H. M. and Moulton, J. D. (December 2010). Morpholinos and their peptide conjugates: therapeutic promise and challenge for Duchenne muscular dystrophy. *Biochim.Biophys.Acta*, Vol.1798,No.12, (December 2010), pp. 2296-2303, ISSN

Moxley, R. T., III and Pandya, S. (August 2011). Weekend high-dosage prednisone: A new option for treatment of Duchenne muscular dystrophy. *Neurology*, Vol.77,No.5, (August 2011), pp. 416-417, ISSN

Nagy, K. and Nagy, I. (November 1990). The effects of idebenone on the superoxide dismutase, catalase and glutathione peroxidase activities in liver and brain homogenates, as well as in brain synaptosomal and mitochondrial fractions. *Arch.Gerontol.Geriatr.*, Vol.11,No.3, (November 1990), pp. 285-291, ISSN

Ohlendieck, K. and Campbell, K. P. (December 1991). Dystrophin-associated proteins are greatly reduced in skeletal muscle from mdx mice. *J.Cell Biol.*, Vol.115,No.6, (December 1991), pp. 1685-1694, ISSN

Ohshima, S., Shin, J. H., Yuasa, K., Nishiyama, A., Kira, J., Okada, T., & Takeda, S. (January 2009). Transduction efficiency and immune response associated with the administration of AAV8 vector into dog skeletal muscle. *Mol.Ther.*, Vol.17,No.1, (January 2009), pp. 73-80, ISSN

Palmer, E., Wilhelm, J. M., & Sherman, F. (January 1979). Phenotypic suppression of nonsense mutants in yeast by aminoglycoside antibiotics. *Nature*, Vol.277,No.5692, (January 1979), pp. 148-150, ISSN

Palumbo, R., Sampaolesi, M., De, M. F., Tonlorenzi, R., Colombetti, S., Mondino, A., Cossu, G., & Bianchi, M. E. (February 2004). Extracellular HMGB1, a signal of tissue damage, induces mesoangioblast migration and proliferation. *J.Cell Biol.*, Vol.164,No.3, (February 2004), pp. 441-449, ISSN

Peault, B., Rudnicki, M., Torrente, Y., Cossu, G., Tremblay, J. P., Partridge, T., Gussoni, E., Kunkel, L. M., & Huard, J. (May 2007). Stem and progenitor cells in skeletal muscle development, maintenance, and therapy. *Mol.Ther.*, Vol.15,No.5, (May 2007), pp. 867-877, ISSN

Politano, L., Nigro, G., Nigro, V., Piluso, G., Papparella, S., Paciello, O., & Comi, L. I. (May 2003). Gentamicin administration in Duchenne patients with premature stop codon. Preliminary results. *Acta Myol.*, Vol.22,No.1, (May 2003), pp. 15-21, ISSN

Prins, K. W., Humston, J. L., Mehta, A., Tate, V., Ralston, E., & Ervasti, J. M. (August 2009). Dystrophin is a microtubule-associated protein. *J.Cell Biol.*, Vol.186,No.3, (August 2009), pp. 363-369, ISSN

Qu, Z., Balkir, L., Van Deutekom, J. C., Robbins, P. D., Pruchnic, R., & Huard, J. (September 1998). Development of approaches to improve cell survival in myoblast transfer therapy. *J.Cell Biol.*, Vol.142,No.5, (September 1998), pp. 1257-1267, ISSN

Qu-Petersen, Z., Deasy, B., Jankowski, R., Ikezawa, M., Cummins, J., Pruchnic, R., Mytinger, J., Cao, B., Gates, C., Wernig, A., & Huard, J. (May 2002). Identification of a novel population of muscle stem cells in mice: potential for muscle regeneration. *J.Cell Biol.*, Vol.157,No.5, (May 2002), pp. 851-864, ISSN

Rando, T. A., Disatnik, M. H., & Zhou, L. Z. (May 2000). Rescue of dystrophin expression in mdx mouse muscle by RNA/DNA oligonucleotides. *Proc.Natl.Acad.Sci.U.S.A*, Vol.97,No.10, (May 2000), pp. 5363-5368, ISSN

Rentschler, S., Linn, H., Deininger, K., Bedford, M. T., Espanel, X., & Sudol, M. (April 1999). The WW domain of dystrophin requires EF-hands region to interact with beta-dystroglycan. *Biol.Chem.*, Vol.380,No.4, (April 1999), pp. 431-442, ISSN

Rodino-Klapac, L. R., Montgomery, C. L., Bremer, W. G., Shontz, K. M., Malik, V., Davis, N., Sprinkle, S., Campbell, K. J., Sahenk, Z., Clark, K. R., Walker, C. M., Mendell, J. R., & Chicoine, L. G. (January 2010). Persistent expression of FLAG-tagged micro dystrophin in nonhuman primates following intramuscular and vascular delivery. *Mol.Ther.*, Vol.18,No.1, (January 2010), pp. 109-117, ISSN

Rybakova, I. N., Amann, K. J., & Ervasti, J. M. (November 1996). A new model for the interaction of dystrophin with F-actin. *J.Cell Biol.*, Vol.135,No.3, (November 1996), pp. 661-672, ISSN

Sampaolesi, M., Blot, S., D'Antona, G., Granger, N., Tonlorenzi, R., Innocenzi, A., Mognol, P., Thibaud, J. L., Galvez, B. G., Barthelemy, I., Perani, L., Mantero, S., Guttinger, M., Pansarasa, O., Rinaldi, C., Cusella De Angelis, M. G., Torrente, Y., Bordignon, C., Bottinelli, R., & Cossu, G. (November 2006). Mesoangioblast stem cells ameliorate muscle function in dystrophic dogs. *Nature*, Vol.444,No.7119, (November 2006), pp. 574-579, ISSN

Schwartz, M. and Duno, M. (2004). Improved molecular diagnosis of dystrophin gene mutations using the multiplex ligation-dependent probe amplification method. *Genet.Test.*, Vol.8,No.4, (2004), pp. 361-367, ISSN

Seale, P., Ishibashi, J., Scime, A., & Rudnicki, M. A. (May 2004). Pax7 is necessary and sufficient for the myogenic specification of CD45+:Sca1+ stem cells from injured muscle. *PLoS.Biol.*, Vol.2,No.5, (May 2004), pp. E130-

Singh, A., Ursic, D., & Davies, J. (January 1979). Phenotypic suppression and misreading Saccharomyces cerevisiae. *Nature*, Vol.277,No.5692, (January 1979), pp. 146-148, ISSN

Skuk, D., Goulet, M., Roy, B., Chapdelaine, P., Bouchard, J. P., Roy, R., Dugre, F. J., Sylvain, M., Lachance, J. G., Deschenes, L., Senay, H., & Tremblay, J. P. (April 2006). Dystrophin expression in muscles of duchenne muscular dystrophy patients after high-density injections of normal myogenic cells. *J.Neuropathol.Exp.Neurol.*, Vol.65,No.4, (April 2006), pp. 371-386, ISSN

Spitali, P., Rimessi, P., Fabris, M., Perrone, D., Falzarano, S., Bovolenta, M., Trabanelli, C., Mari, L., Bassi, E., Tuffery, S., Gualandi, F., Maraldi, N. M., Sabatelli-Giraud, P., Medici, A., Merlini, L., & Ferlini, A. (November 2009). Exon skipping-mediated dystrophin reading frame restoration for small mutations. *Hum.Mutat.*, Vol.30,No.11, (November 2009), pp. 1527-1534, ISSN

Sproat, B. S., Lamond, A. I., Beijer, B., Neuner, P., & Ryder, U. (May 1989). Highly efficient chemical synthesis of 2'-O-methyloligoribonucleotides and tetrabiotinylated derivatives; novel probes that are resistant to degradation by RNA or DNA specific nucleases. *Nucleic Acids Res.*, Vol.17,No.9, (May 1989), pp. 3373-3386, ISSN

Summerton, J. (December 1999). Morpholino antisense oligomers: the case for an RNase H-independent structural type. *Biochim.Biophys.Acta*, Vol.1489,No.1, (December 1999), pp. 141-158, ISSN

Suzuki, A., Yoshida, M., Hayashi, K., Mizuno, Y., Hagiwara, Y., & Ozawa, E. (March 1994). Molecular organization at the glycoprotein-complex-binding site of dystrophin. Three dystrophin-associated proteins bind directly to the carboxy-terminal portion of dystrophin. *Eur.J.Biochem.*, Vol.220,No.2, (March 1994), pp. 283-292, ISSN

Tremblay, J. P., Malouin, F., Roy, R., Huard, J., Bouchard, J. P., Satoh, A., & Richards, C. L. (March 1993). Results of a triple blind clinical study of myoblast transplantations without immunosuppressive treatment in young boys with Duchenne muscular dystrophy. *Cell Transplant.*, Vol.2,No.2, (March 1993), pp. 99-112, ISSN

Van Deutekom, J. C., Bremmer-Bout, M., Janson, A. A., Ginjaar, I. B., Baas, F., den Dunnen, J. T., & van Ommen, G. J. (July 2001). Antisense-induced exon skipping restores dystrophin expression in DMD patient derived muscle cells. *Hum.Mol.Genet.*, Vol.10,No.15, (July 2001), pp. 1547-1554, ISSN

Van Deutekom, J. C., Janson, A. A., Ginjaar, I. B., Frankhuizen, W. S., Aartsma-Rus, A., Bremmer-Bout, M., den Dunnen, J. T., Koop, K., van der Kooi, A. J., Goemans, N. M., de Kimpe, S. J., Ekhart, P. F., Venneker, E. H., Platenburg, G. J., Verschuuren, J. J., & van Ommen, G. J. (December 2007). Local dystrophin restoration with antisense oligonucleotide PRO051. *N.Engl.J.Med.*, Vol.357,No.26, (December 2007), pp. 2677-2686, ISSN

Wagner, K. R., Hamed, S., Hadley, D. W., Gropman, A. L., Burstein, A. H., Escolar, D. M., Hoffman, E. P., & Fischbeck, K. H. (June 2001). Gentamicin treatment of Duchenne and Becker muscular dystrophy due to nonsense mutations. *Ann.Neurol.*, Vol.49,No.6, (June 2001), pp. 706-711, ISSN

Wang, B., Li, J., Qiao, C., Chen, C., Hu, P., Zhu, X., Zhou, L., Bogan, J., Kornegay, J., & Xiao, X. (August 2008). A canine minidystrophin is functional and therapeutic in mdx mice. *Gene Ther.*, Vol.15,No.15, (August 2008), pp. 1099-1106, ISSN

Wang, B., Li, J., & Xiao, X. (December 2000). Adeno-associated virus vector carrying human minidystrophin genes effectively ameliorates muscular dystrophy in mdx mouse model. *Proc.Natl.Acad.Sci.U.S.A*, Vol.97,No.25, (December 2000), pp. 13714-13719, ISSN

Wang, Z., Kuhr, C. S., Allen, J. M., Blankinship, M., Gregorevic, P., Chamberlain, J. S., Tapscott, S. J., & Storb, R. (June 2007). Sustained AAV-mediated dystrophin expression in a canine model of Duchenne muscular dystrophy with a brief course of immunosuppression. *Mol.Ther.*, Vol.15,No.6, (June 2007), pp. 1160-1166, ISSN

Welch, E. M., Barton, E. R., Zhuo, J., Tomizawa, Y., Friesen, W. J., Trifillis, P., Paushkin, S., Patel, M., Trotta, C. R., Hwang, S., Wilde, R. G., Karp, G., Takasugi, J., Chen, G., Jones, S., Ren, H., Moon, Y. C., Corson, D., Turpoff, A. A., Campbell, J. A., Conn, M. M., Khan, A., Almstead, N. G., Hedrick, J., Mollin, A., Risher, N., Weetall, M., Yeh, S., Branstrom, A. A., Colacino, J. M., Babiak, J., Ju, W. D., Hirawat, S., Northcutt, V. J., Miller, L. L., Spatrick, P., He, F., Kawana, M., Feng, H., Jacobson, A., Peltz, S. W., & Sweeney, H. L. (May 2007). PTC124 targets genetic disorders caused by nonsense mutations. *Nature*, Vol.447,No.7140, (May 2007), pp. 87-91, ISSN

Wu, B., Benrashid, E., Lu, P., Cloer, C., Zillmer, A., Shaban, M., & Lu, Q. L. (2011a). Targeted skipping of human dystrophin exons in transgenic mouse model systemically for antisense drug development. *PLoS.One.*, Vol.6,No.5, (2011a), pp. e19906-

Wu, B., Li, Y., Morcos, P. A., Doran, T. J., Lu, P., & Lu, Q. L. (May 2009). Octa-guanidine morpholino restores dystrophin expression in cardiac and skeletal muscles and ameliorates pathology in dystrophic mdx mice. *Mol.Ther.*, Vol.17,No.5, (May 2009), pp. 864-871, ISSN

Wu, B., Lu, P., Benrashid, E., Malik, S., Ashar, J., Doran, T. J., & Lu, Q. L. (January 2010). Dose-dependent restoration of dystrophin expression in cardiac muscle of dystrophic mice by systemically delivered morpholino. *Gene Ther.*, Vol.17,No.1, (January 2010), pp. 132-140, ISSN

Wu, B., Moulton, H. M., Iversen, P. L., Jiang, J., Li, J., Li, J., Spurney, C. F., Sali, A., Guerron, A. D., Nagaraju, K., Doran, T., Lu, P., Xiao, X., & Lu, Q. L. (September 2008). Effective rescue of dystrophin improves cardiac function in dystrophin-deficient mice by a modified morpholino oligomer. *Proc.Natl.Acad.Sci.U.S.A*, Vol.105,No.39, (September 2008), pp. 14814-14819, ISSN

Wu, B., Xiao, B., Cloer, C., Shaban, M., Sali, A., Lu, P., Li, J., Nagaraju, K., Xiao, X., & Lu, Q. L. (March 2011b). One-year treatment of morpholino antisense oligomer improves skeletal and cardiac muscle functions in dystrophic mdx mice. *Mol.Ther.*, Vol.19,No.3, (March 2011b), pp. 576-583, ISSN

Yin, H., Moulton, H. M., Betts, C., Seow, Y., Boutilier, J., Iverson, P. L., & Wood, M. J. (November 2009). A fusion peptide directs enhanced systemic dystrophin exon skipping and functional restoration in dystrophin-deficient mdx mice. *Hum.Mol.Genet.*, Vol.18,No.22, (November 2009), pp. 4405-4414, ISSN

Yin, H., Moulton, H. M., Seow, Y., Boyd, C., Boutilier, J., Iverson, P., & Wood, M. J. (December 2008). Cell-penetrating peptide-conjugated antisense oligonucleotides restore systemic muscle and cardiac dystrophin expression and function. *Hum.Mol.Genet.*, Vol.17,No.24, (December 2008), pp. 3909-3918, ISSN

Yin, H., Saleh, A. F., Betts, C., Camelliti, P., Seow, Y., Ashraf, S., Arzumanov, A., Hammond, S., Merritt, T., Gait, M. J., & Wood, M. J. (July 2011). Pip5 transduction peptides direct high efficiency oligonucleotide-mediated dystrophin exon skipping in heart and phenotypic correction in mdx mice. *Mol.Ther.*, Vol.19,No.7, (July 2011), pp. 1295-1303, ISSN

Yokota, T., Lu, Q. L., Partridge, T., Kobayashi, M., Nakamura, A., Takeda, S., & Hoffman, E. (June 2009). Efficacy of systemic morpholino exon-skipping in Duchenne dystrophy dogs. *Ann.Neurol.*, Vol.65,No.6, (June 2009), pp. 667-676, ISSN

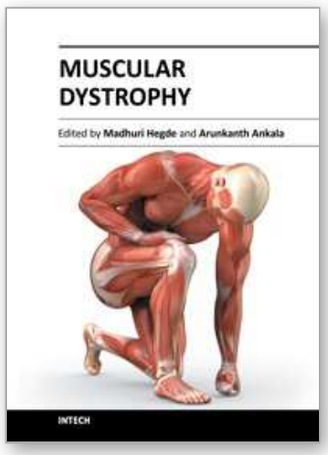
Yoshida, M., Suzuki, A., Yamamoto, H., Noguchi, S., Mizuno, Y., & Ozawa, E. (June 1994). Dissociation of the complex of dystrophin and its associated proteins into several unique groups by n-octyl beta-D-glucoside. *Eur.J.Biochem.*, Vol.222,No.3, (June 1994), pp. 1055-1061, ISSN

Yoshida, M., Yamamoto, H., Noguchi, S., Mizuno, Y., Hagiwara, Y., & Ozawa, E. (July 1995). Dystrophin-associated protein A0 is a homologue of the Torpedo 87K protein. *FEBS Lett.*, Vol.367,No.3, (July 1995), pp. 311-314, ISSN

Yoshizawa, S., Fourmy, D., & Puglisi, J. D. (November 1998). Structural origins of gentamicin antibiotic action. *EMBO J.*, Vol.17,No.22, (November 1998), pp. 6437-6448, ISSN

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With more than 30 different types and subtypes known and many more yet to be classified and characterized, muscular dystrophy is a highly heterogeneous group of inherited neuromuscular disorders. This book provides a comprehensive overview of the various types of muscular dystrophies, genes associated with each subtype, disease diagnosis, management as well as available treatment options. Though each different type and subtype of muscular dystrophy is associated with a different causative gene, the majority of them have overlapping clinical presentations, making molecular diagnosis inevitable for both disease diagnosis as well as patient management. This book discusses the currently available diagnostic approaches that have revolutionized clinical research. Pathophysiology of the different muscular dystrophies, multifaceted functions of the involved genes as well as efforts towards diagnosis and effective patient management, are also discussed. Adding value to the book are the included reports on ongoing studies that show a promise for future therapeutic strategies.

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