patients with late onset Pompe disease were diagnosed in Denmark (population 5.5 million). We conducted a screening program in the largest neuromuscular clinic, and respiratory center in Denmark, as we hypothesised that the condition may be overlooked. The inclusion criteria were age (over 15 years), unexplained hyper-CR-aemia and myopathy, unclassified Limb-girdle muscular dystrophy (LGMD) and unexplained, restrictive respiratory insufficiency. We went through medical journals and 123 patients (67 from the neuromuscular clinic and 56 from the respiratory center) met the inclusion criteria. Forty three neuromuscular and 17 respiratory patients accepted screening, which was conducted using Dried blood spots (DBS). We found three patients, with low activity of GAA, and molecular genetic analysis confirmed pathogenic mutations in all patients. All three patients were from the neuromuscular clinic, and were diagnosed as cases of unclassified LGMD. Among 28 patients with a LGMD phenotype, three had Pompe disease. The results suggest that adult Pompe disease in Denmark is underdiagnosed. DBS is a cheap and effective method to screen for Pompe disease. As treatment is available for Pompe disease, we suggest that a DBS should be taken early in the course of evaluation of a patient with a LGMD phenotype.

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P3.61
A 40 year old female with glycogen storage disease type II according to muscle pathology and clinical features, but normal genetic study and GAA enzyme activity

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Glycogen storage disease type II that also called Pompe disease or acid maltase deficiency is an autosomal recessive inherited disorder due to the deficiency of acid alpha glucosidase. An accumulation of glycogen in the lysosome cause damage of muscle and nerve cells throughout the body. GSD type II has been divided into two groups; infantile onset and late onset. A former type has severe features that including lack of muscle tone, weakness, hepatomegaly and cardiomegaly. Most patient with infantile onset GSD type II die from respiratory or cardiac complications before 2 years of age. Meanwhile late onset GSD type II has more mild phenotype mainly respiratory distress and there can be cardiomyopathy but uncommon. A large number of mutations in the acid alpha glucosidase gene have been described. Also deceased GAA activity in peripheral blood, muscle tissue, skin fibroblast that is helps to confirm GSD type II. We investigated a 40 year old female who had progressive respiratory distress from early 30 and progressive proximal weakness from mid-30s. Her parents and sibling was healthy. Her only son who was diagnosed Pompe disease with muscle pathology was died at 14 months, at that time, GAA activity was 39.7 nmole/h/mg (reference range: 22.9–51.5nmole/h/mg). Also we performed GAA enzyme activity assay from peripheral blood, the result was 39.7 nmole/h/mg (reference range: 22.9–51.5nmole/h/mg). Also we performed sequencing at coding region of GAA gene, there was only 15 kinds of types already known polymorphism. We report a complex case of suspected GSD type II patient but cannot confirmed with enzyme assay and gene study.

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ADVANCES IN THERAPY OF NEUROMUSCULAR DISORDERS 1

T.I.1
Potential for gene therapy in DMD

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Molecular therapies including exon skipping, mutation suppression, and gene therapy are under intense investigation. No single approach is applicable for all muscle diseases. Our focus has been on gene replacement to restore function by replacing or repairing defective genes. In the pre-clinical setting gene transfer appears to have few barriers to success. Translating this to the bedside, however, reveals challenges never fully appreciated in animals. An obvious constraint for adeno-associated virus (AAV)-mediated gene therapy is its limited packaging capacity (~4.8 kb). For clinical translational myologists targeting DMD, packaging restrictions necessitate reducing the very large DMD gene (11 kb) to fit AAV. With some large genes like dysferlin (6.7 kb), we found that homologous recombination permits full length protein expression. For the DMD gene, co-delivery of AAV vectors sharing a central region of a central homologous recombinogenic region enables reconstitution of larger expression cassettes (Chamberlain Laboratory). A potential limiting factor we identified in a clinical gene transfer trial was an immune response provoked by

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mini-dystrophin gene expression in the domain of a deleted endogenous DMD gene. An unanticipated T cell response was encountered by a novel immunogenic epitope expressed on some revertant muscle fibers. We are currently engaged in studies to determine the frequency of immunogenic dystrophin epitopes in DMD boys compared to normal controls. To further advance the field we have used isolated limb perfusion (ILP) through the femoral artery in non-human primates to target specific muscle groups. This approach has advantages: (1) preventing spread of virus to the systemic circulation; (2) permitting efficient AAV transduction to specific muscle groups; and (3) demonstrating levels of gene expression related to binding antibody titers that can be altered with plasma exchange. These translational studies help provide a path forward for future gene transfer studies for DMD.

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T.I.2
Antisense oligonucleotide exon skipping therapy for DMD
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Antisense oligonucleotides (AOs) are able to modulate the splicing of the dystrophin pre-mRNA to correct the aberrant reading frame resulting from mutations in the dystrophin gene and thereby lead to the production of functional amounts of dystrophin protein in patients with Duchenne muscular dystrophy (DMD). A recent dose escalation systemic delivery clinical trial was undertaken using morpholino phosphorodiamidate (PMO) AOs administered intravenously to patients with DMD, carried out by the UK MDEX Consortium in collaboration with AVI BioPharma. The results demonstrated a good safety profile of the drug, dose-dependent restoration dystrophin protein in skeletal muscle tissue and a reduction in the local muscle inflammation observed. Efforts are also underway to improve delivery and activity of PMO AOs with the discovery of high efficiency peptide delivery systems for PMOs (so-called PPMOs). A number of highly active novel PPMOs have been discovered (B-MSP-PMO and Pip-PMOs) with utility for low dose dystrophin restoration in skeletal and for the first time at high efficiency also in cardiac muscle tissues. AO-mediated exon skipping therefore shows significant promise as an experimental therapy for DMD.

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T.I.3
Antisense Oligonucleotide (AON) mediated exon skipping trials in Duchenne Muscular Dystrophy (DMD): Current status, future prospects and challenges
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DMD is a severe progressive neuromuscular disease caused by lack of the cytoskeletal dystrophin protein. AON mediated exon skipping is a therapeutic approach aiming at restoring the disrupted reading frame of the DMD gene during splicing, allowing the production of a partially functional dystrophin. Proof of concept studies with local administration of 2-O-methyl phosphorothioate (2 OMePS) or morpholino (PMO) AON’s, targeting exon 51, showed novel dystrophin expression at the injection site in muscle biopsies of treated DMD subjects. Subcutaneous (2 OMePS,PRO 051/GSK2402968) or intravenous (PMO, A.V.I-4658) administration has been tested in dose ranging studies, confirming the ability of those AON’s to induce exon skipping and novel dystrophin expression. Forty eight-week follow-up data from a phase I/Ia open label extension study of systemic PRO051/GSK2402968 in DMD subjects showed encouraging safety and efficacy results which have to be confirmed by the ongoing larger placebo controlled phase IIb (GSK DMD 114117) and phase III (GSK DMD114044) studies. A phase I/IIa open label dose escalating study targeting exon 44 (PRO044) of the DMD gene is underway. AON targeting exon 45, 43, 46, 53, 52 (Prosensa Therapeutics) and exon 50 (AVI Biopharma) are in late phase pre clinical development. Challenges in the development of AON treatment are the unknown effects of lifelong administration, the mutation-specific approach requiring the development of mutation specific sequences inducing different truncated dystrophins with different functionality and stability, and the limited number of eligible patients impairing the conduct of clinical trials meeting standard regulatory requirements.

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ADVANCES IN THERAPY OF NEUROMUSCULAR DISORDERS 2

O.9
Stem cell therapy of muscular dystrophies using exon skipping approach in GRMD dogs
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In DMD, skeletal and cardiac muscles are affected. A combination of different therapeutic approaches might enhance the possibility of successful therapy. We isolated CD133+ cells from muscle biopsies of GRMD dogs. Two dogs characterized by a mild clinical phenotype and three characterized by a severe clinical phenotype, were treated with their own transduced U7 exon 6–8 cells. Old GRMD dogs are well characterized in term of clinical history. All dogs received two arterial systemic injections through a catheter that was introduced in the left femoralis artery and reached the aortic arch at the level of the left subclavia. We performed three different functional measures: climb stairs (time), swimming, 6 minute walking test (6MWT). After the injection, all treated dogs had a clinical performance improvement. After the first injection, all dogs had no detectable anti-dystrophin antibodies and its circulating lymphocytes did not react to transduced CD133+ cells. All transplanted animals were analysed at different times; most of the biopsies in all muscles had a morphological amelioration when compared to untreated dogs. Dystrophin expression in the biopsies was variable, ranging from 2% to 7% in several biopsies of the injected legs. The percentage of dystrophin expressing fibres ranged from 1% to 7%, in two distant sections of different biopsies each of selected muscles from the dogs. Western blot analysis from different biopsies of the same muscles confirmed the presence of different amount of dystrophin, varying from an undetectable signal to around 6% of a wt canine muscle. Two dogs received their own CD133+ cells without lentiviral transduction. In the group of untreated GRMD dogs, two dogs died of pneumonia and other complications during the follow up. This is the first demonstration of a clinical effect in old GRMD dogs that regained walking ability after autologous transplantation of engineered stem cells.

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