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Gene Therapy for Muscular Dystrophy: Lessons Learned and Path Forward

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Abstract

Our Translational Gene Therapy Center has used small molecules for exon skipping and mutation suppression and gene transfer to replace or provide surrogate genes as tools for molecular-based approaches for the treatment of muscular dystrophies. Exon skipping is targeted at the pre-mRNA level allowing one or more exons to be omitted to restore the reading frame. In Duchenne Muscular Dystrophy (DMD), clinical trials have been performed with two different oligomers, a 2'-O-methyl-ribo-oligonucleoside-phosphorothioate (2'OMe) and a phosphorodiamidate morpholino (PMO). Both have demonstrated early evidence of efficacy. A second molecular approach involves suppression of stop codons to promote readthrough of the *DMD* gene. We have been able to establish proof of principle for mutation suppression using the aminoglycoside, gentamicin. A safer, orally administered, alternative agent referred to as Ataluren (PTC124) has been used in clinical trials and is currently under consideration for approval by the FDA.

Using a gene therapy approach, we have completed two trials and have initiated a third. For DMD, we used a mini-dystrophin transferred in adeno-associated virus (AAV). In this trial an immune response was seen directed against transgene product, a quite unexpected outcome that will help guide further studies. For limb girdle muscular dystrophy 2D (alpha-sarcoglycan deficiency), the transgene was again transferred using AAV but in this study, a muscle specific creatine kinase promoter controlled gene expression that persisted for six months. A third gene therapy trial has been initiated with transfer of the follistatin gene in AAV directly to the quadriceps muscle. Two diseases with selective quadriceps muscle weakness are undergoing gene transfer including sporadic inclusion body myositis (sIBM) and Becker muscular dystrophy (BMD). Increasing the size and strength of the muscle is the goal of this study. Most importantly, no adverse events have been encountered in any of these clinical trials.

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Keywords

exon skipping; mutation suppression; dystrophin; alpha-sarcoglycan; follistatin; adeno-associated virus

Mandate for Treatment of Muscular Dystrophies

DMD is the most common, severe childhood form of muscular dystrophy. Inheritance follows an X-linked recessive pattern. Based on data from worldwide newborn screening studies, birth prevalence is about 1:4087 [31]. The very large size of the *DMD* gene [17] results in spontaneous mutations, and an unending trail of new cases and new carriers, emphasizing the compelling need to find a treatment. Serum creatine kinase (CK) is elevated at birth, and motor and even speech milestones are often delayed. Questions usually begin to surface between ages 3 to 5 regarding reduced motor skills that alert a need for diagnostic evaluation. DMD is relentlessly progressive and the medical need for treatment is undeniable. Quality of life is affected at a young age for DMD boys with inability to keep up with peers and loss of ambulation by age 12 [3]. Improvement in respiratory care can unmask decline in cardiac function dictating a need for treatment of the dilated cardiomyopathy. Quality of life is also diminished by the common, yet under-recognized occurrence of pain in DMD children [48]. The practical need for treatment is further underscored by the enormous life-time financial burden, and limitation of family resources, poorer health status and increased divorce rate of parents related to anxiety and depression [4], and accusations of blame thrust upon maternal inheritance [32].

BMD is a “milder” form of dystrophin deficiency resulting from translation of a truncated dystrophin protein with significantly variant functional properties. Some BMD patients lose the ability to walk as early as late teen-age years, while others ambulate until late middle-life [6], putting added stress on the heart that can be life threatening. BMD patients typically die in the fourth and fifth decades.

The other group of diseases referred to in this review is the limb girdle muscular dystrophies. The full classification of these disorders is based upon the sequential order of linkage analysis; reference to the deficient skeletal muscle protein is ignored in the cataloging of these diseases [20]. Our clinical gene therapy trials have focused on LGMD2D caused by α -sarcoglycan deficiency. This disorder has striking parallels to DMD with the exception of autosomal recessive inheritance [33]. The clinical spectrum with childhood-onset, early loss of ambulation, the need for wheelchair and functional dependency mimics DMD. Others with LGMD2D exhibit prolonged ambulation similar to BMD. As a target entity for skeletal muscle gene therapy, the lack of cardiac involvement of in sarcoglycanopathy patients makes this group potentially more receptive compared to dystrophinopathies.

Current Treatment Options

Only glucocorticoids have consistently demonstrated efficacy in any of the muscular dystrophies, and the benefits are restricted to DMD. The unequivocal proof was established in a randomized, double-blind controlled trial [28]. At six months prednisone-treatment compared to placebo improved muscle strength and pulmonary functions, as well as the time needed to rise from supine to standing, to walk 9 m, and to climb four stairs ($P < 0.001$ for all comparisons). Similar results were later reported with deflazacort, an alternative, sodium-sparing glucocorticoid that was shown to prolong ambulation ($p < 0.005$) [2]. A high-dose, weekend regimen of prednisone (10 mg/kg/wk: half on Saturday and half on Sunday) shows equal efficacy to daily steroids [7].

The side-effect profile of glucocorticoids consistently demonstrates weight gain with a cushingoid appearance. DMD boys on steroids are also at risk for hypertension, cataract formation, loss of bone density, vertebral compression fractures, and long bone fractures. Long-term administration may be limited in some cases by steroid-induced behavioral problems frequently observed with this class of drugs.

Emerging Drug or Small Molecule Therapies

Gene manipulation using small molecules is developing at a rapid pace and is moving forward in clinical trials. Results of studies parallel the progress made by gene replacement therapy. Our Center has been involved in two potentially promising strategies, exon skipping and mutation suppression.

Exon Skipping

Exon skipping is targeted at the pre-mRNA level allowing one or more exons to be omitted to restore the dystrophin reading frame. This is accomplished with splice-switching oligomers, typically 20–30 nucleotides in length and complementary in sequence to regions of the pre-mRNA transcript. Pre-clinical efficacy has been demonstrated in the mdx, dystrophin/utrophin knock-out mouse, and the dystrophin-deficient dog using both 2'-O-methyl-ribo-oligonucleoside-phosphorothioate (2'OMe) and phosphorodiamidate morpholino oligomers (PMOs) [11, 23, 47]. Two proof-of-principle clinical trials in DMD used a 2'OMe oligomer (PRO051/GSK2402968, ProsensaTherapeutics) or a PMO (AVI-4658/Eteplirsen®; AVI BioPharma Inc.) delivered directly to muscle, targeting exon 51 [16, 41]. Evidence favoring exon skipping was validated using either oligomer by RT-PCR and by demonstrating newly synthesized dystrophin protein correctly localized to the sarcolemma. Phase I/II extension studies were performed using systemically delivered oligomers. In the PRO051 trial [10], dose-related efficacy was achieved with evidence of new dystrophin expression in approximately 60–100% of muscle fibers in 10 of 12 patients with modest improvement in the 6-minute walk test. In the AVI phase II open-label study with AVI-4658 (Eteplirsen®) 7 of 19 patients saw a modest response with a mean increase of sarcolemmal dystrophin from 8.9% to 16.4% [5]. The results were variable with one patient responding following treatment with 2 mg/kg, while all other responders received the higher dosing levels of 10 and 20 mg/kg. AVI-4658 is now in testing in a phase IIb randomized, double-blind, placebo-controlled, multiple dose efficacy trial at Nationwide Children's Hospital, Columbus, OH, USA to assess 30 and 50 mg/kg dosing over 24 weeks (<http://www.clinicaltrials.gov>). The results of this trial will influence dosing regimens for a probable multi-clinic phase III clinical trial.

Mutation Suppression

A second molecular approach involves suppression of stop codon mutations of the *DMD* gene that comprise approximately 15% of DMD cases [9, 26]. Efficacious data in pre-clinical mdx mouse studies [1] led to a clinical trial in DMD patients with stop codons, treated weekly or twice weekly for six months using the aminoglycoside, gentamicin. Proof of principle was established based on a significant increase in dystrophin levels, with the highest levels reaching 13 and 15% of normal [22]. Muscle strength was stabilized and a modest increase in forced vital capacity was achieved. Although this study demonstrated the therapeutic potential of gentamicin, higher doses might be necessary to further improve functional outcomes. The known renal toxicity of aminoglycoside antibiotics and the potential limitations of intravenous administration pushed the field in the direction of identifying a safer, orally administered agent.

Ataluren, formerly referred to as PTC124 (PTC Therapeutics), fulfilled the requirement of an orally administered pharmacologic read-through agent for stop codon mutations [45]. A phase I study in healthy volunteers established safety and tolerability at doses exceeding what was required for pre-clinical efficacy [15]. In a phase IIa proof-of-concept 28-day study in DMD/BMD patients, dystrophin appeared to increase post treatment. A randomized, double-blind, placebo-controlled phase IIb trial followed, evaluating safety and efficacy over a 48 week treatment period. PTC, Inc. released preliminary results indicating a very strong safety profile; however, the primary endpoint of the 6 minute walk test did not reach statistical significance [<http://www.clinicaltrials.gov>; Phase 2b Extension Study of Ataluren (PTC124) in Duchenne/Becker Muscular Dystrophy (DMD/BMD)]. Continued subgroup analysis to look at efficacy is currently underway.

Gene Therapy for Duchenne Muscular Dystrophy

More than 20 years ago the *DMD* gene was cloned, defining the molecular basis for the disease [17]. The identification of the dystrophin as the deficient protein followed closely on the heels of this discovery [3]. Dystrophin is a 427kDa cytoskeletal protein required for muscle fiber stability. The histological consequences of the loss of this protein include susceptibility to repeated cycles of necrosis and regeneration, satellite cell depletion, diminished regenerative capacity of the muscle, and finally, fat and connective tissue replacement (fibrosis). This very large protein is translated by the largest gene in the human genome (>2.4 MBP) with an mRNA of 14 kilobases (kb) (11kb coding region of dystrophin from start codon to stop codon) [18]. Preference for the use of adeno-associated virus (AAV) as the vector of choice for gene delivery of the *DMD* gene is based on its known persistence in healthy muscle and absence of pathogenicity. However, its cloning capacity of 5 kb [28–30] presents a potentially limiting obstacle for successful *DMD* gene transfer. Offsetting this unfavorable property are clinical observations that large, in-frame deletions of the central rod domain often lead to mild BMD [6, 46]. The most notable example was a patient with a deletion of exons 17–48 that removed 48% of the coding region of the *DMD* gene, yet ambulation persisted beyond age 61 [6]. Elegant work by two laboratories (Chamberlain and Xiao Xiao) keyed off of this clinical finding in constructing functional mini-dystrophin genes. Methodical studies in the Chamberlain Laboratory dissected functional correlations of small dystrophins in transgenic mdx mice [13] demonstrating protection against the dystrophic process. For gene transfer to muscle using AAV, their studies also showed that mini-dystrophins required preservation of the cysteine rich (CR) domain; further size reduction could be achieved by removal of the C-terminal (CT) without forfeiting function.

In the Xiao Xiao Laboratory, other studies laid the ground work for a clinical trial providing evidence that a mini-dystrophin gene of 3990kb under control of a muscle specific creatine kinase (MCK) promoter or a cytomegalovirus (CMV) promoter packaged in AAV resulted in sustained gene expression (2, 4, and 6 months) upon injection into the adult mdx muscle (gastrocnemius) [43]. In addition, there was protection of the plasma membrane of myofibers shielded from uptake of Evans blue dye. In a later publication, AAV.CMV.Δ3990 was shown to increase isometric force and provide resistance to contraction-induced injury in mdx muscle [44]. These pre-clinical studies set the stage for the first human trial of gene therapy in DMD [27].

In March 2006, six boys with frame-shifting deletions of the dystrophin gene were enrolled in the first clinical gene therapy trial for DMD at Nationwide Children's Hospital (Table 1). At enrollment, four patients were receiving standard glucocorticoid therapy that was continued throughout the course of the study. Asklepios Biopharmaceutical manufactured a modified serotype 2 capsid of AAV with insertion of 5 amino acids from AAV1

(rAAV2.5.CMV.Δ3990) (Fig. 1). Intravenous methylprednisolone (2.0 mg/kg) was given 4 hours prior to vector administration and repeated at 24 and 48 hours to reduce the potential for local inflammation from intramuscular needle insertion. This was a randomized, double blind study with gene transfer to one biceps while the opposite side received saline (cohort 1, n=3) or empty capsids (cohort 2, n =3). Patients 1, 2, and 3 received low dose vector (2.0×10^{10} vector genomes/kg body weight), and Patients 4, 5, and 6 received a dose five-fold higher (1.0×10^{11} vector genomes/kg body weight). Peripheral blood mononuclear cells (PBMCs) were tested in an interferon- γ enzyme-linked immunosorbent spot (ELISpot) assay for reactivity to mini-dystrophin. Three synthetic peptide pools spanning the entire mini-dystrophin sequence (designated MDP1, MDP2, and MDP3) were used to stimulate PBMCs in the ELISpot assay.

Muscle biopsy specimens were assessed on day 42 (Patients 1, 3, 4, and 6) or day 90 (Patients 2 and 5). In all the patients, vector DNA was detected in amounts ranging from 0.01 to 2.56 genome copies per diploid genome in the treated muscles, but none was detected in the untreated contralateral biceps muscles. However, transgene expression was virtually undetectable on the side of treatment. Exceptions included four dystrophin-positive fibers in Patient 3 and one dystrophin-positive fiber in Patient 6 recognized by double-labeling with antibodies positive for N-terminal dystrophin but negative for the C-terminal, an expression pattern predicted by transgene Δ3990. In Cohort 1 (low dose vector), the interferon- γ ELISpot assay in Patient 2 showed a robust T-cell response against MDP2 beginning on the 15th day post gene transfer (Fig. 2). The response peaked on day 30, slowly declined during the next 3 months, and was intermittently positive after day 120. In Cohort 2, Patients 4, and 5 had positive ELISpots to MDP3 [27].

Additional studies revealed that at least two different pathways provoked an immune response to mini-dystrophin encountered in these patients. In Patient 5 the delayed, transient T-cell response detected three discrete dystrophin epitopes in the region of the patient's deletion: an exon 7 epitope recognized by HLA-B*1801-restricted CD8+ T cells; an epitope that localized to exon 8 recognized by CD4+ T cells; and another in exon 6 recognized by CD4+ T cells. Because exons 3 through 17 were deleted from the dystrophin gene in this patient, it was clear that CD4+ and CD8+ T cells were primed by mini-dystrophin that expressed exons 3 through 12 in the region of the patient's deletion (Fig. 3).

In Patient 2, a different and unexpected mechanism of immunity was elicited. An MDP2-specific interferon- γ T cell response was triggered by amino acids 2809 to 2829 of exon 57, downstream from the patient's exon-50 deletion. The patient's mutation predicted a frameshift, thereby impeding dystrophin expression. However, in the muscle biopsy from Patient 2, revertant fibers were visualized with the use of exon specific antibodies against dystrophin expressed from exons 55–56 and 59 (Fig. 4), implying that exon 57 was expressed in the correct reading frame. Thus, T cells were primed from exon 57, and we were able to demonstrate a robust interferon- γ response in PBMCs not only after gene transfer, but prior to treatment as well (Fig. 4). A similar situation was seen in Patient 4 who had a deletion at exons 49 through 54, in whom we found revertant fibers with novel epitopes encoding exon 59, downstream of the mutation. In this case, the ELISpot showed a CD8+ T cell response directed against MDP3 peptides encoded by exons 59 through 70 from spectrin repeat 24, hinge 4, and the cysteine repeat region.

The findings from this study are important as we move forward with gene therapy for DMD. First we learned that we cannot express the mini-dystrophin in a deleted region of the patient's DMD gene (Fig 3). In retrospect, this seems intuitive but it was underappreciated as inclusion/exclusion criteria for this study. The second important lesson learned is that revertant fibers, expressing dystrophin by restoring the reading frame in the DMD gene

through alternative splicing or a second-site mutation, can express an immunogenic novel epitope never before encountered by the patient (Fig 4). This finding was very much unexpected given that most clinicians and scientists predicted that revertant fibers would induce immune tolerance. Validation of this principle was demonstrated in a follow up study conducted in our Center where we encountered dystrophin-specific T cells on a significant number of DMD patients screened using the interferon- γ ELISpot assay (manuscript in preparation). These findings illustrate the importance of pre-screening patients for immunity to dystrophin prior to enrolling in mini-dystrophin gene therapy clinical trials.

Gene Therapy for Limb Girdle Muscular Dystrophy (LGMD)

Our experience with gene transfer for LGMD2D, alpha-sarcoglycan (α -SG) deficiency was dramatically different from DMD [29, 30]. This is the most common form of the sarcoglycanopathies [20, 33]. There were several factors that favored a more satisfactory outcome. The predominant mutation in LGMD2D is a missense mutation, providing a basis for gene expression that reduces the risk of immunogenicity from transgene expression. The full length α -SG cDNA is <2 kb and is well within the packaging capacity of rAAV. We also used a muscle specific creatine kinase (tMCK) promoter [40] for the first time in a clinical trial improving the safety profile of gene transfer targeting muscle. Indications from pre-clinical studies supported the use of AAV1 to achieve sustained gene expression in dystrophic muscle [35]. This was a double blind, randomized controlled study (vector on one side and saline injected in the other). In contrast to the DMD trial, the extensor digitorum brevis (EDB) muscle provided a better target for gene therapy with fewer dystrophic features compared to limb muscles. In addition, the entire EDB muscle could be removed post-gene transfer with no risk of functional loss to the patient and at the same time, provided a comprehensive substrate for analysis post gene transfer.

Six LGMD2D subjects with established SGCA mutations at both alleles were enrolled [29, 30]. A dose escalation study was initially planned but detection of robust gene expression at 6 weeks (patients 1 and 3) and 3 months (patient 2) following the first three gene transfer subjects favored using the identical dose of rAAV1.tMCK.SGCA (3.25×10^{11} vector genomes delivered in a 1.5 ml of saline) for all subjects. Thus, patients 4, 5, and 6 received the same dose, however we extended the time for observations to 6 months post gene transfer.

All subjects in this trial received intravenous methylprednisolone at the same dose and time of administration as in the DMD trial. Targeting muscle for gene delivery was guided by ultrasound and electromyography (37mm Teca Myoject injection recording needle) to ensure that muscle was the destination for the delivered product. Gene transfer using this vector, cassette and mode of delivery proved to be safe, without any adverse effects encountered over a 2 year monitoring period. All analyses of gene expression were done prior to breaking the blind. Relatively consistent findings were seen in five of the six subjects. In patients 1, 2, 3, 4, and 5, robust gene expression was seen on only one side in each case that was easily distinguishable from low level or background dystrophin staining observed on the contralateral side. In addition, restoration of the full sarcoglycan complex was seen on the side of gene transfer (Fig. 5). In two cases, Patient 2 (gene expression assayed at 3 months) and Patient 4 (gene expression assayed at 6 months), muscle fiber size increased in cross sectional diameter in treated compared to untreated sides. An important finding in this study was that through multiple approaches we could validate transgene expression in these patients. Bioquant® Image Analysis and western blots showed a significant increase of α -SG (Fig. 5). In addition, the side of gene transfer was corroborated using PCR amplification of a unique 5' untranslated leader sequence that we specifically incorporated into the transgene cassette to distinguish endogenous from transgene α -SG

expression. The RNase P gene was used as an internal control to normalize for genomic input and confirmed the absence of PCR inhibitors in the sample DNA.

The final participant in this study, Patient 6, was the exception compared to all others in this clinical gene therapy trial. The muscle biopsy showed comparative α -SG gene expression on the treated and untreated sides at the 6-month timepoint (Fig. 5). A one-time muscle biopsy makes it difficult to differentiate between loss of gene expression versus poor muscle transduction at the time of gene transfer. Several features favored poor muscle transduction. The muscle biopsy failed to show expression of major histocompatibility complex I (MHC I) antigen on any muscle fiber (Fig. 6). This is in direct contrast to the findings of all other participants in the clinical trial. The patient was also found to have both early humoral and T-cell responses to AAV1 capsid. The INF- γ ELISpot assay demonstrated T cell activation to AAV1 capsid as early as day 2 following gene transfer (also present on day 7) (Fig. 7). Accompanying the AAV capsid-induced T cell response, we found a very rapid rise in AAV neutralizing antibody titers reaching levels more than 30-times greater than seen in other cases (Table 3). Collectively these findings favor an amnestic response related to pre-existing immunity to AAV.

The lessons learned from this trial, combined with our experience in the DMD clinical gene transfer trial, help refine approaches as we move forward. The LGMD2D study shows us that AAV1 expresses well in muscle over long periods and we can expect robust expression using a muscle specific promoter that will restore gene expression levels to near normal. With proper vector cassette design we can differentiate endogenous versus transgene expressed product, an observation particularly relevant to other autosomal recessive disorders having a high percentage of missense mutations. The study also highlighted that we can never be over confident regarding pre-existing immunity to AAV capsid. The immune system can impede vector transduction, as we encountered in this study by the observation of a rapid rise in both T cell and humoral antibody responses (Figs. 6 and 7).

Surrogates for Gene Replacement using a Muscle Building Strategy

Blocking the myostatin pathway shows dramatic muscle building properties that are highly conserved across species, including mice, sheep, cattle, canines, and humans [24, 25, 34, 39]. Myostatin is a member of the transforming growth factor (TGF)- β superfamily and numerous studies have demonstrated that it is a negative regulator of skeletal muscle growth. An antibody to myostatin (MYO-29) was not beneficial to patients in a clinical trial [42]. Follistatin is a powerful inhibitor of myostatin plus having the advantage of controlling muscle mass through pathways independent of the myostatin signaling cascade [21]. In studies of the myostatin knockout mice crossed to mice carrying a follistatin transgene there was a quadrupling of muscle mass compared with the doubling of muscle mass that is observed from lack of myostatin alone.

In preparation for taking this to clinical trial, we identified an alternatively spliced isoform of follistatin (FS344) that can be packaged in AAV1. Upon intramuscular injection, FS344 undergoes post-translational modification and generates a stable, FS315 isoform with minimal toxicity. In normal C57Bl/6 mice and in the *mdx* mouse, intramuscular injections of AAV1 carrying the FS344 gene increased muscle mass (histological measure) and quantitative strength, as assessed by hind- and forelimb grip and measures of force generation and protection against eccentric contractions [12]. The benefits persist for more than two years, and in older mice not treated until 210 days of age, muscle strength increased for 60 days post gene delivery and persisted long-term, throughout the 560 days of evaluation. Study of the muscle and multiple organs from these mice revealed no significant abnormalities. In addition, we have tested the safety and efficacy of AAV1.CMV.FS344 in

non-human primates showing no organ system toxicity for as long as 15 months and an ability to increase quadriceps muscle size and strength following direct muscle injection [19]. This occurred without adverse effects. Necropsies showed no abnormalities in heart, lungs, liver, kidneys, or gonads. We also monitored clinical chemistries and hormone levels related to gonadal function including follicle stimulating hormone, luteinizing hormone, testosterone, and estradiol. No abnormalities were encountered.

We have now taken this to clinical trial in two patient groups who could specifically benefit by increasing size and strength of the quadriceps muscle similar to our proof of principle studies in mdx mice and cynomolgus macaques (<http://clinicaltrials.gov/>). In Becker muscular dystrophy and in sporadic inclusion body myositis the quadriceps muscle is targeted by the disease process leading to frequent falls and ultimately loss of ambulation. The gene delivery protocol approved by the FDA calls for injection of 12 sites in the quadriceps muscle divided evenly between rectus femoris, vastus medialis, and vastus lateralis. Vector administration includes a low dose cohort receiving intramuscular injections on one side only (2×10^{11} vg/kg); intermediate dosing (6×10^{11} vg divided between extremities) and high level dosing (1.2×10^{12} vg/kg divided between extremities). Two patients in the low dose cohort have been injected at the time of this writing. No adverse effects have been encountered. Muscle biopsies will be done at 6 months in all patients to delineate fiber size. MRI of the quadriceps muscle will also be used to assess muscle volume. Additional testing includes the 6 minute walk test, climbing 4 standard steps, timed to get up from a chair, and strength of knee extensors using quantitative myometry.

The Path Forward for Gene Therapy for Muscle Disease

The foundation for gene therapy for muscle disease has been established based on our own experience in clinical trials in DMD [27], LGMD2D [29, 30], BMD, and sIBM and reinforced by the French intramuscular γ -sarcoglycan gene transfer trial in LGMD2C [14]. Our future plans are focused on vascular delivery in DMD and LGMD2D. For these studies, we have designed a stepwise program with the rationale that isolated limb perfusion (ILP) would provide the potential for achieving a clinically meaningful outcome and accommodate safe viral delivery with limited exposure beyond the intended muscle groups targeted for gene therapy. Two different approaches have been designed to provide widespread muscle extremity transduction through delivery based upon transvenous [8] or transarterial [36–38] approaches. Studies in seven patients with muscular dystrophy (including BMD, LGMD, and Emery Dreifuss muscular dystrophy) have demonstrated a safe and feasible venous approach carried out through the saphenous vein at rate of 80 ml/minute at a volume not to exceed 20% of limb volume [8]. For these studies a tourniquet is placed above the knee and inflated to 310 mm Hg pressure. There is a transient depression of limb tissue oximetry and transient elevation of muscle compartment pressures but no symptoms or signs of nerve, muscle, or vascular damage. This method will have to be further tested in clinical trial with delivery of a specific transgene to establish transduction efficiency.

We have preferred an arterial approach with catheter placement in the femoral artery. We have studied this method extensively in mice [36] and monkeys [37, 38]. Following catheter placement, a tourniquet is positioned above the site of vessel entry. The vessel is perfused with saline to remove blood, platelet products and other debris in the vessel. A distal tourniquet is then placed at the ankle. The vector (AAV6 or AAV8) and transgene (microdystrophin, α -SG or eGFP) of choice is then delivered through this isolated vessel. After a ten minute dwell time, we are able to achieve very high transduction efficiency with greater than 50% of muscle fibers expressing the transgene. We have discussed this approach with the FDA in a pre-IND meeting and mapped out a plan for clinical delivery of α -SG and

micro-dystrophin for both DMD and LGMD2D. We believe based upon lessons learned in the previous and ongoing clinical trials and pre-clinical vascular gene delivery studies in rodents and non-human primates [8, 36–38], the future of gene therapy for muscular dystrophy presents a favorable outlook.

References

1. Barton-Davis ER, Cordier L, Shoturma DI, Leland SE, Sweeney HL. Aminoglycoside antibiotics restore dystrophin function to skeletal muscles of mdx mice. *J Clin Invest*. 1999; 104:375–381. [PubMed: 10449429]
2. Biggar WD, Harris VA, Eliasoph L, Alman B. Long-term benefits of deflazacort treatment for boys with Duchenne muscular dystrophy in their second decade. *Neuromuscul Disord*. 2006; 16:249–255. [PubMed: 16545568]
3. Brooke MH, Fenichel GM, Griggs RC, Mendell JR, Moxley R, Miller JP, Province MA. Clinical investigation in Duchenne dystrophy: 2. Determination of the “power” of therapeutic trials based on the natural history. *Muscle Nerve*. 1983; 6:91–103. [PubMed: 6343858]
4. Chen JY, Clark MJ. Family function in families of children with Duchenne muscular dystrophy. *Fam Community Health*. 2007; 30:296–304. [PubMed: 17873636]
5. Cirak S, Arechavala-Gomez V, Guglieri M, Feng L, Torelli S, Anthony K, Abbs S, Garralda ME, Bourke J, Wells DJ, Dickson G, Wood MJ, Wilton SD, Straub V, Kole R, Shrewsbury SB, Sewry C, Morgan JE, Bushby K, Muntoni F. 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*. 2011; 378:595–605. [PubMed: 21784508]
6. England SB, Nicholson LV, Johnson MA, Forrest SM, Love DR, Zubrzycka-Gaarn EE, Bulman DE, Harris JB, Davies KE. Very mild muscular dystrophy associated with the deletion of 46% of dystrophin. *Nature*. 1990; 343:180–182. [PubMed: 2404210]
7. Escolar DM, Hache LP, Clemens PR, Cnaan A, McDonald CM, Viswanathan V, Kornberg AJ, Bertorini TE, Nevo Y, Lotze T, Pestronk A, Ryan MM, Monasterio E, Day JW, Zimmerman A, Arrieta A, Henricson E, Mayhew J, Florence J, Hu F, Connolly AM. Randomized, blinded trial of weekend vs daily prednisone in Duchenne muscular dystrophy. *Neurology*. 2011; 77:444–452. [PubMed: 21753160]
8. Fan Z, Kocis K, Valley R, Howard JF, Chopra M, An H, Lin W, Muenzer J, Powers W. Safety and feasibility of high-pressure transvenous limb perfusion with 0.9% saline in human muscular dystrophy. *Mol Ther*. 2012; 20:456–461. [PubMed: 21772257]
9. Flanigan KM, von Niederhausern A, Dunn DM, Alder J, Mendell JR, Weiss RB. Rapid direct sequence analysis of the dystrophin gene. *Am J Hum Genet*. 2003; 72:931–939. [PubMed: 12632325]
10. Goemans NM, Tulinius M, van den Akker JT, Burm BE, Ekhart PF, Heuvelmans N, Holling T, Janson AA, Platenburg GJ, Sipkens JA, Sitsen JM, Aartsma-Rus A, van Ommen GJ, Buyse G, Darin N, Verschuuren JJ, Campion GV, de Kimpe SJ, van Deutekom JC. Systemic administration of PRO051 in Duchenne’s muscular dystrophy. *N Engl J Med*. 2011; 364:1513–1522. [PubMed: 21428760]
11. Goyenvallé A, Babbs A, Powell D, Kole R, Fletcher S, Wilton SD, Davies KE. Prevention of dystrophic pathology in severely affected dystrophin/utrophin-deficient mice by morpholino-oligomer-mediated exon-skipping. *Mol Ther*. 2010; 18:198–205. [PubMed: 19844193]
12. Haidet AM, Rizo L, Handy C, Umapathi P, Eagle A, Shilling C, Boue D, Martin PT, Sahenk Z, Mendell JR, Kaspar BK. Long-term enhancement of skeletal muscle mass and strength by single gene administration of myostatin inhibitors. *Proceedings of the National Academy of Sciences of the United States of America*. 2008; 105:4318–4322. [PubMed: 18334646]
13. Harper SQ, Hauser MA, DelloRusso C, Duan D, Crawford RW, Phelps SF, Harper HA, Robinson AS, Engelhardt JF, Brooks SV, Chamberlain JS. Modular flexibility of dystrophin: implications for gene therapy of Duchenne muscular dystrophy. *Nature medicine*. 2002; 8:253–261.
14. Herson S, Hentati F, Rigolet A, Behin A, Romero NB, Leturcq F, Laforet P, Maissonobe T, Amouri R, Haddad H, Audit M, Montus M, Masurier C, Gjata B, Georger C, Cherai M, Carlier P, Hogrel JY, Herson A, Allenbach Y, Lemoine FM, Klatzmann D, Sweeney HL, Mulligan RC, Eymard B,

- Caizergues D, Voit T, Benveniste O. A phase I trial of adeno-associated virus serotype 1-gamma-sarcoglycan gene therapy for limb girdle muscular dystrophy type 2C. *Brain*. 2012; 135:483–492. [PubMed: 22240777]
15. Hirawat S, Welch EM, Elfring GL, Northcutt VJ, Paushkin S, Hwang S, Leonard EM, Almstead NG, Ju W, Peltz SW, Miller LL. 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*. 2007; 47:430–444. [PubMed: 17389552]
 16. Kinali M, Arechavala-Gomez V, Feng L, Cirak S, Hunt D, Adkin C, Guglieri M, Ashton E, Abbs S, Nihoyannopoulos P, Garralda ME, Rutherford M, McCulley C, Popplewell L, Graham IR, Dickson G, Wood MJ, Wells DJ, Wilton SD, Kole R, Straub V, Bushby K, Sewry C, Morgan JE, Muntoni F. 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*. 2009; 8:918–928. [PubMed: 19713152]
 17. Koenig M, Hoffman EP, Bertelson CJ, Monaco AP, Feener C, Kunkel LM. Complete cloning of the Duchenne muscular dystrophy (DMD) cDNA and preliminary genomic organization of the DMD gene in normal and affected individuals. *Cell*. 1987; 50:509–517. [PubMed: 3607877]
 18. Koenig M, Monaco AP, Kunkel LM. The complete sequence of dystrophin predicts a rod-shaped cytoskeletal protein. *Cell*. 1988; 53:219–228. [PubMed: 3282674]
 19. Kota J, Handy CR, Haidet AM, Montgomery CL, Eagle A, Rodino-Klapac LR, Tucker D, Shilling CJ, Therfall WR, Walker CM, Weisbrode SE, Janssen PM, Clark KR, Sahenk Z, Mendell JR, Kaspar BK. Follistatin gene delivery enhances muscle growth and strength in nonhuman primates. *Sci Transl Med*. 2009; 1:6ra15.
 20. Laval SH, Bushby KM. Limb-girdle muscular dystrophies--from genetics to molecular pathology. *Neuropathol Appl Neurobiol*. 2004; 30:91–105. [PubMed: 15043707]
 21. Lee SJ. Quadrupling muscle mass in mice by targeting TGF-beta signaling pathways. *PLoS One*. 2007; 2:e789. [PubMed: 17726519]
 22. Malik V, Rodino-Klapac LR, Viollet L, Wall C, King W, Al-Dahhak R, Lewis S, Shilling CJ, Kota J, Serrano-Munuera C, Hayes J, Mahan JD, Campbell KJ, Banwell B, Dasouki M, Watts V, Sivakumar K, Bien-Willner R, Flanigan KM, Sahenk Z, Barohn RJ, Walker CM, Mendell JR. Gentamicin-induced readthrough of stop codons in Duchenne muscular dystrophy. *Ann Neurol*. 2010; 67:771–780. [PubMed: 20517938]
 23. Mann CJ, Honeyman K, Cheng AJ, Ly T, Lloyd F, Fletcher S, Morgan JE, Partridge TA, Wilton SD. Antisense-induced exon skipping and synthesis of dystrophin in the mdx mouse. *Proc Natl Acad Sci U S A*. 2001; 98:42–47. [PubMed: 11120883]
 24. McPherron AC, Lawler AM, Lee SJ. Regulation of skeletal muscle mass in mice by a new TGF-beta superfamily member. *Nature*. 1997; 387:83–90. [PubMed: 9139826]
 25. McPherron AC, Lee SJ. Double muscling in cattle due to mutations in the myostatin gene. *Proc Natl Acad Sci U S A*. 1997; 94:12457–12461. [PubMed: 9356471]
 26. Mendell JR, Buzin CH, Feng J, Yan J, Serrano C, Sangani DS, Wall C, Prior TW, Sommer SS. Diagnosis of Duchenne dystrophy by enhanced detection of small mutations. *Neurology*. 2001; 57:645–650. [PubMed: 11524473]
 27. Mendell JR, Campbell K, Rodino-Klapac L, Sahenk Z, Shilling C, Lewis S, Bowles D, Gray S, Li C, Galloway G, Malik V, Coley B, Clark KR, Li J, Xiao X, Samulski J, McPhee SW, Samulski RJ, Walker CM. Dystrophin immunity in Duchenne's muscular dystrophy. *N Engl J Med*. 2010; 363:1429–1437. [PubMed: 20925545]
 28. Mendell JR, Moxley RT, Griggs RC, Brooke MH, Fenichel GM, Miller JP, King W, Signore L, Pandya S, Florence J, et al. Randomized, double-blind six-month trial of prednisone in Duchenne's muscular dystrophy. *N Engl J Med*. 1989; 320:1592–1597. [PubMed: 2657428]
 29. Mendell JR, Rodino-Klapac LR, Rosales-Quintero X, Kota J, Coley BD, Galloway G, Craenen JM, Lewis S, Malik V, Shilling C, Byrne BJ, Conlon T, Campbell KJ, Bremer WG, Viollet L, Walker CM, Sahenk Z, Clark KR. Limb-girdle muscular dystrophy type 2D gene therapy restores alpha-sarcoglycan and associated proteins. *Ann Neurol*. 2009; 66:290–297. [PubMed: 19798725]

30. Mendell JR, Rodino-Klapac LR, Rosales XQ, Coley BD, Galloway G, Lewis S, Malik V, Shilling C, Byrne BJ, Conlon T, Campbell KJ, Bremer WG, Taylor LE, Flanigan KM, Gastier-Foster JM, Astbury C, Kota J, Sahenk Z, Walker CM, Clark KR. Sustained alpha-sarcoglycan gene expression after gene transfer in limb-girdle muscular dystrophy, type 2D. *Ann Neurol*. 2010; 68:629–638. [PubMed: 21031578]
31. Mendell JR, Shilling C, Leslie ND, Flanigan KM, al-Dahhak R, Gastier-Foster J, Kneile K, Dunn DM, Duval B, Aoyagi A, Hamil C, Mahmoud M, Roush K, Bird L, Rankin C, Lilly H, Street N, Chandrasekar R, Weiss RB. Evidence based path to newborn screening for duchenne muscular dystrophy. *Annals of Neurology*. 2012:n/a–n/a.
32. Mendell JR, Vaughn AJ. Duchenne muscular dystrophy: ethical and emotional considerations in long-term management. *Semin Neurol*. 1984; 4:98–103. [PubMed: 11649674]
33. Moore SA, Shilling CJ, Westra S, Wall C, Wicklund MP, Stolle C, Brown CA, Michele DE, Piccolo F, Winder TL, Stence A, Barresi R, King N, King W, Florence J, Campbell KP, Fenichel GM, Stedman HH, Kissel JT, Griggs RC, Pandya S, Mathews KD, Pestronk A, Serrano C, Darvish D, Mendell JR. Limb-girdle muscular dystrophy in the United States. *J Neuropathol Exp Neurol*. 2006; 65:995–1003. [PubMed: 17021404]
34. Mosher DS, Quignon P, Bustamante CD, Sutter NB, Mellers CS, Parker HG, Ostrander EA. A mutation in the myostatin gene increases muscle mass and enhances racing performance in heterozygote dogs. *PLoS Genet*. 2007; 3:e79. [PubMed: 17530926]
35. Rabinowitz JE, Rolling F, Li C, Conrath H, Xiao W, Xiao X, Samulski RJ. Cross-packaging of a single adeno-associated virus (AAV) type 2 vector genome into multiple AAV serotypes enables transduction with broad specificity. *J Virol*. 2002; 76:791–801. [PubMed: 11752169]
36. Rodino-Klapac LR, Janssen PM, Montgomery CL, Coley BD, Chicoine LG, Clark KR, Mendell JR. A translational approach for limb vascular delivery of the micro-dystrophin gene without high volume or high pressure for treatment of Duchenne muscular dystrophy. *Journal of translational medicine*. 2007; 5:45. [PubMed: 17892583]
37. Rodino-Klapac LR, Montgomery CL, Bremer WG, Shontz KM, Malik V, Davis N, Sprinkle S, Campbell KJ, Sahenk Z, Clark KR, Walker CM, Mendell JR, Chicoine LG. Persistent expression of FLAG-tagged micro dystrophin in nonhuman primates following intramuscular and vascular delivery. *Mol Ther*. 2010; 18:109–117. [PubMed: 19904237]
38. Rodino-Klapac LR, Montgomery CL, Mendell JR, Chicoine LG. AAV-mediated gene therapy to the isolated limb in rhesus macaques. *Methods Mol Biol*. 2011; 709:287–298. [PubMed: 21194036]
39. Schuelke M, Wagner KR, Stolz LE, Hubner C, Riebel T, Komen W, Braun T, Tobin JF, Lee SJ. Myostatin mutation associated with gross muscle hypertrophy in a child. *N Engl J Med*. 2004; 350:2682–2688. [PubMed: 15215484]
40. Shield MA, Haugen HS, Clegg CH, Hauschka SD. E-box sites and a proximal regulatory region of the muscle creatine kinase gene differentially regulate expression in diverse skeletal muscles and cardiac muscle of transgenic mice. *Mol Cell Biol*. 1996; 16:5058–5068. [PubMed: 8756664]
41. van Deutekom JC, Janson AA, Ginjaar IB, Frankhuizen WS, Aartsma-Rus A, Bremner-Bout M, den Dunnen JT, Koop K, van der Kooij AJ, Goemans NM, de Kimpe SJ, Ekhart PF, Venneker EH, Platenburg GJ, Verschuuren JJ, van Ommen GJ. Local dystrophin restoration with antisense oligonucleotide PRO051. *N Engl J Med*. 2007; 357:2677–2686. [PubMed: 18160687]
42. Wagner KR, Fleckenstein JL, Amato AA, Barohn RJ, Bushby K, Escolar DM, Flanigan KM, Pestronk A, Tawil R, Wolfe GI, Eagle M, Florence JM, King WM, Pandya S, Straub V, Juneau P, Meyers K, Csimma C, Araujo T, Allen R, Parsons SA, Wozney JM, Lavallie ER, Mendell JR. A phase I/II trial of MYO-029 in adult subjects with muscular dystrophy. *Ann Neurol*. 2008; 63:561–571. [PubMed: 18335515]
43. Wang B, Li J, Xiao X. Adeno-associated virus vector carrying human minidystrophin genes effectively ameliorates muscular dystrophy in mdx mouse model. *Proc Natl Acad Sci U S A*. 2000; 97:13714–13719. [PubMed: 11095710]
44. Watchko J, O'Day T, Wang B, Zhou L, Tang Y, Li J, Xiao X. Adeno-associated virus vector-mediated minidystrophin gene therapy improves dystrophic muscle contractile function in mdx mice. *Hum Gene Ther*. 2002; 13:1451–1460. [PubMed: 12215266]

45. Welch EM, Barton ER, Zhuo J, Tomizawa Y, Friesen WJ, Trifillis P, Paushkin S, Patel M, Trotta CR, Hwang S, Wilde RG, Karp G, Takasugi J, Chen G, Jones S, Ren H, Moon YC, Corson D, Turpoff AA, Campbell JA, Conn MM, Khan A, Almstead NG, Hedrick J, Mollin A, Risher N, Weetall M, Yeh S, Branstrom AA, Colacino JM, Babiak J, Ju WD, Hirawat S, Northcutt VJ, Miller LL, Spatrick P, He F, Kawana M, Feng H, Jacobson A, Peltz SW, Sweeney HL. PTC124 targets genetic disorders caused by nonsense mutations. *Nature*. 2007; 447:87–91. [PubMed: 17450125]
46. Yazaki M, Yoshida K, Nakamura A, Koyama J, Nanba T, Ohori N, Ikeda S. Clinical characteristics of aged Becker muscular dystrophy patients with onset after 30 years. *Eur Neurol*. 1999; 42:145–149. [PubMed: 10529540]
47. Yokota T, Lu QL, Partridge T, Kobayashi M, Nakamura A, Takeda S, Hoffman E. Efficacy of systemic morpholino exon-skipping in Duchenne dystrophy dogs. *Ann Neurol*. 2009; 65:667–676. [PubMed: 19288467]
48. Zebracki K, Drotar D. Pain and activity limitations in children with Duchenne or Becker muscular dystrophy. *Dev Med Child Neurol*. 2008; 50:546–552. [PubMed: 18611207]

Highlights

- Molecular therapeutic approaches to treat muscular dystrophies are discussed
- Exon skipping targets pre-mRNA allowing one or more exons to be omitted
- Proof of principle for readthrough of stop codons has been established in DMD
- Mini-dystrophin gene transfer resulted in an immune response, an unexpected outcome
- Alpha-sarcoglycan gene transfer showed sustained gene expression for six months

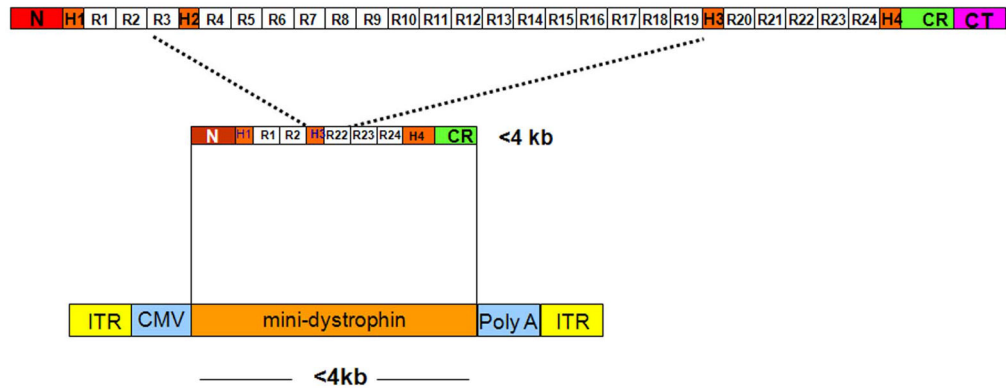


Figure 1. Full dystrophin protein

Amino-terminal (N), cysteine-rich (CR) domain, C-terminal (CT), and rod domain seen with 24 spectrin repeats (R1–R24) and four hinges (H1, H2, H3, H4). The mini-dystrophin represents a truncated peptide with N-terminal, CR domain and rod domain with reduced number of spectrin repeats (R1, R2, R22, R23, R24) and 3 hinges (H1, H3, H4). The mini-dystrophin cDNA with its Poly A tail is under control of the cytomegalovirus (CMV) promoter flanked by inverted terminal repeats (ITRs).

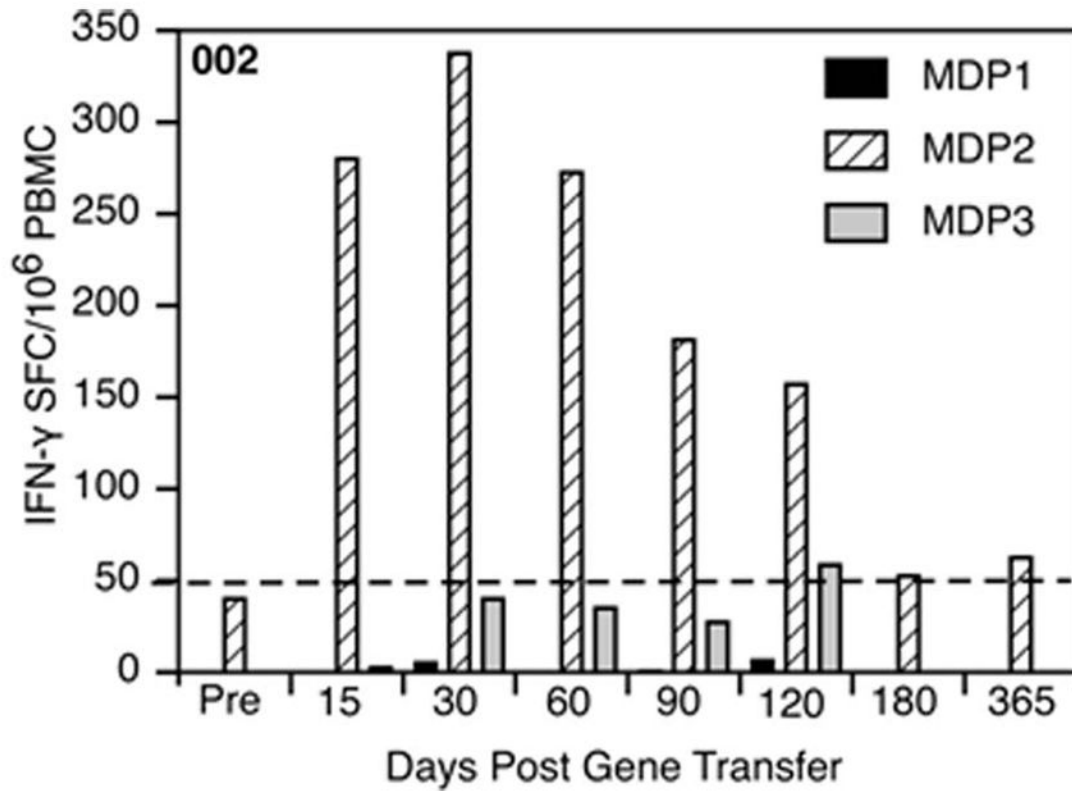


Figure 2. T cell responses clinical *DMD* gene therapy trial

Peripheral blood mononuclear cells (PBMC) were collected on the indicated days and cultured with three pools of synthetic peptides (MDP1, MDP2, MDP3) spanning the mini-dystrophin expressed by the transgene. Interferon- γ production was assessed 36 hours later with use of an interferon- γ enzyme-linked immunosorbent spot (ELISPOT) assay. Data are presented as interferon- γ spot forming cells (SFC) per 1 million PBMCs. The dashed line represents the threshold for a positive assay response (50 SFCs per 1 million PBMCs).

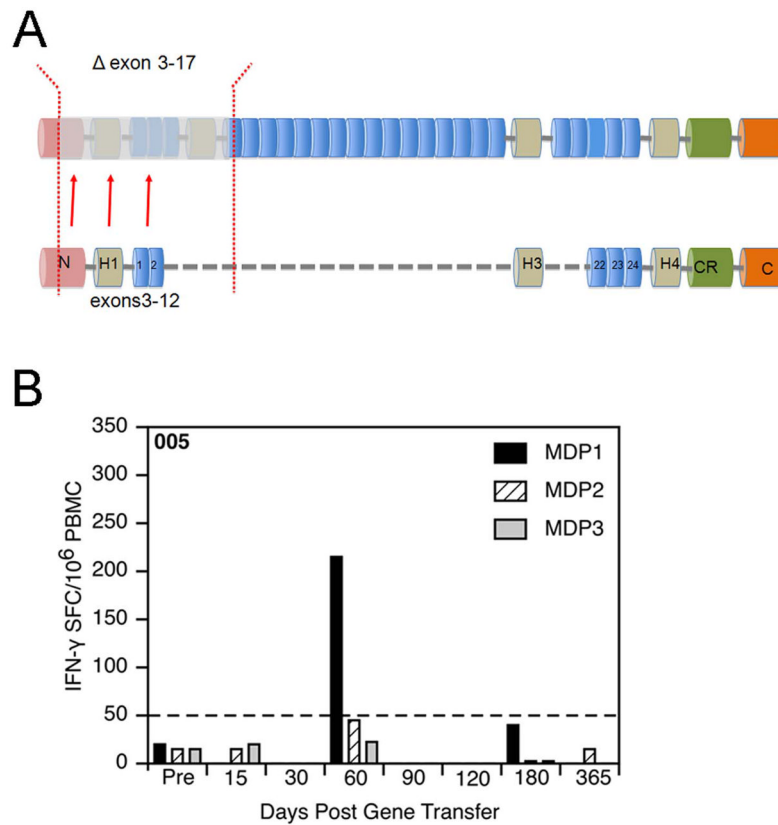


Figure 3. Patient's deleted dystrophin peptide and transgene product

A) Depiction of dystrophin peptide resulting from deletion of exons 3 to 17 of *DMD* gene. Below is the truncated peptide translated from mini-dystrophin. The peptide expresses in the region of the patient's deletion. The consequences are shown in B) where an immune response is demonstrated by ELISPOT assay to mini-dystrophin peptide 1 (MDP-1) composed of amino acids (aa) 1–469. There are no T cell responses generated by culturing with MDP2 or MDP3.

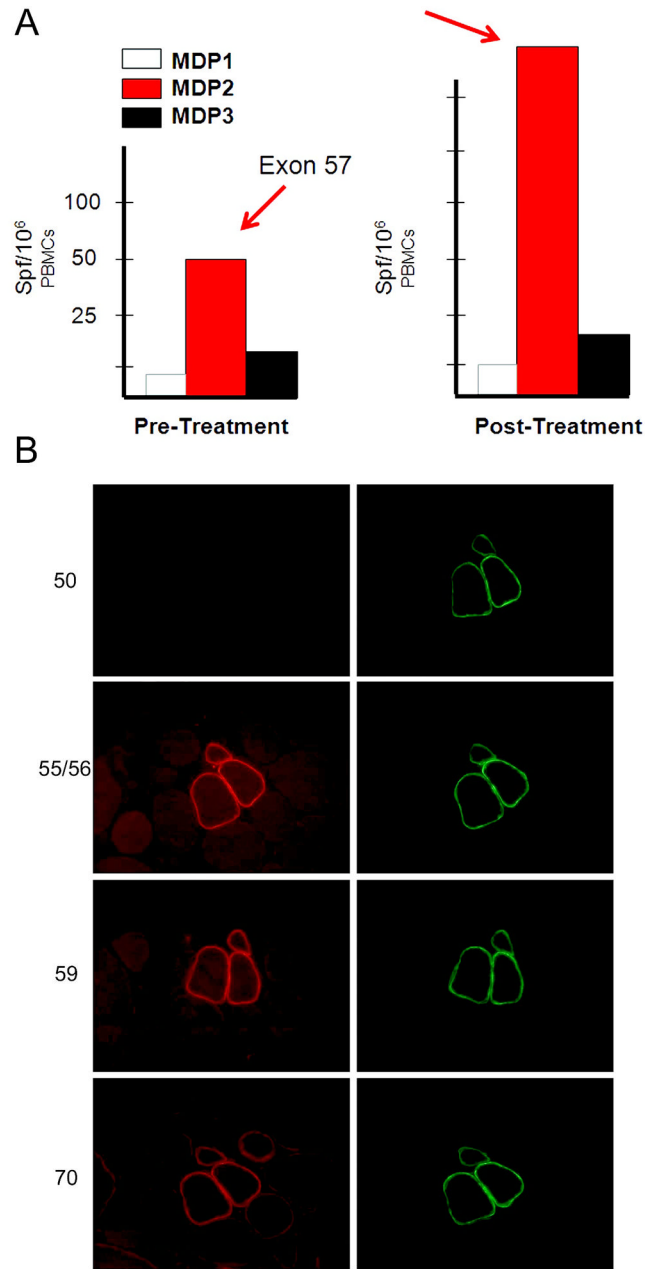


Figure 4. ELISPOT assay pre- and post-gene therapy

A) T cell response of PBMCs is stimulated by an amino acid fragment translated by exon 57. There is a robust T cell response seen post-treatment and minimal but suggestive immune response even pre-treatment. B) Revertant fiber cluster (3 positive fibers) is seen using C-terminal antibody (right panel). On the left, there is no staining at exon 50 corresponding to the patient's deletion. Starting at exon 55/56 the revertant cluster begins to express dystrophin using exon specific antibodies kindly supplied by Glen Morris. Similar findings are seen with antibodies directed at exons 59 and 70. This confirms that the revertant cluster expresses dystrophin from exon 57 (although antibody to this exon was not available).

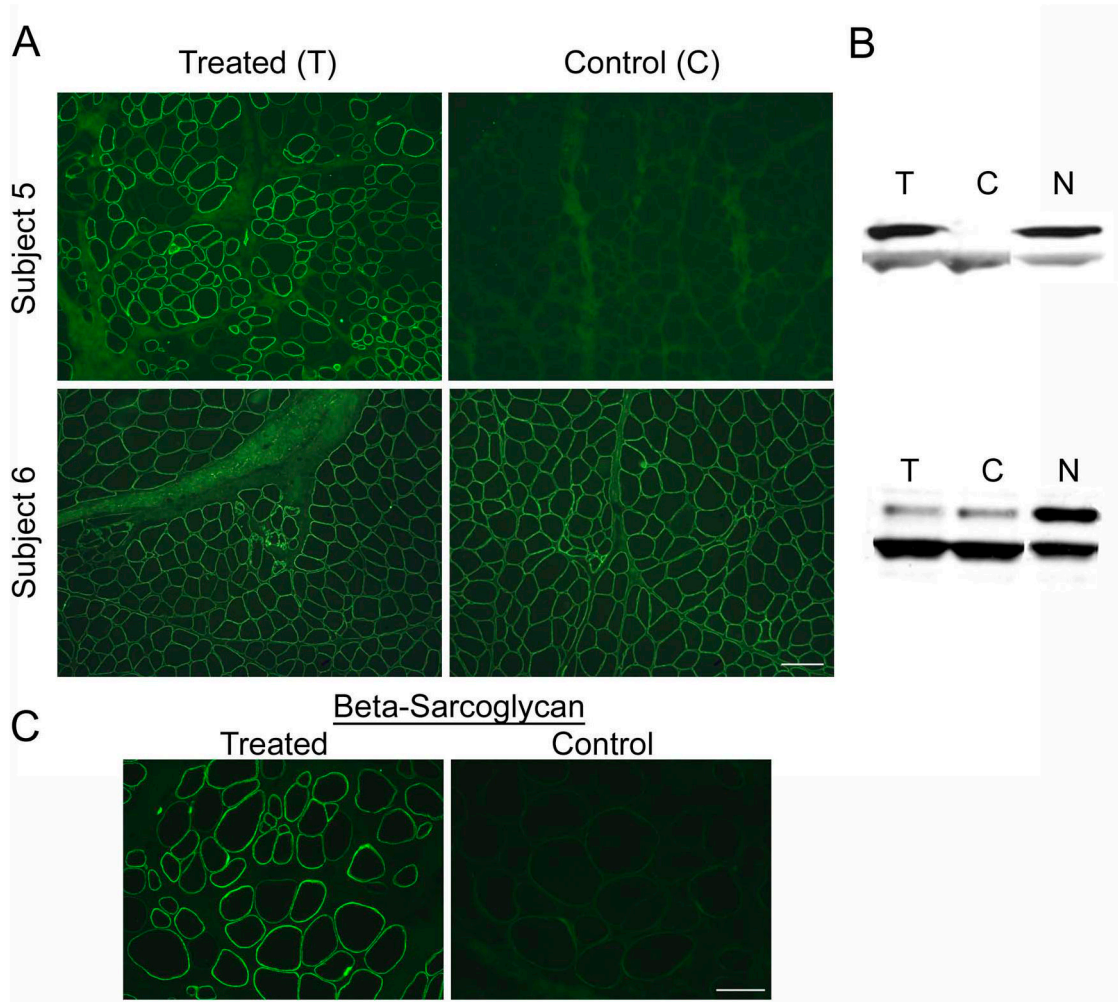


Figure 5. Gene transfer muscle sections from EDB stained with antibody to alpha-sarcoglycan (α SG)

(A) Subjects 5 showed increased staining on the gene transfer (Treated) compared to the placebo-treated side (control). Subject 6 showed no difference in α -SG staining intensity before or after gene transfer (findings verified by Bioquant Image Analysis) (scale bar = 150 μ m). (B) Western blots (WB) from subject 5 shows increased α -SG gene expression on the side of gene transfer compared to the control side. Subject 6 showed no increase in α -SG expression between treated side and control side after transfer. WBs are normalized to actin (lower band) and each is compared with normal (N) muscle for comparison. (C) β -SG was restored on the treated side but not on the control side. Other sarcoglycans (delta and gamma) were also restored (not shown). (scale bar = 150 μ m).

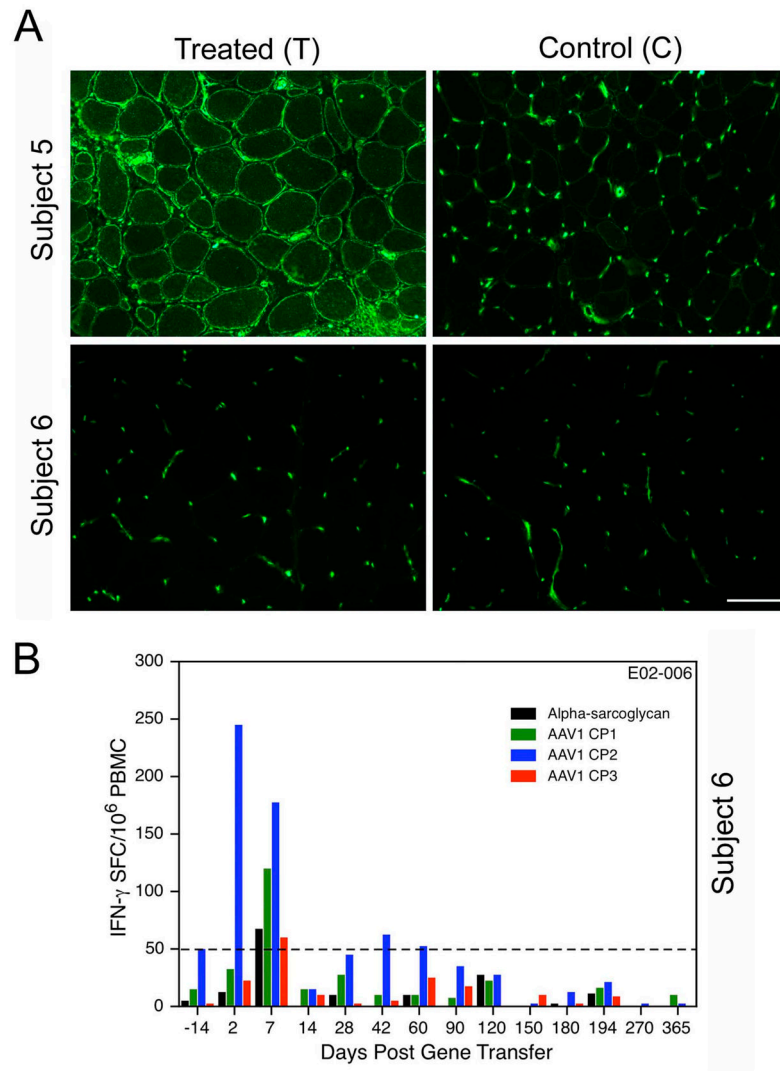


Figure 6. Major histocompatibility complex (MHC) I staining of sarcolemmal membrane following gene transfer

Muscle on side of gene transfer (Treated) of Subject 5 shows MHC I staining on the treated side and not the control side. Subject 6 shows no MHC I staining on the treated side or the control side following gene transfer (scale bar = 100 μ m). Microvascular circulation is positive for MHC I on both sides in all subjects. B) Subject 6 of the LGMD2D gene transfer study shows that IFN- γ ELISpot assays were negative to α -SG stimulation (black) before and after gene transfer. However, this patient showed a robust AAV1 capsid response as early as day 2 (Blue) that was again positive on day 7. This was distinctly earlier than other patients undergoing gene transfer indicative of an amnestic response suggesting pre-existing immunity to AAV.

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Table 1

Description of Cohorts receiving DMD gene therapy. The vector dose is shown for Cohort 1 (subjects 001,002,003) and Cohort 2 (subjects 004,005,006). The age of the patients, their DMD gene mutations, and glucocorticoid treatment [prednisone (Pred) or deflazacort] is shown

Vector Dose	Subject	Age (yrs)	Deletion	Glucocorticoid	DOSE (mg)	FREQ
2×10^{10} vg/kg	001	8	45	Deflazacort	18	QD
	002	9	50	Pred	18	QD
	003	9	46-50	None	N/A	N/A
1×10^{11} vg/kg	004	5	49-54	None	N/A	N/A
	005	11	3-17	Pred	150	2xwk
	006	9	46-52	Pred	22.5	QD

Table 2

ELISA assay for neutralizing antibody titers against AAV1 pre- and post-gene transfer comparing results for Subject 6 with other patients. By day 7 the neutralizing antibody titers rose >30-fold compared to other subjects

Time Post Injection	Subject #4	Subject # 5	Subject #6
Pre-treatment	1:800	<1:50	1:1600
Week 1 (Day 7)	1:1600	1:3200	>1:102400
Week 2	1:25600	1:6400	>1:102400
Week 6	1:25600	1:6400	>1:102400
Week 12	1:51200	1:12800	>1:102400
Week 26	>1:102400	1:12800	>1:102400