We are IntechOpen,
the world's leading publisher of
Open Access books
Built by scientists, for scientists

6,900
Open access books available

186,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS
BOOK CITATION INDEX
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Progress and Challenges in AAV-Mediated Gene Therapy for Duchenne Muscular Dystrophy

Takashi Okada and Shin'ichi Takeda
National Institute of Neuroscience, National Center of Neurology and Psychiatry
Japan

1. Introduction

Duchenne muscular dystrophy (DMD) is the most common form of childhood muscular dystrophy. DMD is an X-linked recessive disorder with an incidence of one in 3500 live male births (Emery, 1991). DMD causes progressive degeneration and regeneration of skeletal and cardiac muscles due to mutations in the *dystrophin* gene, which encodes a 427-kDa subsarcolemmal cytoskeletal protein (Hoffman et al., 1987). DMD is associated with severe, progressive muscle weakness and typically leads to death between the ages of 20 and 35 years. Due to recent advances in respiratory care, much attention is now focused on treating the cardiac conditions suffered by DMD patients.
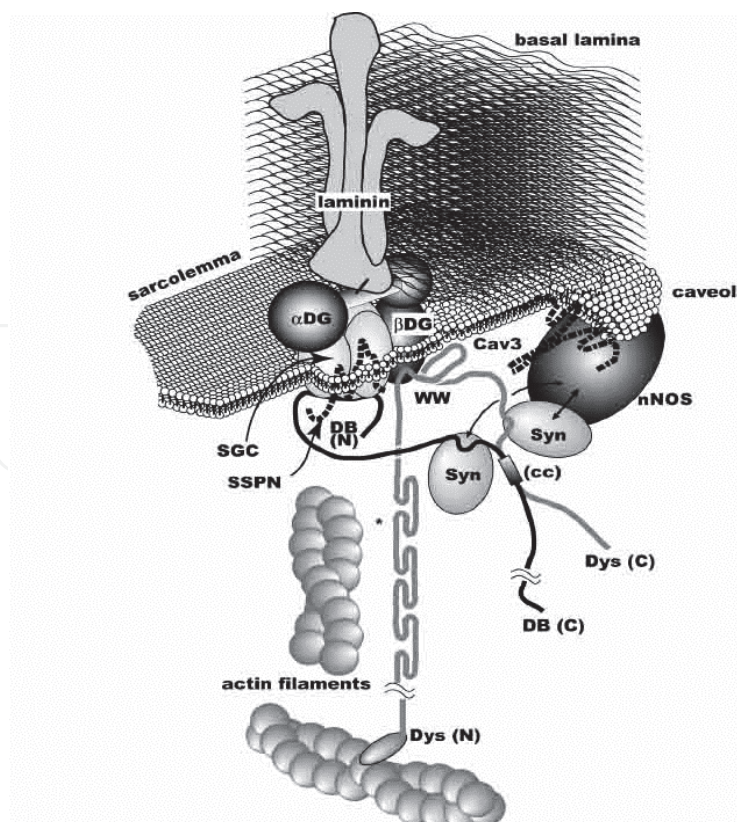

Fig. 1. Dystrophin-glycoprotein complex.

Molecular structure of the dystrophin-glycoprotein complex and related proteins superimposed on the sarcolemma and subsarcolemmal actin network (redrawn from Yoshida *et al.* (Yoshida et al., 2000), with modifications). cc, coiled-coil motif on dystrophin (Dys) and dystrobrevin (DB); SGC, sarcoglycan complex; SSPN, sarcospan; Syn, syntrophin; Cav3, caveolin-3; N and C, the N and C termini, respectively; G, G-domain of laminin; asterisk indicates the actin-binding site on the dystrophin rod domain; WW, WW domain.

The approximately 2.5-megabase *dystrophin* gene is the largest gene identified to date, and because of its size, it is susceptible to a high sporadic mutation rate. Absence of dystrophin and the dystrophin-glycoprotein complex (DGC) from the sarcolemma leads to severe muscle wasting (Figure 1). Whereas DMD is characterized by the absence of functional protein, Becker muscular dystrophy, which is commonly caused by in-frame deletions of the *dystrophin* gene, results in the synthesis of a partially functional protein.

2. Gene-replacement strategies using virus vectors

2.1 Choice of vector

Successful therapy for DMD requires the restoration of dystrophin protein in skeletal and cardiac muscles. While various viral vectors have been considered for the delivery of genes to muscle fibers, the adeno-associated virus (AAV)-based vector is emerging as the gene transfer vehicle with the most potential for use in DMD gene therapies. The advantages of the AAV vector include the lack of disease associated with a wild-type virus, the ability to transduce non-dividing cells, and the long-term expression of the delivered transgenes (Okada et al., 2002). Serotypes 1, 6, 8 and 9 of recombinant AAV (rAAV) exhibit a potent tropism for striated muscles (Inagaki et al., 2006). Since a 5-kb genome is considered to be the upper limit for a single AAV virion, a series of rod-truncated micro-dystrophin genes is used in this treatment (Yuasa et al., 1998).

Due to ingenious cloning and preparation techniques, adenovirus vectors are efficient delivery systems of episomal DNA into eukaryotic cell nuclei (Okada et al., 1998). The utility of adenovirus vectors has been increased by capsid modifications that alter tropism, and by the generation of hybrid vectors that promote chromosomal insertion (Okada et al., 2004). Also, gutted adenovirus vectors devoid of all adenoviral genes allow for the insertion of large transgenes, and trigger fewer cytotoxic and immunogenic effects than do those only deleted in the E1 regions (from bases 343 to 2270) (Hammerschmidt, 1999). Human artificial chromosomes (HACs) have the capacity to deliver a large gene (roughly 6-10 megabases) into host cells without integrating the gene into the host genome, thereby preventing the possibility of insertional mutagenesis and genomic instability (Hoshiya et al., 2008).

A goal in clinical gene therapy is to develop gene transfer vehicles that can integrate exogenous therapeutic genes at specific chromosomal loci, so that insertional oncogenesis is prevented. AAV can insert its genome into a specific locus, designated AAVS1, on chromosome 19 of the human genome (Kotin et al., 1992). The AAV Rep78/68 proteins and the Rep78/68-binding sequences are the trans- and cis-acting elements needed for this reaction. A dual high-capacity adenovirus-AAV hybrid vector with full-length human dystrophin-coding sequences flanked by AAV integration-enhancing elements was tested for targeted integration (Goncalves et al., 2005).

Gene correction is a process whereby sequence alterations in genes can be corrected by homologous recombination-mediated gene conversion between the recipient target locus

and a donor construct encoding the correct sequence (Klug, 2005). The introduction of a corrective sequence together with a site-specific nuclease to induce a double-stranded break (DSB) at sites responsible for monogenic disorders would activate gene correction. Pairs of designated zinc-finger protein with tandem DNA binding sites fused to the cleavage domain of the Fok1 protein were introduced into model systems or cell lines and produced corrections in 10–30% of cases tested (Porteus and Baltimore, 2003).

2.2 Modification of the dystrophin gene and promoter

Due to the large deletion in its genome, the gutted adenovirus vector can package 14-kb of full-length *dystrophin* cDNA. Multiple proximal muscles of seven-day-old utrophin/dystrophin double knockout mice (*dko* mice), which typically show symptoms similar to human DMD, were effectively transduced with the gutted adenovirus bearing full-length murine *dystrophin* cDNA (Kawano et al., 2008). However, further improvements are needed to regulate the virus-associated host immune response before clinical trials can be performed.
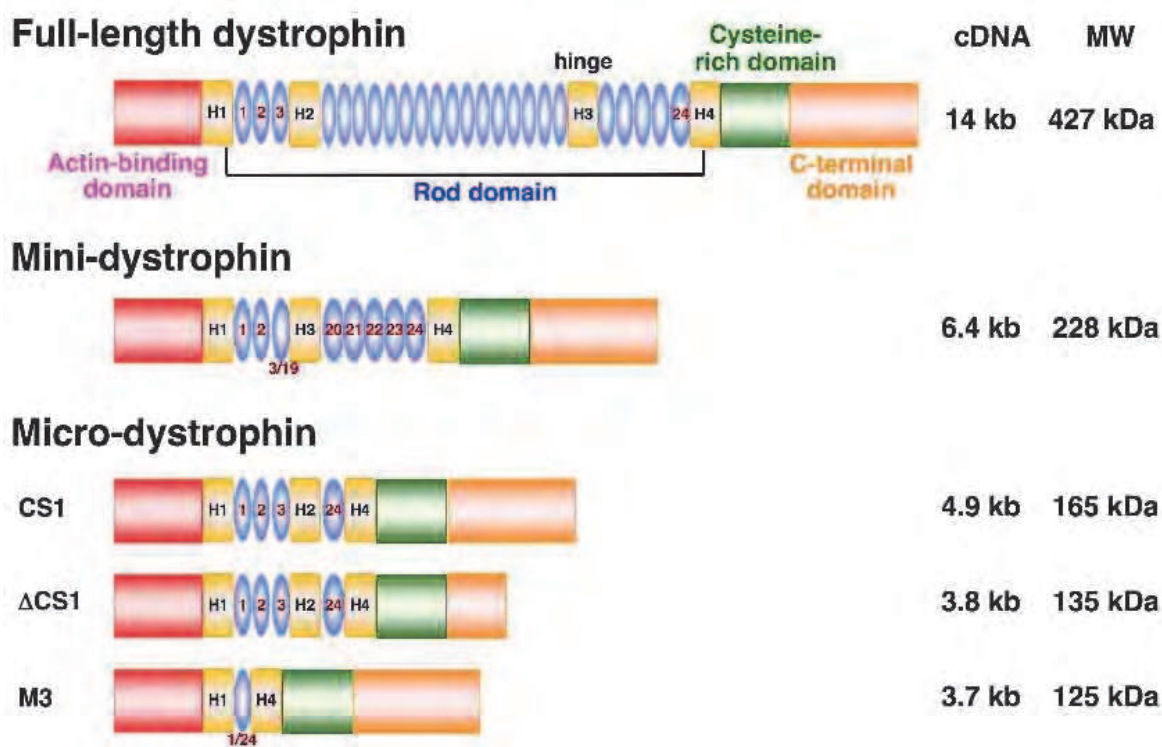

Fig. 2. Structures of full-length and truncated dystrophin.

Helper-dependent adenovirus vector can package 14-kb of full-length dystrophin cDNA because of the large-sized deletion in its genome. A mini-dystrophin is cloned from a patient with Becker muscular dystrophy, which is caused by in-frame deletions resulting in the synthesis of partially functional protein. A series of truncated micro-dystrophin cDNAs harboring only four rod repeats with hinge 1, 2, and 4 (CS1); the same components, except that the C-terminal domain is deleted (delta CS1); or one rod repeat with hinge 1 and 4 (M3), are constructed to be packaged in the AAV vector.

A series of truncated *dystrophin* cDNAs containing rod repeats with hinge 1, 2, and 4 were constructed (Figure 2) (Yuasa et al., 1998). Although AAV vectors are too small to package

the full-length *dystrophin* cDNA, AAV vector-mediated gene therapy using a rod-truncated *dystrophin* gene provides a promising approach (Wang et al., 2000). The structure and, particularly, the length of the rod are crucial for the function of micro-dystrophin (Sakamoto et al., 2002). An AAV type 2 vector expressing micro-dystrophin (DeltaCS1) under the control of a muscle-specific MCK promoter was injected into the tibialis anterior (TA) muscles of dystrophin-deficient *mdx* mice (Yoshimura et al., 2004), and resulted in extensive and long-term expression of micro-dystrophin that exhibited improved force generation.

The impact of codon usage optimization on micro-dystrophin expression and function in the *mdx* mouse was assessed to compare the function of two different configurations of codon-optimized *micro-dystrophin* genes under the control of a muscle-restrictive promoter (Spc5-12) (Foster et al., 2008). Codon optimization of micro-dystrophin significantly increased micro-dystrophin mRNA and protein levels after intramuscular and systemic administration of plasmid DNA or rAAV8. By randomly assembling myogenic regulatory elements into synthetic promoter recombinant libraries, several artificial promoters were isolated whose transcriptional potencies greatly exceed those of natural myogenic and viral gene promoters (Li et al., 1999).

2.3 Use of surrogate genes

An approach using a surrogate gene would bypass the potential immune responses associated with the delivery of exogenous dystrophin. Methods to increase expression of utrophin, a dystrophin paralog, show promise as a treatment for DMD. rAAV6 harboring a murine codon-optimized micro-utrophin transgene was intravenously administered into adult *dko* mice to alleviate the pathophysiological abnormalities (Odom et al., 2008). The paralogous gene efficiently acted as a surrogate for *dystrophin*. Myostatin is extensively documented as being a negative regulator of muscle growth. Systemic gene delivery of myostatin propeptide, a natural inhibitor of myostatin, enhanced body-wide skeletal muscle growth in both normal and *mdx* mice (Qiao et al., 2008). The delivery of various growth factors, such as insulin-like growth factor-I (IGF-I), has been successful in promoting skeletal muscle regeneration after injury (Schertzer and Lynch, 2006).

Matrix metalloproteinases (MMPs) are key regulatory molecules in the formation, remodeling and degradation of all extracellular matrix (ECM) components in pathological processes. MMP-9 is involved predominantly in the inflammatory process during muscle degeneration (Fukushima et al., 2007). In contrast, MMP-2 is associated with ECM remodeling during muscle regeneration and fiber growth.

3. AAV-mediated transduction of animal models

3.1 Vector production

When adenovirus helper plasmid is co-transfected into human embryonic kidney 293 cells along with a vector plasmid encoding the AAV vector and an AAV packaging plasmid harboring *rep-cap* genes, the AAV vector is produced as efficiently as when using adenovirus infection. A large-scale transduction method to produce AAV vectors with an active gassing system makes use of large culture vessels for labor- and cost-effective transfection in a closed system. Samples containing vector particles are further purified with a two-tier CsCl gradient or dual ion-exchange chromatography to obtain highly purified vector particles.
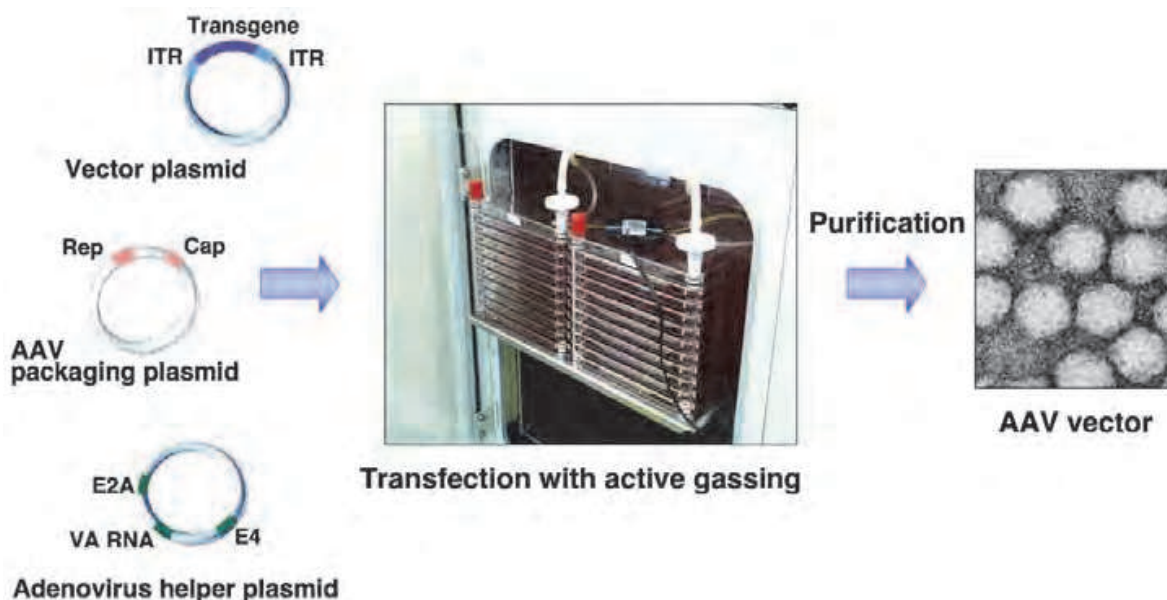
Fig. 3. A scalable triple plasmid transfection system using active gassing.

To gain acceptance as a medical treatment with a dose of over 1×10^{13} genome copies (g.c.)/kg body weight, AAV vectors require a scalable and economical production method. A production protocol of AAV vectors in the absence of a helper virus (Matsushita et al., 1998) is widely employed for triple plasmid transduction of human embryonic kidney 293 cells (Okada et al., 2002). The adenovirus regions that mediate AAV vector replication (namely, the VA, E2A and E4 regions) were assembled into a helper plasmid. When this helper plasmid is co-transfected into human embryonic kidney 293 cells along with plasmids encoding the AAV vector genome and *rep-cap* genes, the AAV vector is produced as efficiently as when using adenovirus infection (Figure 3). Importantly, contamination of most adenovirus proteins can be avoided in AAV vector stock made by this helper virus-free method. Samples containing vector particles are further purified with a two-tier CsCl gradient or dual ion-exchange chromatography to obtain highly purified vector particles (Okada et al., 2002).

Despite improvements in vector production, including the development of packaging cell lines expressing Rep/Cap, and of methods that induce the expression and regulation of Rep/Cap (Okada et al., 2001), maintaining such cell lines remains difficult, as the early expression of Rep proteins is toxic to cells. A scalable method, using active gassing and large culture vessels, was developed to transfect rAAV in a closed system, in a labor- and cost-effective manner (Okada et al., 2005). This vector production system achieved a yield of more than 5×10^{13} g.c./flask by improving gas exchange to maintain the physiological pH in the culture medium. Recent developments in ion-exchange chromatography also suggest that vector production using transduction culture supernatant would be compatible with current good manufacturing practice and production on an industrial scale (Okada et al., 2009). Moreover, AAV vector production in insect cells would be compatible with current good manufacturing practice production on an industrial scale (Cecchini et al., 2008).

3.2 Animal models for the gene transduction study

Dystrophin-deficient canine X-linked muscular dystrophy was found in a golden retriever with a 3' splice-site point mutation in intron 6 (Valentine et al., 1988). The clinical and

pathological characteristics of dystrophic dogs are more similar to those of DMD patients than are those of *mdx* mice. A beagle-based model of canine X-linked muscular dystrophy, which is smaller and easier to handle than the golden retriever-based muscular dystrophy dog (GRMD) model, has been established in Japan, and is referred to as CXMD$_J$ (Shimatsu et al., 2005). The limb and temporal muscles of CXMD$_J$ are affected by two-month-old, which is the age corresponding to the second peak of serum creatine kinase.

Interestingly, we found extensive lymphocyte-mediated immune responses to rAAV2-*lacZ* after direct intramuscular injection into CXMD$_J$ dogs, despite successful delivery of the same viral construct into mouse skeletal muscle (Yuasa et al., 2007). In contrast to rAAV2, rAAV8-mediated transduction of canine skeletal muscles produced significantly higher transgene expression with less lymphocyte proliferation than rAAV2 (Ohshima et al., 2008).

It is increasingly important to develop strategies to treat DMD that consider the effect on cardiac muscle. The pathology of the conduction system in CXMD$_J$ was analyzed to establish the therapeutic target for DMD (Urasawa et al., 2008). Although dystrophic changes of the ventricular myocardium were not evident at the age of 1 to 13 months, Purkinje fibers showed remarkable vacuolar degeneration when dogs were as young as four-months-old. Furthermore, degeneration of Purkinje fibers was coincident with overexpression of Dp71 at the sarcolemma. The degeneration of Purkinje fibers could be associated with the distinct deep Q waves present in ECGs and the fatal arrhythmias seen in cases of dystrophin deficiency (Urasawa et al., 2008).

3.3 Immunological Issues of rAAV

Neo-antigens introduced by AAV vectors evoke significant immune reactions in DMD muscle, since increased permeability of sarcolemma allows leakage of the transgene products from the dystrophin-deficient muscle fibers (Yuasa et al., 2002). rAAV2 transfer into skeletal muscles of normal dogs resulted in low and transient expression, together with intense cellular infiltration, and the marked activation of cellular and humoral immune responses (Yuasa et al., 2007). Furthermore, an *in vitro* interferon-gamma release assay showed that canine splenocytes respond to immunogens or mitogens more strongly than do murine splenocytes. In fact, co-administration of immunosuppressants, cyclosporine (CSP) and mycophenolate mofetil (MMF) improved rAAV2 transduction. The AAV2 capsids can induce a cellular immune response via MHC class I antigen presentation with a cross-presentation pathway, and rAAV2 has also been proposed to have an effect on human dendritic cells (DCs). In contrast, other serotypes, such as rAAV8, induced T-cell activation to a lesser degree (Ohshima et al., 2008). Immunohistochemical analysis revealed that the rAAV2-injected muscles showed higher rates of infiltration of CD4$^+$ and CD8$^+$ T lymphocytes in the endomysium than the rAAV8-injected muscles (Ohshima et al., 2008).

Resident antigen-presenting cells, such as DCs, myoblasts, myotubes and regenerating immature myofibers, might play a role in the immune response. A recent study also showed that mRNA levels of MyD88 and co-stimulating factors, such as CD80, CD86 and type I interferon, are elevated in both rAAV2- and rAAV8-transduced dog DCs *in vitro* (Ohshima et al., 2008). A brief course of immunosuppression with a combination of anti-thymocyte globulin (ATG), CSP and MMF was effective in permitting AAV6-mediated, long-term and robust expression of a canine micro-dystrophin in the skeletal muscle of a dog DMD model (Wang et al., 2007).

3.4 Intravascular vector administration by limb perfusion

Although recent studies suggest that vectors based on AAV are capable of body-wide transduction in rodents, translating this finding into large animals remains a challenge. Intravascular delivery can be performed as a form of limb perfusion, which might bypass the immune activation of DCs in the injected muscle (Ohshima et al., 2008). We performed limb perfusion-assisted intravenous administration of rAAV8-lacZ into the hind limb of normal dogs and rAAV8-micro-dystrophin into the hind limb of CXMD$_J$ dogs (Figure 4) (Ohshima et al., 2008). Administration of rAAV8-micro-dystrophin by limb perfusion produced extensive transgene expression in the distal limb muscles of CXMD$_J$ dogs without obvious immune responses for as long as eight weeks after injection.
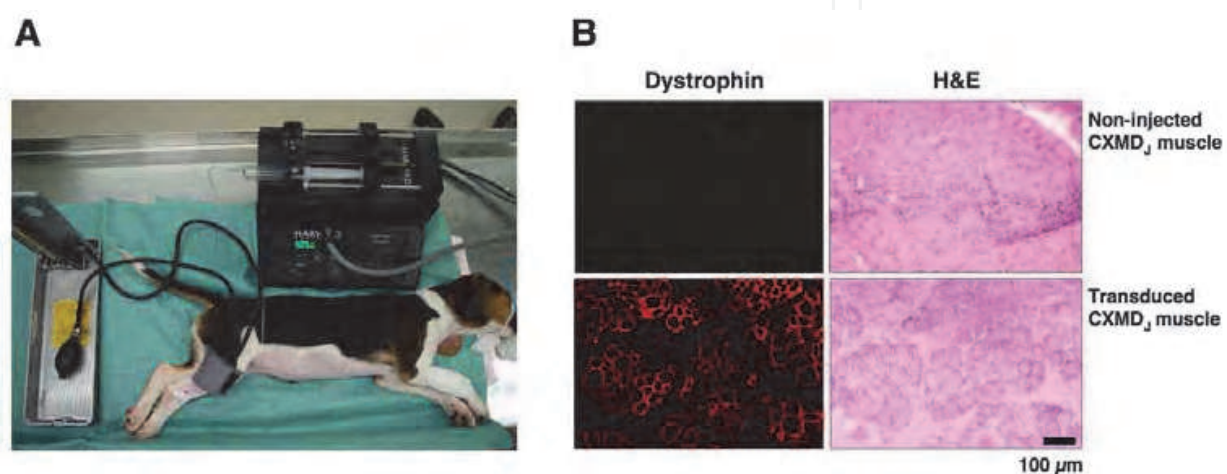

Fig. 4. Intravascular vector administration by limb perfusion.

(A) A blood pressure cuff is applied just above the knee of an anesthetized CXMD$_J$ dog. A 24-gauge intravenous catheter is inserted into the lateral saphenous vein, connected to a three-way stopcock, and flushed with saline. With a blood pressure cuff inflated to over 300 mmHg, saline (2.6 ml/kg) containing papaverine (0.44 mg/kg, Sigma-Aldrich, St. Louis, MO) and heparin (16 U/kg) is injected by hand over a 10 second period. The three-way stopcock is connected to a syringe containing rAAV8 expressing micro-dystrophin (1 x 10^{14} vg/kg, 3.8 ml/kg). The syringe is placed in a PHD 2000 syringe pump (Harvard Apparatus, Edenbridge, UK). Five minutes after the papaverine/heparin injection, rAAV8 is injected at a rate of 0.6 ml/sec. (B) Administration of rAAV8-micro-dystrophin by limb perfusion produces extensive transgene expression in the distal limb muscles of CXMD$_J$ dogs without obvious immune responses at four weeks after injection.

3.5 Global muscle therapies

In comparison with fully dystrophin-deficient animals, targeted transgenic repair of skeletal muscle, but not cardiac muscle, paradoxically elicits a five-fold increase in cardiac injury and dilated cardiomyopathy (Townsend et al., 2008). Because the dystrophin-deficient heart is highly sensitive to increased stress, increased activity by the repaired skeletal muscle provides the stimulus for heightened cardiac injury and heart remodeling. In contrast, a single intravenous injection of AAV9 vector expressing micro-dystrophin efficiently transduces the entire heart in neonatal *mdx* mice, thereby ameliorating cardiomyopathy (Bostick et al., 2008).

Since a number of muscular dystrophy patients can be identified through newborn screening, neonatal transduction may lead to an effective early intervention in DMD patients. After a single intravenous injection, robust skeletal muscle transduction with AAV9 vector throughout the body was observed in neonatal dogs (Yue et al., 2008). Systemic transduction was achieved in the absence of pharmacological intervention or immune suppression and lasted for at least six months, whereas cardiac muscle was barely transduced in the dogs.

4. Safety and potential impact of clinical trials

The initial clinical studies lay the foundation for future studies, providing important information about vector dose, viral serotype selection, and immunogenicity in humans. The first virus-mediated gene transfer for muscle disease was carried out for limb-girdle muscular dystrophy type 2D using rAAV1. The study, consisting of intramuscular injection of virus into a single muscle, was discharged to establish the safety of this procedure in phase I clinical trials (Rodino-Klapac et al., 2007). The first clinical gene therapy trial for DMD began in March 2006 (Mendell et al., 2010). This was a Phase I/IIa study in which an AAV vector was used to deliver micro-dystrophin to the biceps of boys with DMD. The study was conducted on six boys with DMD, each of whom was transduced with mini-dystrophin genes in a muscle of one arm in the absence of serious adverse events. Interestingly, dystrophin-specific T cells were detected after treatment, providing evidence of transgene expression. The potential for T-cell immunity to self and nonself dystrophin epitopes should be considered in designing and monitoring experimental therapies for this disease.

While low immunogenicity was considered a major strength supporting the use of rAAV in clinical trials, a number of observations have recently provided a more balanced view of this procedure (Manno et al., 2006). An obvious barrier to AAV transduction is the presence of circulating neutralizing antibodies that prevent the virion from binding to its cellular receptor (Scallan et al., 2006). This potential threat can be reduced by prescreening patients for AAV serotype-specific neutralizing antibodies or by performing procedures such as plasmapheresis before gene transfer. Another challenge recently revealed is the development of a cell-mediated cytotoxic T-cell (CTL) response to AAV capsid peptides. In the human factor IX gene therapy trial in which rAAV was delivered to the liver, only short-term transgene expression was achieved and levels of therapeutic protein declined to baseline levels 10 weeks after vector infusion (Manno et al., 2006). This was accompanied by elevation of serum transaminase levels and a CTL response toward specific AAV capsid peptides. To overcome this response, transient immunosuppression may be required until AAV capsids are completely cleared. Additional findings suggest that T-cell activation requires AAV2 capsid binding to the heparan sulfate proteoglycan (HSPG) receptor, which would permit virion shuttling into a DC pathway, as cross-presentation (Vandenberghe et al., 2006). Exposure to vectors from other AAV clades, such as AAV8, did not activate capsid-specific T-cells.

5. Challenges and limitations of related strategies

5.1 Design of read-through drugs

To suppress premature stop codon mutations, treatments involving aminoglycosides and other agents have been attempted. PTC124, a novel drug capable of suppressing premature

termination and selectively inducing ribosomal read-through of premature, but not normal, termination codons, was recently identified using nonsense-containing reporters (Welch et al., 2007). The selectivity of PTC124 for premature termination codons, its oral bioavailability and its pharmacological properties indicate that this drug may have broad clinical potential for the treatment of a large group of genetic disorders with limited or no therapeutic options.

5.2 Modification of mRNA splicing

By inducing the skipping of specific exons during mRNA splicing, antisense compounds against exonic and intronic splicing regulatory sequences were shown to correct the open reading frame of the DMD gene and thus to restore truncated yet functional dystrophin expression *in vitro* (Takeshima et al., 1995). Intravenous infusion of an antisense phosphorothioate oligonucleotide created an in-frame *dystrophin* mRNA via exon skipping in a 10-year-old DMD patient possessing an out-of-frame exon 20 deletion of the *dystrophin* gene (Takeshima et al., 2006). Moreover, the adverse-event profile and local dystrophin-restoring effect of a single intramuscular injection of an antisense 2'-O-methyl phosphorothioate oligonucleotide, PRO051, in patients with DMD were explored (van Deutekom et al., 2007). Four patients received a dose of 0.8 mg of PRO051 in the TA muscle. Each patient showed specific skipping of exon 51 of dystrophin in 64 to 97% of myofibers, without clinically apparent adverse side effects.

The efficacy and toxicity of intravenous injection of stable morpholino phosphorodiamidate (morpholino)-induced exon skipping were tested using CXMD$_J$ dogs, and widespread rescue of dystrophin expression to therapeutic levels was observed (Yokota et al., 2009). Furthermore, a morpholino oligomer with a designed cell-penetrating peptide can efficiently target a mutated *dystrophin* exon in cardiac muscles (Wu et al., 2008).

Long-term benefits can be obtained through the use of viral vectors expressing antisense sequences against regions within *dystrophin* gene. The sustained production of dystrophin at physiological levels in entire groups of muscles as well as the correction of muscular dystrophy were achieved by treatment with exon-skipping AAV1-U7 (Goyenvalle et al., 2004).

5.3 *Ex vivo* gene therapy

Transplantation of genetically corrected autologous myogenic cells is a possible treatment for DMD. Freshly isolated satellite cells transduced with lentiviral vectors expressing micro-dystrophin were transplanted into the TA muscles of *mdx* mice, and these cells efficiently contributed to the regeneration of muscles with micro-dystrophin expression at the sarcolemma (Ikemoto et al., 2007). Mesoangioblasts are vessel-associated stem cells and might be candidates for future stem cell therapy for DMD (Sampaolesi et al., 2006). Intra-arterial delivery of wild-type canine mesoangioblasts resulted in the extensive recovery of *dystrophin* expression, normal muscle morphology and function in the GRMD. Multipotent mesenchymal stromal cells (MSCs) are less immunogenic and have the potential to differentiate and display a myogenic phenotype (Dezawa et al., 2005).

6. Future perspectives

6.1 Pharmacological Intervention

The use of a histone deacetylase (HDAC) inhibitor would likely enhance the utility of rAAV-mediated transduction strategies in the clinic (Okada et al., 2006). In contrast to adenovirus-

mediated transduction, the improved transduction with rAAV induced by the HDAC inhibitor is due to enhanced transgene expression rather than to increased viral entry. The enhanced transduction was proposed to be related to the histone-associated chromatin form of the rAAV concatemer in transduced cells. Since various HDAC inhibitors are currently being tested in clinical trials for many diseases, the use of such agents in rAAV-mediated DMD gene therapy is theoretically and practically reasonable.

6.2 Capsid modification

A DNA shuffling-based approach for developing cell type-specific vectors is an intriguing possibility to achieve altered tropism. Capsid genomes of AAV serotypes 1-9 were randomly reassembled using PCR to generate a chimeric capsid library (Li et al., 2008). A single infectious clone (chimeric-1829) containing genome fragments from AAV1, 2, 8, and 9 was isolated from an integrin minus hamster melanoma cell line previously shown to have low permissiveness to AAV.

7. Conclusion

DMD remains an untreatable genetic disease that severely limits motility and life expectancy in affected children. The systemic delivery of rAAV to transduce truncated dystrophin is predicted to ameliorate the symptoms of DMD patients in the future. To translate gene transduction technologies into clinical practice, development of an effective delivery system with improved vector constructs as well as efficient immunological modulation must be established. A novel protocol that considers all of these issues would help improve the therapeutic benefits of DMD gene therapy.

8. Acknowledgment

9. References

Bostick, B.; Yue, Y.; Lai, Y.; Long, C.; Li, D. & Duan, D. (2008). Adeno-associated virus serotype-9 microdystrophin gene therapy ameliorates electrocardiographic abnormalities in mdx mice. *Hum Gene Ther*, Vol.19, No.8, pp. 851-856, ISSN 1043-0342

Cecchini, S.; Negrete, A. & Kotin, R.M. (2008). Toward exascale production of recombinant adeno-associated virus for gene transfer applications. *Gene Ther*, Vol.15, No.11, pp. 823-830, ISSN 0969-7128

Dezawa, M.; Ishikawa, H.; Itokazu, Y.; Yoshihara, T.; Hoshino, M.; Takeda, S.; Ide, C. & Nabeshima, Y. (2005). Bone marrow stromal cells generate muscle cells and repair muscle degeneration. *Science*, Vol.309, No.5732, pp. 314-317, ISSN 0193-4511

Emery, A.E. (1991). Population frequencies of inherited neuromuscular diseases--a world survey. *Neuromuscul Disord*, Vol.1, No.1, pp. 19-29, ISSN 0960-8966

Foster, H.; Sharp, P.S.; Athanasopoulos, T.; Trollet, C.; Graham, I.R.; Foster, K.; Wells, D.J. & Dickson, G. (2008). Codon and mRNA sequence optimization of microdystrophin transgenes improves expression and physiological outcome in dystrophic mdx mice following AAV2/8 gene transfer. *Mol Ther*, Vol.16, No.11, pp. 1825-1832, ISSN 1525-0016

Fukushima, K.; Nakamura, A.; Ueda, H.; Yuasa, K.; Yoshida, K.; Takeda, S. & Ikeda, S. (2007). Activation and localization of matrix metalloproteinase-2 and -9 in the skeletal muscle of the muscular dystrophy dog (CXMDJ). *BMC Musculoskelet Disord*, Vol.8, pp. 54, ISSN 1471-2474

Goncalves, M.A.; Van Nierop, G.P.; Tijssen, M.R.; Lefesvre, P.; Knaan-Shanzer, S.; Van Der Velde, I.; Van Bekkum, D.W.; Valerio, D. & De Vries, A.A. (2005). Transfer of the full-length dystrophin-coding sequence into muscle cells by a dual high-capacity hybrid viral vector with site-specific integration ability. *J Virol*, Vol.79, No.5, pp. 3146-3162, ISSN 1098-5514

Goyenvalle, A.; Vulin, A.; Fougerousse, F.; Leturcq, F.; Kaplan, J.C.; Garcia, L. & Danos, O. (2004). Rescue of dystrophic muscle through U7 snRNA-mediated exon skipping. *Science*, Vol.306, No.5702, pp. 1796-1799, ISSN 0193-4511

Hammerschmidt, D.E. (1999). Development of a gutless vector. *J Lab Clin Med*, Vol.134, No.3, pp. C3, ISSN 0022-2143

Hoffman, E.P.; Brown, R.H., Jr. & Kunkel, L.M. (1987). Dystrophin: the protein product of the Duchenne muscular dystrophy locus. *Cell*, Vol.51, No.6, pp. 919-928, 0092-8674

Hoshiya, H.; Kazuki, Y.; Abe, S.; Takiguchi, M.; Kajitani, N.; Watanabe, Y.; Yoshino, T.; Shirayoshi, Y.; Higaki, K.; Messina, G.; Cossu, G. & Oshimura, M. (2008). A highly Stable and Nonintegrated Human Artificial Chromosome (HAC) Containing the 2.4 Mb Entire Human Dystrophin Gene. *Mol Ther*, pp. 309-317, ISSN 1525-0016

Ikemoto, M.; Fukada, S.; Uezumi, A.; Masuda, S.; Miyoshi, H.; Yamamoto, H.; Wada, M.R.; Masubuchi, N.; Miyagoe-Suzuki, Y. & Takeda, S. (2007). Autologous transplantation of SM/C-2.6(+) satellite cells transduced with micro-dystrophin CS1 cDNA by lentiviral vector into mdx mice. *Mol Ther*, Vol.15, No.12, pp. 2178-2185, ISSN 1525-0016

Inagaki, K.; Fuess, S.; Storm, T.A.; Gibson, G.A.; Mctiernan, C.F.; Kay, M.A. & Nakai, H. (2006). Robust systemic transduction with AAV9 vectors in mice: efficient global cardiac gene transfer superior to that of AAV8. *Mol Ther*, Vol.14, No.1, pp. 45-53, ISSN 1525-0016

Kawano, R.; Ishizaki, M.; Maeda, Y.; Uchida, Y.; Kimura, E. & Uchino, M. (2008). Transduction of full-length dystrophin to multiple skeletal muscles improves motor performance and life span in utrophin/dystrophin double knockout mice. *Mol Ther*, Vol.16, No.5, pp. 825-831, ISSN 1525-0016

Klug, A. (2005). Towards therapeutic applications of engineered zinc finger proteins. *FEBS Lett*, Vol.579, No.4, pp. 892-894, ISSN 0014-5793

Kotin, R.M.; Linden, R.M. & Berns, K.I. (1992). Characterization of a preferred site on human chromosome 19q for integration of adeno-associated virus DNA by non-homologous recombination. *Embo J*, Vol.11, No.13, pp. 5071-5078, ISSN 0261-4189

Li, W.; Asokan, A.; Wu, Z.; Van Dyke, T.; Diprimio, N.; Johnson, J.S.; Govindaswamy, L.; Agbandje-Mckenna, M.; Leichtle, S.; Redmond, D.E., Jr.; Mccown, T.J.; Petermann, K.B.; Sharpless, N.E. & Samulski, R.J. (2008). Engineering and selection of shuffled AAV genomes: a new strategy for producing targeted biological nanoparticles. *Mol Ther*, Vol.16, No.7, pp. 1252-1260, ISSN 1525-0016

Li, X.; Eastman, E.M.; Schwartz, R.J. & Draghia-Akli, R. (1999). Synthetic muscle promoters: activities exceeding naturally occurring regulatory sequences. *Nat Biotechnol*, Vol.17, No.3, pp. 241-245, ISSN 1087-0156

Manno, C.S.; Pierce, G.F.; Arruda, V.R.; Glader, B.; Ragni, M.; Rasko, J.J.; Ozelo, M.C.; Hoots, K.; Blatt, P.; Konkle, B.; Dake, M.; Kaye, R.; Razavi, M.; Zajko, A.; Zehnder, J.; Rustagi, P.K.; Nakai, H.; Chew, A.; Leonard, D.; Wright, J.F.; Lessard, R.R.; Sommer, J.M.; Tigges, M.; Sabatino, D.; Luk, A.; Jiang, H.; Mingozzi, F.; Couto, L.; Ertl, H.C.; High, K.A. & Kay, M.A. (2006). Successful transduction of liver in hemophilia by AAV-Factor IX and limitations imposed by the host immune response. *Nat Med*, Vol.12, No.3, pp. 342-347, ISSN 1078-8956

Matsushita, T.; Elliger, S.; Elliger, C.; Podsakoff, G.; Villarreal, L.; Kurtzman, G.J.; Iwaki, Y. & Colosi, P. (1998). Adeno-associated virus vectors can be efficiently produced without helper virus. *Gene Ther*, Vol.5, No.7, pp. 938-945, ISSN 0969-7128

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. (2010). Dystrophin immunity in Duchenne's muscular dystrophy. *N Engl J Med*, Vol.363, No.15, pp. 1429-1437, ISSN 0028-4793

Odom, G.L.; Gregorevic, P.; Allen, J.M.; Finn, E. & Chamberlain, J.S. (2008). Microutrophin delivery through rAAV6 increases lifespan and improves muscle function in dystrophic dystrophin/utrophin-deficient mice. *Mol Ther*, Vol.16, No.9, pp. 1539-1545, ISSN 1525-0016

Ohshima, S.; Shin, J.H.; Yuasa, K.; Nishiyama, A.; Kira, J.; Okada, T. & Takeda, S. (2008). Transduction Efficiency and Immune Response Associated With the Administration of AAV8 Vector Into Dog Skeletal Muscle. Mol Ther, pp. 73-91, ISSN 1525-0016

Okada, T.; Caplen, N.J.; Ramsey, W.J.; Onodera, M.; Shimazaki, K.; Nomoto, T.; Ajalli, R.; Wildner, O.; Morris, J.; Kume, A.; Hamada, H.; Blaese, R.M. & Ozawa, K. (2004). In situ generation of pseudotyped retroviral progeny by adenovirus-mediated transduction of tumor cells enhances the killing effect of HSV-tk suicide gene therapy in vitro and in vivo. *J Gene Med*, Vol.6, No.3, pp. 288-299, ISSN 1521-2254

Okada, T.; Mizukami, H.; Urabe, M.; Nomoto, T.; Matsushita, T.; Hanazono, Y.; Kume, A.; Tobita, K. & Ozawa, K. (2001). Development and characterization of an antisense-mediated prepackaging cell line for adeno-associated virus vector production. *Biochem Biophys Res Commun*, Vol.288, No.1, pp. 62-68., ISSN 1090-2104

Okada, T.; Nomoto, T.; Yoshioka, T.; Nonaka-Sarukawa, M.; Ito, T.; Ogura, T.; Iwata-Okada, M.; Uchibori, R.; Shimazaki, K.; Mizukami, H.; Kume, A. & Ozawa, K. (2005). Large-scale production of recombinant viruses by use of a large culture vessel with active gassing. *Hum Gene Ther*, Vol.16, No.10, pp. 1212-1218, ISSN 1043-0342

Okada, T.; Nonaka-Sarukawa, M.; Uchibori, R.; Kinoshita, K.; Hayashita-Kinoh, H.; Nitahara-Kasahara, Y.; Takeda, S. & Ozawa, K. (2009). Scalable purification of adeno-associated virus serotype 1 (AAV1) and AAV8 vectors, using dualion-exchange adsorptive membranes. *Hum Gene Ther*, Vol.20, No.9, pp. 1013-1021, ISSN 1043-0342

Okada, T.; Ramsey, J.; Munir, J.; Wildner, O. & Blaese, M. (1998). Efficient directional cloning of recombinant adenovirus vectors using DNA-protein complex. *Nucleic Acids Res.*, Vol.26, No.8, pp. 1947-1950, ISSN 0305-1048

Okada, T.; Shimazaki, K.; Nomoto, T.; Matsushita, T.; Mizukami, H.; Urabe, M.; Hanazono, Y.; Kume, A.; Tobita, K.; Ozawa, K. & Kawai, N. (2002). Adeno-associated viral vector-mediated gene therapy of ischemia-induced neuronal death. *Methods Enzymol*, Vol.346, pp. 378-393., ISSN 0076-6879

Okada, T.; Uchibori, R.; Iwata-Okada, M.; Takahashi, M.; Nomoto, T.; Nonaka-Sarukawa, M.; Ito, T.; Liu, Y.; Mizukami, H.; Kume, A.; Kobayashi, E. & Ozawa, K. (2006). A histone deacetylase inhibitor enhances recombinant adeno-associated virus-mediated gene expression in tumor cells. *Mol Ther*, Vol.13, No.4, pp. 738-746, ISSN 1525-0016

Porteus, M.H. & Baltimore, D. (2003). Chimeric nucleases stimulate gene targeting in human cells. *Science*, Vol.300, No.5620, pp. 763, ISSN 0193-4511

Qiao, C.; Li, J.; Jiang, J.; Zhu, X.; Wang, B.; Li, J. & Xiao, X. (2008). Myostatin propeptide gene delivery by adeno-associated virus serotype 8 vectors enhances muscle growth and ameliorates dystrophic phenotypes in mdx mice. *Hum Gene Ther*, Vol.19, No.3, pp. 241-254, ISSN 1043-0342

Rodino-Klapac, L.R.; Chicoine, L.G.; Kaspar, B.K. & Mendell, J.R. (2007). Gene therapy for duchenne muscular dystrophy: expectations and challenges. *Arch Neurol*, Vol.64, No.9, pp. 1236-1241, ISSN 0003-9942

Sakamoto, M.; Yuasa, K.; Yoshimura, M.; Yokota, T.; Ikemoto, T.; Suzuki, M.; Dickson, G.; Miyagoe-Suzuki, Y. & Takeda, S. (2002). Micro-dystrophin cDNA ameliorates dystrophic phenotypes when introduced into mdx mice as a transgene. *Biochem Biophys Res Commun*, Vol.293, No.4, pp. 1265-1272, ISSN 1090-2104

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. (2006). Mesoangioblast stem cells ameliorate muscle function in dystrophic dogs. *Nature*, Vol.444, No.7119, pp. 574-579, ISSN 0028-0836
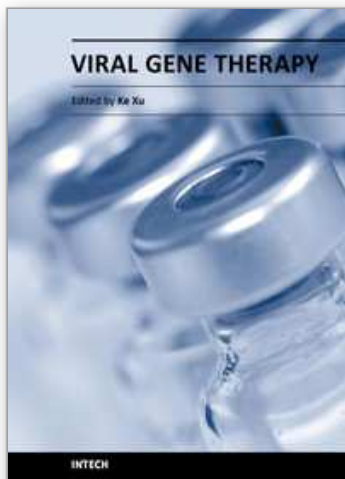
Scallan, C.D.; Jiang, H.; Liu, T.; Patarroyo-White, S.; Sommer, J.M.; Zhou, S.; Couto, L.B. & Pierce, G.F. (2006). Human immunoglobulin inhibits liver transduction by AAV vectors at low AAV2 neutralizing titers in SCID mice. *Blood*, Vol.107, No.5, pp. 1810-1817, ISSN 0006-4971

Schertzer, J.D. & Lynch, G.S. (2006). Comparative evaluation of IGF-I gene transfer and IGF-I protein administration for enhancing skeletal muscle regeneration after injury. *Gene Ther*, Vol.13, No.23, pp. 1657-1664, ISSN 0969-7128

Shimatsu, Y.; Yoshimura, M.; Yuasa, K.; Urasawa, N.; Tomohiro, M.; Nakura, M.; Tanigawa, M.; Nakamura, A. & Takeda, S. (2005). Major clinical and histopathological characteristics of canine X-linked muscular dystrophy in Japan, CXMDJ. *Acta Myol*, Vol.24, No.2, pp. 145-154, ISSN 1128-2460

Takeshima, Y.; Nishio, H.; Sakamoto, H.; Nakamura, H. & Matsuo, M. (1995). Modulation of in vitro splicing of the upstream intron by modifying an intra-exon sequence which is deleted from the dystrophin gene in dystrophin Kobe. *J Clin Invest*, Vol.95, No.2, pp. 515-520, ISSN 0021-9738

Takeshima, Y.; Yagi, M.; Wada, H.; Ishibashi, K.; Nishiyama, A.; Kakumoto, M.; Sakaeda, T.; Saura, R.; Okumura, K. & Matsuo, M. (2006). Intravenous infusion of an antisense oligonucleotide results in exon skipping in muscle dystrophin mRNA of Duchenne muscular dystrophy. *Pediatr Res*, Vol.59, No.5, pp. 690-694, ISSN 0031-3998

Townsend, D.; Yasuda, S.; Li, S.; Chamberlain, J.S. & Metzger, J.M. (2008). Emergent dilated cardiomyopathy caused by targeted repair of dystrophic skeletal muscle. *Mol Ther*, Vol.16, No.5, pp. 832-835, ISSN 1525-0016

Urasawa, N.; Wada, M.R.; Machida, N.; Yuasa, K.; Shimatsu, Y.; Wakao, Y.; Yuasa, S.; Sano, T.; Nonaka, I.; Nakamura, A. & Takeda, S. (2008). Selective vacuolar degeneration in dystrophin-deficient canine Purkinje fibers despite preservation of dystrophin-associated proteins with overexpression of Dp71. *Circulation*, Vol.117, No.19, pp. 2437-2448, ISSN 0009-7322

Valentine, B.A.; Cooper, B.J.; De Lahunta, A.; O'quinn, R. & Blue, J.T. (1988). Canine X-linked muscular dystrophy. An animal model of Duchenne muscular dystrophy: clinical studies. *J Neurol Sci*, Vol.88, No.1-3, pp. 69-81, ISSN 1878-5883

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. (2007). Local dystrophin restoration with antisense oligonucleotide PRO051. *N Engl J Med*, Vol.357, No.26, pp. 2677-2686, ISSN 0028-4793

Vandenberghe, L.H.; Wang, L.; Somanathan, S.; Zhi, Y.; Figueredo, J.; Calcedo, R.; Sanmiguel, J.; Desai, R.A.; Chen, C.S.; Johnston, J.; Grant, R.L.; Gao, G. & Wilson, J.M. (2006). Heparin binding directs activation of T cells against adeno-associated virus serotype 2 capsid. *Nat Med*, Vol.12, No.8, pp. 967-971, ISSN 1078-8956

Wang, B.; Li, J. & Xiao, X. (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, pp. 13714-13719, ISSN 0027-8424

Wang, Z.; Kuhr, C.S.; Allen, J.M.; Blankinship, M.; Gregorevic, P.; Chamberlain, J.S.; Tapscott, S.J. & Storb, R. (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, pp. 1160-1166, ISSN 1525-0016

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. (2007). PTC124 targets genetic disorders caused by nonsense mutations. *Nature*, Vol.447, No.7140, pp. 87-91, ISSN 0028-0836

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. (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, pp. 14814-14819, ISSN 0027-8424

Yokota, T.; Lu, Q.; Partridge, T.; Kobayashi, M.; Nakamura, A.; Takeda, S. & Hoffman, E.P. (2009). Efficacy of systemic morpholino exon-skipping in Duchenne dystrophy dogs. *Annals Neurol*, pp. 667-676, ISSN 0364-5134

Yoshida, M.; Hama, H.; Ishikawa-Sakurai, M.; Imamura, M.; Mizuno, Y.; Araishi, K.; Wakabayashi-Takai, E.; Noguchi, S.; Sasaoka, T. & Ozawa, E. (2000). Biochemical evidence for association of dystrobrevin with the sarcoglycan-sarcospan complex as a basis for understanding sarcoglycanopathy. *Hum Mol Genet*, Vol.9, No.7, pp. 1033-1040, ISSN 0964-6906

Yoshimura, M.; Sakamoto, M.; Ikemoto, M.; Mochizuki, Y.; Yuasa, K.; Miyagoe-Suzuki, Y. & Takeda, S. (2004). AAV vector-mediated microdystrophin expression in a relatively small percentage of mdx myofibers improved the mdx phenotype. *Mol Ther*, Vol.10, No.5, pp. 821-828, ISSN 1525-0016

Yuasa, K.; Miyagoe, Y.; Yamamoto, K.; Nabeshima, Y.; Dickson, G. & Takeda, S. (1998). Effective restoration of dystrophin-associated proteins in vivo by adenovirus-mediated transfer of truncated dystrophin cDNAs. *FEBS Lett*, Vol.425, No.2, pp. 329-336, ISSN 0014-5793

Yuasa, K.; Sakamoto, M.; Miyagoe-Suzuki, Y.; Tanouchi, A.; Yamamoto, H.; Li, J.; Chamberlain, J.S.; Xiao, X. & Takeda, S. (2002). Adeno-associated virus vector-mediated gene transfer into dystrophin-deficient skeletal muscles evokes enhanced immune response against the transgene product. *Gene Ther*, Vol.9, No.23, pp. 1576-1588, ISSN 0969-7128

Yuasa, K.; Yoshimura, M.; Urasawa, N.; Ohshima, S.; Howell, J.M.; Nakamura, A.; Hijikata, T.; Miyagoe-Suzuki, Y. & Takeda, S. (2007). Injection of a recombinant AAV serotype 2 into canine skeletal muscles evokes strong immune responses against transgene products. *Gene Ther*, Vol.14, No.17, pp. 1249-1260, ISSN 0969-7128

Yue, Y.; Ghosh, A.; Long, C.; Bostick, B.; Smith, B.F.; Kornegay, J.N. & Duan, D. (2008). A single intravenous injection of adeno-associated virus serotype-9 leads to whole body skeletal muscle transduction in dogs. *Mol Ther*, Vol.16, No.12, pp. 1944-1952, ISSN 1525-0016

Viral Gene Therapy

Edited by Dr. Ke Xu

The development of technologies that allow targeting of specific cells has progressed substantially in recent years for several types of vectors, particularly viral vectors, which have been used in 70% of gene therapy clinical trials. Particular viruses have been selected as gene delivery vehicles because of their capacities to carry foreign genes and their ability to efficiently deliver these genes associated with efficient gene expression. This book is designed to present the most recent advances in viral gene therapy

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Shin'Ichi Takeda and Takashi Okada (2011). Progress and Challenges in AAV-Mediated Gene Therapy for Duchenne Muscular Dystrophy, Viral Gene Therapy, Dr. Ke Xu (Ed.), ISBN: 978-953-307-539-6, InTech, Available from: http://www.intechopen.com/books/viral-gene-therapy/progress-and-challenges-in-aav-mediated-gene-therapy-for-duchenne-muscular-dystrophy

INTECH
open science | open minds