Clinical development on the frontier: gene therapy for duchenne muscular dystrophy

Damon R. Asher, Khampaseuth Thapa, Sachi D. Dharia, Navid Khan, Rachael A. Potter, Louise R. Rodino-Klapac & Jerry R. Mendell


To link to this article: https://doi.org/10.1080/14712598.2020.1725469

© 2020 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.

Accepted author version posted online: 07 Feb 2020.
Published online: 12 Feb 2020.

Submit your article to this journal

Article views: 554

View related articles

View Crossmark data
Clinical development on the frontier: gene therapy for duchenne muscular dystrophy

Damon R. Asher, Khampaseuth Thapa, Sachi D. Dharia, Navid Khan, Rachael A. Potter, Louise R. Rodino-Klapac and Jerry R. Mendell

© 2020 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.

ABSTRACT

Introduction: The development of adeno-associated virus (AAV) vectors as safe vehicles for in vivo delivery of therapeutic genes has been a major milestone in the advancement of gene therapy, enabling a promising strategy for ameliorating a wide range of diseases, including Duchenne muscular dystrophy (DMD).

Areas covered: Based on experience with the development of a gene transfer therapy agent for DMD, we discuss ways in which gene therapy for rare disease challenges traditional clinical development paradigms, and recommend a step-wise approach for design and evaluation to support broader applicability of gene therapy.

Expert opinion: The gene therapy development approach should intentionally design the therapeutic construct and the clinical study to systematically evaluate agent delivery, safety, and efficacy. Rigorous preclinical work is essential for establishing an effective gene delivery platform and determining the efficacious dose. Clinical studies should thoroughly evaluate transduction, on-target transgene expression at the tissue and cellular level, and functional efficacy.

1. Introduction

Gene transfer therapy is designed to treat the underlying genetic cause of a disease. The intent is to deliver a transgene that will compensate for a disease-causing mutation in patients. The concept is straightforward but clinical implementation of the idea is complex. Realization of the promise of gene therapy has awaited the development of safe methods to deliver transgenes to relevant tissues and specifically express them there. Further complications are introduced by factors specific to rare diseases and the single-dose nature of current gene transfer treatments. Here, we will review considerations for the construction of a gene transfer therapy agent and the evaluation of its effectiveness, guided by our experience in developing a gene therapy to treat Duchenne muscular dystrophy (DMD).

Functional gene copies can be delivered via vector delivery systems by one of two routes: ex vivo or in vivo. Ex vivo gene transfer introduces a transgene into cells of interest that have been isolated, maintained, and expanded in culture, after which the modified cells are reintroduced to the patient [1]. In contrast, in vivo methods include gene transfer and gene editing through direct delivery into the body either systemically or locally [1].

After almost 50 years since gene therapy was proposed by Theodore Friedman as a viable therapeutic strategy for human disease [2], the first clinical trials were initiated in the 1990s. Viral vectors provide a promising system for gene delivery due to their efficiency and specificity. However, hard lessons were learned about the importance of vector choice for patient safety in these early trials. Much has been reported about the tragic case of Jesse Gelsinger, an 18-year old with ornithine transcarbamylase deficiency, who was treated with adenoviral gene therapy in 1999 and subsequently died as a result of a massive inflammatory response [3,4]. This case highlighted the criticality of accounting for the patient’s immune response to the vector used. Similarly, cases of leukemias following retroviral-mediated gene therapy underscored the need to consider safety issues related to insertion mutations when identifying novel viral vectors [5–9].

Consequently, adeno-associated virus (AAV) vectors have emerged as the vehicle of choice for systemic gene transfer therapy. AAV is a small (25-nm) virus from the Parvoviridae family that consists of a non-enveloped icosahedral capsid (protein shell) containing a linear single-stranded DNA genome of about 4.7 Kb [10,11]. AAV vectors are the preferred delivery system for in vivo application, as they can be delivered systemically via the vascular system, are nonpathogenic and can infect a broad range of dividing and non-dividing cells and the various serotypes display differential tissue tropism [10,12]. AAV vectors have essentially replaced adenovirus for in vivo gene delivery with the initiation of a large number of clinical trials (>100 trials either recruiting or active/not recruiting in the U.S. [13]). In contrast to adenovirus vectors used in early gene therapy studies, AAVs elicit a milder course of innate immune reactions, and this is a key determinant of their favorable safety profile and low toxicity [14]. Furthermore, as opposed to retroviruses that carry a risk of oncogenesis, transgenes carried by AAV vectors infrequently
integrate into chromosomes, thereby reducing the chances of insertional mutagenesis that has been observed with retroviral vectors [15–17]. Moreover, recombinant AAVs (rAAVs) are available in many serotypes derived from different species and phylogenetic clades, with varying tissue tropisms and immunogenic profiles [18]. This provides a toolbox of vectors for specific delivery routes and target tissues.

Gene therapy treatments have revolutionary potential to be effective for a wide array of diseases, including hematological, ocular, neurodegenerative diseases, and several cancers [19]. Specifically, gene therapies have the potential to provide long-lasting, one-time treatments for diseases that were previously untreatable or for which only symptomatic and suboptimal treatments were available. Despite historical challenges and setbacks, there have been recent examples of successful development and US Food and Drug Administration (FDA) approval of gene therapies including voretigene neparvovec-rzyl (Luxturna®) [20] and onasemnogene abeparvovec-xioi (Zolgensma®) [21]. Clinical research on gene transfer therapy has demonstrated positive outcomes including improvements in muscle function, developmental milestones, and survival in spinal muscular atrophy [22]; restoration of vision in patients who were blind; eradication of blood cancers for patients not responding to other treatments; correction of hemoglobinopathies and coagulation factor deficiencies; and immune system restoration in children born with primary immune deficiency [19,23,24]. Notably, many clinical studies report successful results with gene therapy agents in pediatric patients [25–28].

The rapid rise of gene therapy products entering early development reflects the significant scientific advancement and the clinical promise of AAV-mediated gene transfer. Based on an assessment of current cell and gene therapy pipelines and the clinical success rates of these products, the FDA anticipates receiving more than 200 investigational new drug (IND) applications per year by 2020, and approval of 10 to 20 cell and gene therapy products by 2025 [29]. This anticipated surge of regulatory filings has been a driver for efforts to evolve regulatory guidance to advance the development of safe and effective cell and gene therapies [29–31]. Best practices for clinical development of gene transfer therapies are currently emerging as well. Here we review considerations for gene transfer therapy for rare diseases and their application to specific choices made in development of a treatment for DMD.

2. Clinical development for conventional small-molecule drugs vs. gene therapy

Drug development is a lengthy and complex journey based on longstanding design principles, many of which are challenged by the nature of gene therapy for rare diseases. Consequently, clinical development paradigms for gene therapies are emerging that differ from those of more conventional treatments, and it is informative to discuss the reasons for these differences and potential solutions for novel challenges presented by these new genetic therapies (Figure 1).

2.1. Essential elements of conventional small-molecule clinical trial design

In the earliest phase, before safety studies can be done in animals, drug developers need to establish manufacturing consistency between lots and define a control strategy to ensure process performance and drug substance quality. In the preclinical phase, laboratory and animal studies are conducted to assess preliminary safety, efficacy, toxicology, pharmacokinetics, and pharmacodynamics. Wide dose ranges of the drug are evaluated using in vitro and in vivo studies and sometimes computer models of established drug–target interactions. Following IND filing, Phase 1 trials are designed to determine safety, efficacy, and correct dosage in humans. The human equivalent dose (HED) is typically capped at the highest (scaled to body surface area) safe dose tested in the animal toxicology studies [32]. Often the HED is further reduced by a factor of 10, creating a margin of safety for initial dosing in humans [32]. Typically, these studies are conducted in small groups of healthy volunteers and in a controlled environment [33]. If there are no serious events, the dose is escalated for the next set of healthy volunteers, leading to evaluation of multiple doses. During this initial study period, drug parameters including absorption, distribution, metabolism, and excretion in humans are also determined. Phase 2 clinical trials are then conducted to assess efficacy, safety, and tolerability of the drug in larger populations of patients with a specific disease or condition [33]. Phase 3 trials are designed to establish safety, efficacy, and superiority over the current standard of care in large patient populations and are generally used by regulators to determine if a drug should be approved for use in patients [33]. On average, this process takes approximately 12 years from the preclinical phase to approval for small-molecule drugs [34].

2.2. Challenges in designing clinical trials for rare disease and for gene therapy

These conventional small-molecule clinical study paradigms are challenged when applied in the development of therapeutics for rare diseases. As established by the Orphan Drug Act of 1983, a drug qualifies for orphan status if it is intended to treat
a rare disease or condition affecting less than 200,000 persons in the U.S. [35]. Many life-threatening rare diseases primarily impact pediatric patients, highlighting both the challenges and urgency of effective drug development [36]. Sparse rare disease patient populations, disease heterogeneity, and geographic dispersion create difficulties in enrolling representative, homogenous cohorts for clinical studies [30]. Adding further complexity are challenges specifically associated with gene therapies. For example, development of antibodies to the vector upon gene therapy administration serves as a barrier to receiving future gene therapies derived from that vector and may also exclude them from participation in other clinical trials. This has implications to the conventional practice of dosing healthy volunteers as these volunteers may then be precluded from receiving gene therapies should a future need arise. Similarly, conventional dose escalation studies need to be reexamined in the context of gene therapy given that patients receiving sub efficacious doses may still develop immunity that would block future doses of a vector. This not only raises an ethical imperative to make the best efforts to give all patients a potentially efficacious dose, but it also puts an especially high burden on the preclinical work to establish an optimal dose range for safety and efficacy.

2.3. Significance of preclinical studies for the development of gene therapies

Preclinical studies should be adequately designed and conducted to provide insights into the safety profile and efficacy of the gene therapy product. These data should be used to inform decisions related to potential future clinical trials in humans. Preclinical work must be designed to identify the effective dose range that will translate from relevant animal models to the target disease population (e.g., infants, children, and/or adults) through identifying the minimally effective dose, the optimal biological dose, and the maximally tolerated dose [37]. In addition, the optimal route and timing of administration must be determined. Comprehensive toxicology assessments, including immunological assessments of viral and transgene T cell responses, should establish the initial safety profile of the therapy. Complete necropsies should provide important insights into potential adverse events and efficacy measures. A comprehensive analysis of safety, tissue-specific viral transduction, transgene expression, protein localization where appropriate, cellular impact, and relevant functional measures should all be performed. The preclinical work should include a thorough assessment of biodistribution to confirm effective transduction and delivery of vector to the relevant tissues. Similarly, expression of the transgene should also be assessed in target and non-target tissues to confirm the desired activity of the promoter only where intended. Effects on molecular and cellular function should be analyzed, and where possible the impact on overall functional measures should be assessed in the animal model. Studies in large animal models may be required to adequately assess safety and vector tropism.
2.4. Essential elements of gene therapy clinical trial design

Following preclinical studies showing promise from both a safety and efficacy perspective, rigorous clinical trials should be designed to evaluate all aspects of the gene therapy’s safety and efficacy including a comprehensive analysis and reporting of viral transduction, transgene expression, protein localization and cell stabilization where appropriate, and any functional measures [38]. The preclinical work should inform the types of safety signals that should be anticipated, and these should include assessments of any immunological responses. The target tissues to be evaluated, and specific cellular and functional measures will vary depending on the target indication and the specific design is informed by knowledge of the disease state and the intended action of the gene transfer therapy on the target tissues.

3. DMD disease overview and targeted patient subtype

DMD is a rare, X-linked, fatal, degenerative, neuromuscular disease that is estimated to affect 1 in 5,000 newborn boys worldwide [39–42]. It is caused by mutations in a single gene, the DMD gene, resulting in a lack of functional dystrophin protein. As a critical component of the dystrophin-associated protein complex (DAPC) in muscle cells, dystrophin is not only essential for the maintenance of muscle fiber stiffness [43], but also for protection against mechanical stress during muscle contraction (Figure 2(a)) [44–47]. Dystrophin provides critical structural support for muscle cell sarcolemma through the attachment of specific regions to anchor points, including the intracellular actin cytoskeleton, the extracellular matrix via the DAPC, and the sarcolemma itself. The absence of functional dystrophin protein leads to loss of sarcolemma structural support, failure of DAPC assembly, and consequent decrease in sarcolemma integrity. This results in continual contraction-induced injury to muscle tissues, which ultimately exhausts the components necessary for myofiber repair and regeneration, resulting in irreversible loss of muscle function [48]. This impacts all skeletal muscles including the diaphragm as well as cardiac muscle and heart function.

Given the essential role of dystrophin in protecting skeletal and cardiac muscle cells from mechanical stress, patients with DMD experience a loss of critical functions resulting from progressive muscle tissue degeneration with motor symptoms (Figure 3) [49,50]. Delays in early developmental milestones, such as sitting, walking, and talking often prompt diagnosis between 3 and 5 years of age. Enlarged calf muscles are also apparent in these early years and usually ignored or misinterpreted. In the early ambulatory phase, at 5–7 years of age, patients with DMD begin to manifest symptoms including motor delays, and difficulty standing. Motor delays are followed by a functional decline, resulting in loss of ambulation in the early teen years, wheelchair dependency, and an inability to perform activities of daily living (e.g. self-feeding) in the teen years. In addition, loss of diaphragm function can result in increasing respiratory impairment, cardiac dysfunction, and the added burden of ventilator-assisted breathing in the teens and twenties. Ultimately, disease progression leads to cardiomyopathy, respiratory failure, and premature death [51]. Gene transfer therapy for DMD is intended to address the underlying genetic cause of the disease by specifically restoring functional dystrophin to the key tissue targets of skeletal muscle including diaphragm and the heart (Figure 3) by introducing a functional gene to compensate for the mutated or absent dystrophin gene.

4. Special considerations for DMD gene therapy

Gene therapy offers new hope for the treatment of rare, monogenetic diseases such as DMD. DMD is well-suited to gene transfer because it is a disease caused by mutation of a single gene, DMD. Furthermore, because muscle cells are non-dividing and long-lived, there is potential that a one-time treatment could have long-term and perhaps even life-long benefits. In fact, preclinical and clinical studies in neuromuscular diseases to date indicate no loss in durability [25,52–55]. The objective of gene therapy for DMD is to deliver a gene encoding a functional version of dystrophin systemically to all

Figure 2. Dystrophin protein. Adapted from [47] with permission from Oxford university press.
target tissues involved in DMD pathology and thereby ameliorate the progression of the disease.

Despite some distinctions between subtypes, AAVs generally have favorable safety profiles compared to adenoviruses and retroviruses. Many subtypes of AAV vectors are available, and these vary in their biodistribution and interaction with innate and adaptive immune response. Optimal treatment of DMD requires selection of an AAV vector with high tropism for skeletal, diaphragm, and cardiac muscles. To reach all these muscles, the vector must be administered systemically. This requires high doses, making the safety profile of the vector selected all the more critical. The focus on skeletal and cardiac muscle also necessitates the selection of a promoter that expresses at high levels in these tissues, and minimally in off-target organs. The use of tissue-specific promoters restricts transgene expression; however, AAV serotypes used clinically to date have significant biodistribution in the liver. The presence of the viral capsid does elicit an immune response in some cases that manifests with liver enzyme elevations \[22,56\]. Elevation of liver enzymes can be managed with the introduction or increase of glucocorticoids such as prednisone, but careful monitoring is critical.

While AAV vectors have numerous advantages, they do have an important limitation when it comes to treating DMD. AAVs are relatively small and have limited packaging capacity, able to contain only about 5kb of DNA \[10,37\]. This is not an issue for many genes but does present a particular challenge for DMD. The dystrophin gene is one of the largest in the human genome, spanning 2.3 megabases, and the size of the cDNA for the DMD gene (~14 kb) greatly exceeds the packing capacity of AAV vectors. Therefore, thought must be put into the design of the dystrophin transgene. In addition to fitting within AAV, the transgene must encode a protein that is stable, functional, properly localized to the sarcolemma, successful in restoration of the DAPC, effective in preserving cell integrity, and able to ultimately improve clinical function.

These requirements necessitate a rational, intentional design approach to developing gene therapy for DMD. This specifically involves careful selection of the right vector, promoter, and transgene to minimize potential safety issues and maximize therapeutic benefits (Figure 4). Furthermore, gene transfer therapy should be evaluated in a way that best provides meaningful data to patients, families, and clinicians. DMD gene therapy development programs should include step-wise assessment of safety; delivery of the transgene DNA to the target cell (transduction); expression of the transgene protein; localization of the transgene protein within intended tissues; transgene function at the molecular and cellular level; and impact on clinical functions (Figure 5). Systematic analyses of these data should inform subsequent steps in the clinical development of DMD gene therapies. For example, any dose-escalation decisions should be informed by transduction data, as low levels of transduction should be the main reason to dose-escalate. Adequate transduction followed by low expression levels would signify issues with the promoter and/or transgene and therefore dose escalation would be inappropriate until proper redesign of the construct. It is crucial that all pertinent data are analyzed and reported, so potential clinical trial participants can make informed decisions in evaluating therapies prior to enrolling in a clinical trial, given that retreatment with a different AAV gene therapy is not possible at present.

5. Case study – clinical development of a novel gene transfer therapy for DMD

Here, we discuss the rationale for our clinical trial design for the assessment of a novel gene transfer therapy in patients with DMD. Our construct (SRP-9001 micro-dystrophin) uses AAVrh74 as the vector, a muscle-specific MHCK7 promoter with a cardiac enhancer region, and a micro-dystrophin transgene including spectrin repeats 1–3 and 24, and hinges 1, 2 and 4 (Figure 2(c), Figure 4).

Isolated at Nationwide Children’s Hospital from rhesus monkeys \[57\], AAVrh74 was chosen as the delivery vector for
DMD gene transfer therapy. Of the familiar numbered AAV strains 1–9, AAVrh74 has most homology to AAV8, another virus in the same phylogenetic clade (referring to a common ancestry), but is distinguished by its transduction efficiency and seroprevalence characteristics [58]. Utilization of a vector of non-human origin avoiding prior human infection was intended to minimize the potential for preexisting immunity [59,60]. Indeed, the seroprevalence of AAVrh74 was found to be relatively low (15–20%) in the DMD patient population currently targeted by initial clinical trials (boys 4–7 years of age) [61]. A study that examined the prevalence of antibodies against a range of AAV serotypes found AAVrh74 to be among the types with the lowest rates of preexisting immunity [62]. The low seroprevalence of AAVrh74 potentially allows for application in more patients.

The MHCK7 promoter was chosen to drive expression of SRP-9001 micro-dystrophin. The regulatory element consists of an α-myosin heavy chain (α-MHC) enhancer and muscle creatine kinase (MCK) enhancer/promoter region [63] (Figure 4). This promoter was chosen to be most suitable for DMD gene therapy given that the MHCK7 promoter induces strong transgene expression in all skeletal muscles, including, critically, the diaphragm, as well as cardiac muscle with minimal expression in off-target tissues. In addition, the α-MHC enhancer portion of the regulatory element dramatically boosts transgene expression in the heart while also driving increased expression in skeletal muscles [63]. Finally, the MHCK7 promoter is space-efficient, including the minimal elements needed, which is particularly important given the packaging size constraints of AAVs [63].

The unusually large size of the natural DMD gene necessitated the strategic, economical design of the transgene to fit within the AAVrh74 vector. The solution chosen was to construct a shortened version of the dystrophin gene, called a micro-dystrophin. The SRP-9001 micro-dystrophin transgene was designed to encode only the most critical parts of the dystrophin protein. The rationale for the use of a micro-dystrophin was based upon a naturally occurring shortened dystrophin in a patient with Becker muscular dystrophy (BMD) [37,64] (Figure 2(b)). BMD, like DMD, is
caused by mutations in the DMD gene. The difference is that DMD mutations result in a failure to produce significant amounts of functional dystrophin, whereas mutations in BMD produce shortened, partially functional dystrophin proteins. BMD severity can vary dramatically, but the patient in this case had a very mild dystrophinopathy despite the fact that his dystrophin was half the size of normal [64]. In fact, this man was ambulatory into his sixties, and a younger male relative with the same mutation was also very mild, with a history of weight training. Notably, the dystrophin in these patients maintained the critical anchor regions at the N-terminus for actin, at the C-distal end for the DAPC, and the R1-R3 region that is known to interact directly with the sarcolemma [47]. The central portion of dystrophin was missing and was evidently not critical for the preservation of a large degree of function in these patients.

The SRP-9001 micro-dystrophin transgene we utilized was modeled upon this natural example. The SRP-9001 micro-dystrophin transgene was further shortened by removing additional central domains, but the three critical anchor regions were preserved. Three hinge domains were also included to maintain molecular flexibility [65]. An additional design principle was to minimize the number of unnatural junctions to reduce the potential for protein instability and creation of immunogenic peptides. SRP-9001 micro-dystrophin has only one of these junctions. The resulting SRP-9001 micro-dystrophin is intended to be highly functional while being compact enough to fit within an AAV vector (Figure 2(c)). While other designs can also be rationalized, the decision was made that these elements represented the best use of available space with the belief that this would maximize the potential for clinical benefit. Preclinical studies with SRP-9001 in the mdx mouse model of DMD demonstrated safety and efficacy measured by marked improvements in histology (fibrosis and normalization of fiber size), reduction in creatine kinase and function (specific force and resistance to contraction-induced injury in the tibialis anterior and diaphragm) [66].

In our initial safety and pivotal trials (ClinicalTrials.gov NCT03375164 and NCT03769116), we have focused on 4- to 7-year-olds. This population corresponds to the age at which most patients are commonly diagnosed with DMD, and thus represents the first opportunity to treat. Furthermore, given that muscle degeneration is largely in its early stages at this age range [67–71], there is relatively more muscle to preserve with treatment. Some boys within this age range may be progressing on functional measures albeit likely at a delayed rate [49]; decline in function is characteristic at age 7 and above, and some may make that transition over the course of the study. The potential range in functional status requires careful outcome measurement and determination of cohort sizes to detect treatment effects across different trajectories.

Treatment with a stable dose of corticosteroids for at least 30 days prior to gene transfer therapy was another key inclusion criterion for our studies (ClinicalTrials.gov NCT03375164 and NCT03769116). In addition to suppressing the immune reaction to the treatment, steroids may also impart some degree of functional benefit [72]. Therefore, it is important to normalize corticosteroid treatment across all patients and study arms.

DMD mutation type also represented another key inclusion criterion (ClinicalTrials.gov NCT03375164 and NCT03769116). Specifically, patients with frameshift (deletion or duplication) or a premature stop codon mutation between exons 18 to 58 of the DMD gene were included. The micro-dystrophin gene is not encoded by these exons and this conservative window was implemented to minimize any potential for immune reactions to the SRP-9001 micro-dystrophin protein in this first trial [73]. However, micro-dystrophin gene therapy is thought to be equally applicable to all DMD mutations, and the intent is to widen these criteria in future studies.

A key exclusion criterion was anti-AAVrh74 antibody titers greater than 1:400, a threshold identified in preclinical studies in nonhuman primates [74,75]. This was implemented to minimize potential that the vector will be cleared or neutralized, preventing muscle transduction.

Upcoming trials plan to expand the inclusion criteria in terms of extending the age range and DMD gene mutation types. We also plan to include non-ambulatory patients given that micro-dystrophin gene transfer therapy has the potential to preserve muscular, respiratory, and cardiac functions at all stages of DMD.

In our program, we have also included a placebo control arm (ClinicalTrials.gov NCT03769116) and this can be a concern for some patients and their caregivers. Given the progressive nature of DMD, and, therefore, the urgency for rapid treatment, lack of control groups makes evaluation of the clinical impact less straightforward from a regulatory and payor perspective and may delay access to the treatment. Importantly, all patients in the placebo arms of our trials will be given the opportunity to cross over into an open-label extension study and ultimately receive treatment.

Our approach to evaluation of the micro-dystrophin gene transfer therapy is in line with our recommendations for a systematic and step-wise evaluation of safety, transduction, expression, localization, cellular impact, and clinical function (Figure 5).

5.1. Safety

In clinical trials, AAV vectors have consistently shown a favorable safety profile, which may be due to their lower immunogenicity compared to adenovirus vectors (i.e., reduced innate immune response activation) [76]. However, recent work suggests safety signals such as complement activation and thrombocytopenia may be associated with AAV capsids like AAV9 [76–78]. In contrast, no toxicity was seen in preclinical testing of AAVrh74 in mice and nonhuman primates, even at the highest doses tested (6E14 vg/kg), which is three times the current dose. Moreover, AAV vectors maintain the transgene as a nonintegrating, stable, extrachromosomal episome [79]. Because AAV vectors integrate into chromosome only at a very low rate, the potential for oncogenesis is minimized. Furthermore, the safety profile and tolerability of this AAVrh74 vector have also been shown in human studies [80]. The safety profile of AAVrh74 will continue to be evaluated
across multiple gene transfer clinical development programs using this vector, including programs targeting various LGMD subtypes [80,81].

5.2. Transduction

The purpose of the vector is to efficiently deliver the transgene to target tissues. For DMD, it is essential that the vector delivers the transgene broadly to skeletal muscle, including the diaphragm, as well as cardiac muscle. This enables the therapy to address the major pathologic effects of DMD: loss of muscle strength and mobility, declining respiratory function, and cardiomyopathy. We selected AAVrh74 due to its high transduction efficiency for skeletal muscle (including the diaphragm) and cardiac muscle.

To properly evaluate that vector is functioning as intended, it is critical to measure and report transduction efficiency (i.e., the level of transgene copies per nucleus) in target tissues. Transduction is evaluated by extracting DNA from a muscle biopsy and performing quantitative PCR [82], which amplifies vector-specific DNA, to determine the number of transgene copies relative to the total amount of DNA. Subsequent calculation of the number of transgenes per nucleus will determine if most cells are receiving the transgene, keeping in mind that muscle cells are multi-nucleated with up to a few hundred nuclei per cell [83]. Specifically, the rAAVrh74 capsid induces high-level transduction of both cardiac and skeletal muscle [54,84].

5.3. Expression and localization

For DMD, promoters that specifically drive expression in skeletal muscle, including the diaphragm, as well as cardiac muscle are particularly attractive. Because mortality due to heart failure is a key consideration in DMD, we utilized the MHCK7 promoter with the intent of driving especially strong expression in the heart.

To rigorously evaluate the effectiveness of the promoter in driving transgene expression, complementary analyses are required to determine the level of protein expression in the overall target tissue. Western blot analysis enables quantification of the protein produced from the transgene using a standard for normal dystrophin levels as well as visualization of the protein size. Extensive work has been done to establish a quantitative test with high precision and reproducibility [85,86]. Done properly, Western blot analysis is accurate with a good standard deviation (coefficient of variation of ~20%).

Concurrent with quantifying transgene expression in muscle by Western blot analysis, it is critical to measure the proportion of muscle cells that express micro-dystrophin via immunohistochemistry (IHC) to confirm distribution throughout the muscle. Cross-sectional samples are prepared from a muscle biopsy and stained using anti-dystrophin antibodies. Image analysis then allows determination of the percentage of muscle fibers expressing the micro-dystrophin protein. IHC is also used to demonstrate proper localization of the expressed micro-dystrophin to the sarcolemma. Immunofluorescence is to visualize restoration of other DAPC proteins such as betasarcoglycan [57]. In addition to the percentage of fibers expressing the transgene, complementary measurement and reporting of total fluorescence (i.e., intensity) allow monitoring of how much transgene expression is distributed across tissue samples. Based on unpublished data from our clinical trials, following gene delivery, we achieved robust muscle dystrophin levels as measured by muscle biopsies with an increase in the number of positive muscle fibers with a comparable intensity of gene expression by IHC at the sarcolemma [56,87,88].

5.4. Muscle cell stabilization

Beyond the characterization of the effectiveness of the vector and promoter, the impact of micro-dystrophin on muscle cell stabilization must be assessed. Loss of muscle cell integrity in DMD leads to rises in serum CK, an early sign of the disease [89]. Conversely, a sustained drop in CK following gene therapy is suggestive of muscle membrane protection by microdystrophin. Though CK levels tend to fluctuate in patients with DMD depending on the amount of physical activity, in unpublished findings from our clinical trials, we observed an overall, sustained reduction [56,87,88].

5.5. Functional outcome measures

Long-term assessments of the impact of micro-dystrophin expression on ambulatory and lower limb function are examined using carefully chosen methods, including the comprehensive North Star Ambulatory Assessment (NSAA) [70,90]. Designed to measure disease progression, NSAA is currently accepted by regulatory bodies in the FDA and European Medicines Agency (EMA) as valid for the assessment of functional endpoints. Individual components of NSAA are currently being evaluated to determine their sensitivity at detecting ambulatory and lower limb function relative to the composite score. As these data are collected from treatment and placebo groups, we will be able to make assessments of the functional effects of the micro-dystrophin gene transfer therapy.

6. Conclusion

The development of AAV as a vehicle for in vivo gene transfer has been a major milestone in the advancement of gene therapy for rare genetic diseases such as DMD. Emerging therapies for rare diseases are shifting the paradigms for how to conduct clinical programs under challenging conditions including variable disease course, evaluation of historical controls, and risk of underdosing. Given the urgent, unmet need for patients with DMD, our clinical development program was designed to firmly establish safety and efficacy in the shortest time possible, requiring navigation of unchartered terrain. However, scientific rigor cannot be compromised for the sake of speed, and we have striven to implement a DMD gene therapy development program that thoroughly evaluates whether the rationally designed construct is functioning as intended. We hope that application of lessons learned from our experience from the development of gene therapy for DMD will lead to more rigorous preclinical and clinical study designs that can overcome the barriers to the broader applicability of gene therapy.
7. Expert opinion

We have learnt that high-dose viral vector gene delivery is critical to achieving success in clinical outcomes. Though initial trials at these doses were frowned upon because of potential risks, our studies (including spinal muscular atrophy gene therapy [22]) have shown these high doses can be well tolerated and achieve sufficient transgene expression. Rigorous preclinical studies are essential for determining the proper balance of safety and efficacy and to lay the best foundation for the success of gene therapy clinical trials.

Clinical trials thus far using AAVrh74 have shown relatively low-risk adverse events in patients, with the most common being a transient elevation in liver enzymes. Rarely, this has been accompanied by transient bilirubin increases. The observed frequency of liver toxicity is notably less than seen in many pharmacologic treatments used, in large part because liver abnormalities following gene transfer can be suppressed through the use of corticosteroids as an anti-inflammatory agent. Thus, far, there has been no reason to use other forms of immune suppression with this particular therapy. Transient nausea, loss of appetite, and vomiting have also been observed. This is common over the first few weeks of treatment and the occurrence may be related to acid reflux from increased corticosteroid dosing to protect the liver from inflammation. More severe events reported with AAV vectors demand continued vigilance [91–93].

A significant barrier to gene therapy is preexisting immunity to AAV that renders patients ineligible for treatment. One of the largest advancements will be the ability to circumvent AAV antibodies to allow the administration of gene therapy to these patients. This is an area of active investigation, with the application of methods such as plasmapheresis or T-cell suppression using medications like rituximab and rapamycin [74]. While we are hopeful, it is too early to predict whether these methods will allow us to overcome the barrier of preexisting immunity.

AAV vectors are currently the favored tool for systemic gene transfer due to their established safety profile and ability to target muscle, but the limited packaging capacity of AAV has necessitated the development of SRP-9001 microdystrophin protein. SRP-9001 micro-dystrophin design was based on naturally occurring shortened dystrophin in highly functioning BMD patients, and will hopefully dramatically improve the course of DMD disease. While it is unclear whether a full-length dystrophin gene therapy would confer any benefit over the current micro-dystrophin approaches, research into restoration of full-length dystrophin could further evolve the field. Potential methods for this are new vectors that can transfer the full dystrophin cDNA sequence or use of gene editing tools to repair the native dystrophin gene. However, it will be some time until these approaches are ready for clinical application, whereas AAV micro-dystrophin is currently in late-stage clinical trials. The AAV approach may also be modified in future programs through the development of vectors with further reduced immunogenicity.

Advancements in gene therapy will necessitate progress in other aspects of health care to best capitalize on the potential of this new treatment modality. The rapid advancement of the field dictates continued education of patients, HCPs, payors, and policymakers on the progress that has been made since early trials. We are hopeful that SRP-9001 micro-dystrophin clinical trials will lead to treatment for one of the most devastating muscle diseases affecting children worldwide, and it will be important that all stakeholders have a strong understanding of the potential benefits and limitations of this treatment to inform decisions. Another key challenge post-approval will be payor access. Unique payor models may be required to support access to gene therapies with the potential to be one-time treatments. This is something that we anticipate will evolve over time as new therapies are approved. A further result of new gene therapies will likely be an expansion of newborn screening protocols. Early diagnosis of genetic conditions could enable the most effective application of new gene therapies to alter the course of disease, hopefully prolonging life and function. Ultimately, gene therapy program success hinges on preclinical and clinical trial design that accounts for the unique aspects of gene transfer for rare diseases. Rational and rigorous application of appropriate design principles is needed to convincing establish safety and efficacy in bringing these treatments to patients.

References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

Declaration of interest

The authors are employees of Sarepta Therapeutics, Inc., and may have stock options. JR Mendell has received research funding from Sarepta Therapeutics and has a service agreement with Sarepta Therapeutics to provide training on ongoing studies. In addition, he is the co-inventor of AAVrh74.MhCK7.micro-dys technology (patent pending) which is exclusively licensed to Sarepta Therapeutics. LR Rodino-Klapac is an employee of Sarepta Therapeutics, has received grant support from Sarepta Therapeutics and the Parent Project Muscular Dystrophy, as well as financial consideration from Sarepta Therapeutics and the Myonexus Therapeutics (now acquired by Sarepta Therapeutics). In addition, she is the co-inventor of AAVrh74.MhCK7.micro-dys technology (patent pending) which is exclusively licensed to Sarepta Therapeutics. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Reviewer Disclosures

Peer reviewers on this manuscript have no relevant financial relationships or otherwise to disclose.


**Provides a nice overview of the use of AAV in gene therapy.**


**Provides a nice overview of gene therapy.**


**Discusses how clinical trials differ in gene therapy compared to conventional small molecules.**


• Provides rationale for transgene design and key dystrophin components.
53. Audentes Therapeutics presents new positive data from ASPIRO, a translational approach for limb vascular delivery of the MHCK7 promoter. [PubMed: E1017].

Provides rationale for use of a vector derived from nonhuman primates.

• Provides rationale for the use of the MHCK7 promoter.
58. Provides rationale for transgene design and key dystrophin components.

• Provides rationale for transgene design and key dystrophin components.


