MDCC | Muscular Dystrophy Coordinating Committee

2025 MDCC Action Plan for the Muscular Dystrophies
Preclinical Translational Research on the Muscular Dystrophies
Draft Priorities

Introduction

Preclinical models of muscular dystrophies (MDs) have been instrumental to define mechanisms of disease and assess the utility of potential clinical therapeutic agents. Over the last decade, there has been expansion in the number of animal models, both large and small, providing a wealth of information on primary genetic defects, secondary genetic modifiers, and response to intervention. There has been some success in establishing standard protocols that more accurately predict meaningful features of some human MD subtypes. MD subtypes differ in disease trajectory and even which muscle groups are affected. To promote therapeutic opportunities for all of those living with MD, preclinical models are needed for all MD subtypes. In the next decade, tissue engineering is expected to complement work being performed in animal models. As with animal models, reproducible, reliable and predictive engineered tissue models are needed to translate discovery into therapy. Overall, the goal for preclinical models is to more rapidly inform whether an approach is efficacious to treat MD, this includes identifying potential adverse outcomes of the test agent, although this latter area is often overlooked. The design and evaluation of agents in the preclinical settings should consider both potential efficacy and toxicity.

Priority Topic 1: Develop effective gene agnostic therapies by targeting common pathways implicated in pathogenesis across many different forms of MD.

Further investigations into mechanistic pathways that underlie normal muscle health and MD pathogenesis are required for development of gene agnostic therapies that can be applied more efficiently and for likely lower cost to larger numbers of patients across a spectrum of MDs. Examples of key mechanistic and pathogenic pathways are: muscle maintenance and wasting, muscle fiber membrane integrity and repair, chronic muscle inflammation, muscle regeneration, and fibrofatty infiltration of diseased muscle. In addition, improved understanding of how the heart and other affected organs are differentially affected in MDs will be required for optimal treatments and improved patient outcomes.

In the MDs, there is progressive loss of functional muscle mass. Muscle loss arises from multiple etiologies including instability of muscle fiber integrity, inefficient regenerative capacity, and an imbalance between anabolic and catabolic processes that drives muscle wasting at the expense of muscle maintenance and growth. Developing therapeutic strategies to prevent pathologic loss of functional muscle and/or to enhance muscle formation and growth will provide benefit across the spectrum of MDs. Potential therapeutic targets include (1) enhancing membrane repair complexes or sarcolemmal stability; (2) optimizing the interaction among satellite cells, immune cells, and fibroadipogenic progenitors (FAPs) to restore functional muscle fibers surrounded by a healthy extracellular matrix; (3) promoting healthy muscle protein turnover and preventing excessive protein degradation in muscle; (4) induction of new muscle mass. Although potentially applicable across multiple MD subtypes,

some applications may be preferred for loss-of-function MDs versus gain of function pathogenesis resulting from toxic or misfolded proteins.

Even with gene replacement therapy, the goal of a cure is limited by lack of targeting enough myofibers and muscle stem cells. This partial treatment is unlikely to allow muscle to fully withstand the subclinical injury that accompanies normal muscle use. Normal muscle growth in children may increase the amount of untreated muscle without targeting muscle stem cells. Even low levels of continuing cycles of degeneration and regeneration will lead to inflammation, and fibrotic and fatty replacement of functional muscle tissue. Emerging data suggests complex crosstalk between cell types in the injured muscle microenvironment and impact on repair and regeneration. Mitigating inflammation and fibrosis, and promoting regeneration are synergistic with genetic therapies. A cocktail of complementary therapies will likely be required to treat all aspects of disease, and carefully conducted testing in preclinical models could reveal treatment synergies more efficiently than human clinical trials.

As gene agnostic pathways are identified, their effects on the heart should also be evaluated. Like skeletal muscle, cardiomyopathy in many MDs is also characterized by an inflammatory and fibrofatty infiltrate leading to impaired function and arrhythmias. Treating skeletal muscle without adequately treating the heart may exacerbate cardiac outcomes. Key differences between heart and skeletal muscle may be highly relevant when understanding common pathways across MDs. Treatments that promote skeletal muscle regeneration will likely display no cardiac efficacy due to limited regenerative potential in the heart. Cardiac metabolism also differs from skeletal muscle, and pathways aimed at shifting metabolism, contraction, or relaxation may have differential effects between cardiac and skeletal muscle. These differences must be considered in both design and testing of agents aimed at these pathways. The cardiac conduction system can be compromised by some MD mutations, leading to both abnormally slow and fast heart rhythms. Cardiac fibrosis itself can increase arrhythmia risk, and so reducing inflammation and fibrosis may also improve arrhythmias.

Current gene therapy approaches focus primarily on restoration of striated muscles. However, non-muscle tissues may also suffer primary consequences from mutations in genes that are expressed more globally. For example, mutations in dystrophin can disrupt expression of non-muscle isoforms in brain, smooth muscle, retina, and kidney. Therefore, the need to expand knowledge on the multifaceted nature of MDs with respect to non-muscle tissue will become greater as correction of mutations in striated muscles becomes more successful. Ultimately, complementary and synergistic therapies will be required for optimal treatment of MDs.

Summary points for Topic 1:

- Gene agnostic pathways are likely to be useful across many different MDs.
- Reducing inflammation and fibrosis, enhancing regeneration and growth, and promoting more efficient metabolism are among the many approaches to be considered.
- The effects outside of muscle must be considered when evaluating these treatments.
- A cocktail strategy may prove most useful to effectively treat MD across the lifespan.

Priority Topic 2: Improve delivery, safety, efficacy and durability of genetic therapies.
Genetic correction has the potential to address the underlying genetic lesions that cause recessive and dominant forms of MDs. Strategies include gene replacement, exon skipping, genome editing, and modulation of gene expression. A bottleneck for the clinical translation of these promising treatments relates to systemic delivery of the tools required to perform the desired correction. Current delivery approaches are broadly classified as either viral or non-viral methods, each with its characteristic set of limitations and room for improvement.

Adeno-associated viral (AAV) capsids are widely employed for systemic delivery of a range of genetic cargo, but high doses are required to overcome a threshold effect needed to achieve therapeutic muscle transduction. Immune responses to high dose AAV, as well as the variability in uptake and expression of AAV-based therapies collectively pose limitations in safety, efficacy and durability of gene therapy. Improved vector designs and manufacturing processes should also be investigated as avenues of reducing immune responses. More detailed analyses on the mechanism of vector uptake and processing should enable using lower, and safer doses. These approaches will be enhanced by improved understanding of the immune mechanisms that limit gene transfer.

New classes of AAVs engineered to improve muscle targeting and liver de-targeting have the potential to enhance safety and efficacy of gene therapies in target tissues. Due to their increased efficiency at lower doses, such next generation myotropic AAVs may facilitate strategies to administer multiple AAV vectors to deliver more than one transgene, or to split a large gene cargo for reconstitution in vivo. Non-AAV vectors that are less immunogenic and allow for packaging of a larger genetic cargo (e.g. lentiviruses) can provide alternatives to AAVs but will require characterization for appropriate biodistribution and safety for systemic applications. Development of vectors from alternate viruses could also enhance delivery options.

Non-viral delivery (e.g. lipid nanoparticles, biopolymers, extracellular vesicles) of genetic manipulation tools can overcome some challenges associated with viral-based delivery and may be more suited to certain applications like gene editing. Gene-editors can induce base changes, insertions, and deletions, and it is possible to modulate gene expression by silencing or upregulation, but these approaches require further investigation. More knowledge is needed regarding the long-term immune consequences and durability of expressing bacterially derived gene-editing proteins. Further investigation is required to demonstrate the viability of multiple dosing rounds with non-viral delivery in terms of safety and improved efficacy.

Antisense oligonucleotides (ASOs) are a widely employed non-viral genetic manipulation method to induce exon skipping. Different backbone chemistries and antibody/ligand conjugates are currently under investigation for improved skeletal and cardiac muscle uptake and subsequent endosomal escape for nuclear targeting. Multi-exon skipping cocktails have been proposed to skip a range of exons simultaneously to increase the candidate patient population amenable to a single therapy. Similarly for gene-editing methods that target the removal and/or insertion of genomic sequences flanking mutation hotspot regions.

Summary points for Topic 2:

- Improve delivery to the target tissue while de-targeting tissues and organs associated with dose limited toxicity (e.g. liver, kidney).
- Define and reduce immune response to delivery vehicle to promote safety and redosing.
- Enhance capacity for on target delivery.

Priority Topic 3: Improve animal models to achieve specific goals in therapy development, including those that approximate the hallmarks of human MDs and their responses to treatment.

Pre-clinical testing is a key stage in the development of new therapies. There are several ways in which animal models can inform the subsequent regulatory pathway: (1) they provide insights into disease mechanisms, (2) they enable identification or pre-clinical evaluation of potential therapies, and (3) they support biomarker discovery. Endpoints (both functional and biomarkers) should be chosen based on their correlation with clinical outcomes and determined in partnership with clinicians and patients. The efficacy studies should demonstrate that the therapeutic has a meaningful effect on the phenotype. Finally, pre-clinical efficacy studies in animal models should address the durability of a given therapy.

The most appropriate animal model should be chosen for the specific purpose of the experiment. Factors such as phenotypic relevance, metabolism, immune response, and genetics should be considered. For many preclinical MD studies, further investigation is needed to ensure that the models accurately mimic human disease and that the model is predictive of human outcomes. Data from humans, especially clinical studies that can provide critically relevant samples, should inform the utility of any model, such that there is a clear and relevant relationship between humans and preclinical models. The stage of disease when humans can optimally receive a treatment is better informed by animal models that mirror the disease course and trajectory being targeted. For example, models relevant to the human immune response to AAV vectors and other therapeutic modalities are needed to optimize safety and efficacy of gene therapies.

There is a need for animal models that approximate the human immune response to gene and cell therapies or emulate the inflammatory component of muscle disorders. The immune response to disease in MDs is an important, yet poorly understood, disease modifier. Modeling the complexities of these immunological responses to MD is essential to fully realize the potential of immunomodulatory therapies to limit the dystrophic process. In addition, improved modeling of the human immune response to AAV is important to uncover, since the efficacy and safety of AAV-based delivery systems are limited by these immune responses. Comparison studies that determine the elements of murine and primate immune systems that accurately emulate human immune responses to disease and following AAV therapies are needed, relying on patient samples and/or natural history studies. Using these comparison studies, reference data sets should be developed and made available to the public.

Summary points for Topic 3:

- Determine animal models to accurately emulate each MD subtype. Animal models can mirror specific aspects of disease (e.g. fibrosis or cardiac involvement). Meaningful measures of disease indices should be established that are indicative of human outcomes and therapeutic benefit, and some outcomes may be MD subtype specific.
- Generate reference data sets of human and murine biomarkers; these data sets may need to be specific to MD subtypes.
- Develop animal models that accurately represent the human immune response in both MD pathogenesis and its response to treatments.

Priority Topic 4: Establish predictive human cell/tissue models and relevant endpoints that more faithfully represent human disease with minimal technical variability.

The MDs are genetically diverse diseases, arising from pathogenic variants on autosomes and the X chromosome. For each genetic form of MD, there are few "hot spot" mutations. Instead, a multitude of rare and ultrarare pathogenic variants underlie the MDs. Gene replacement is viable for genetic mutations where the protein's function has been lost. However, many pathogenic genetic changes are more suited to gene editing since this approach, in principle, better corrects and restores function. Gene editing tools are rapidly expanding. Moreover, the noncoding regions of the genome require more attention, since these regions may also harbor pathogenic genetic changes. Noncoding regions are also well suited for upregulation and downregulation strategies mediated by CRISPR activation or inhibition. Finally, an important subset of certain MDs arises from repeat expansion disorders, which have been challenging to establish and maintain in animal models. For these reasons, it is not feasible, nor in many cases even possible, to generate animal models of each human pathogenic myopathic variant. Human cells and tissue engineering have the potential to fill this gap.

Cellular reprogramming methods enable the generation of human cell models of skeletal and cardiac muscle. These "personalized" models are ideal to establish the molecular effectiveness of genetic correction. Human induced pluripotent stem cells (hIPSCs) can be created from blood, skin, or urine cells and avoid the ethical issues associated with embryonic stem cells. The use of cells, rather than animals, is consistent with FDA 2.0 Modernization Act 2022. hIPSCs bespoke models are useful for determining genetic pathogenicity, defining pathological mechanisms of disease, and testing gene-targeted and other treatments. hIPSC-derived cellular models are not fully mature, nor do they include the three-dimensional architecture of human tissues. Improving the efficacy, rigor and reproducibility of maturation methods, especially for skeletal muscle, will allow for physiological endpoint measurements that translate better to human muscle outcomes. Similarly, improving cardiac differentiation and maturation protocols will provide better models to assess the cardiac outcomes in MD. Bioengineering platforms and organoid approaches that incorporate additional cell types to more effectively mimic myopathic processes should promote better human translation.

Although mice have been a mainstay of preclinical assessment, there are fundamental differences between mice and humans. For example, mouse skeletal muscle is primarily composed of fast fibers, while human skeletal muscle has a greater contribution of slow fibers.

Future engineering methods should address fiber type representation since this may be important for functional outcomes.

An added benefit of using human cells as engineered models is their ability to reflect the full human genome context, which can and may need to be personalized for evaluating the efficacy of certain preclinical treatments. Human genomes harbor more diversity across coding and noncoding regions, and incorporating this genomic diversity into human skeletal muscle models is imperative to achieve clinical efficacy of treatments.

Summary points for Topic 4:

- Create reproducible models of rare and ultrarare muscle diseases.
- Develop improved tissue models with physiological endpoints that reflect the human disease context.
- As human tissue engineering is a newer field, criteria for rigor and reproducibility are especially relevant to reduce technical variability and better define true biological variability.

Metrics for Success / Anticipated outcomes

Priority Topic 1: Develop effective gene agnostic therapies by targeting common pathways implicated in pathogenesis across many different forms of MD.
Priority Topic 2: Improve delivery, safety, efficacy and durability of genetic therapies.
Priority Topic 3: Enhance use of animal models to achieve specific goals in therapy development, including those that approximate human immune responses, and establish standard operating procedures for their consistent and transparent use.
Priority Topic 4: Establish predictive human cell/tissue models and relevant endpoints that more faithfully represent human disease with minimal technical variability.

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